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Effect of different farming sites and techniques on the growing performances and quality of Pacific oysters (*Crassostrea gigas*, Thunberg)

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ABSTRACT

This research deals with the effects that different farming sites, containers and techniques produce on the growing performances, some quality parameters and shelf-life evolution of Pacific oysters (*Crassostrea gigas*).

Aims of this research were:

- to develop a proper experimental protocol useful for oysters commercial and in-conservation quality evaluation
- to evaluate the suitability for oysters growing of two farming sites on the Tuscany coast (Orbetello Lagoon and open sea near Porto Ercole)
- to evaluate the performance of oysters farmed in sea site utilising different kinds of containers
- to evaluate the feasibility of a finishing period in the open sea (Porto Ercole) for oysters previously grown in the Orbetello Lagoon.

Three trials were performed:

- trial 1 was a preliminary study on the quality characteristics and on their changes during 10 days of 4 °C refrigerated storage of commercial sized oysters with different Italian origin (Orbetello Lagoon and open sea near Manfredonia, Foggia);

- trial 2 was an experiment on the effect of different farming sites (Orbetello Lagoon and open sea near Porto Ercole) and different container typology (*poche*, lanterns, baskets) on oysters growing performances and commercial characteristics from spat to adult stage along a period of 14 months;

- trial 3 was performed by transferring in the open sea site commercial sized oysters previously grown in the lagoon, using two different containers (baskets and lanterns); the growing performances and the commercial characteristics of the oysters as well as their quality characteristics and conservation performances during 10 days of 4°C refrigerated storage were evaluated.

The best results for the growing performances and commercial characteristics were achieved by the oysters farmed in the lagoon, while the best container for growing in sea site resulted to be the basket. During the finishing period in the open sea site a general worsening of the commercial characteristics of the oysters

occurred, while the fatty acids profile quality improved. The best container for oysters in-open-sea finishing resulted to be the lantern. Lagoon oysters reacted in a better way to the storage conditions, presenting a higher resistance to spoilage factors. They also have been judged by a panel test with a higher score both for appearance and flavour.

Further research should be conducted to try other sea farming sites, more suitable for oysters farming and finishing, in order to improve the quality characteristics of the oysters farmed in the lagoon and obtain a high quality product.

INTRODUCTION

1 Elements of anatomy and physiology of bivalve molluscs and oysters

The deep knowledge of the anatomy and physiology of oysters, as for every zootechnic species, is fundamental to understand the reproductive and growing mechanisms that characterize every species, in order to plan a rational management of the farming cycle.

Oysters belong to the *phylum* Mollusca*,* class Bivalvia*,* order Filibranchia*,* family Ostreidae*.* The family Ostreidae is composed by the genera *Crassostrea, Ostrea, Saccostrea* and *Picnodonta.* Oysters generally live between the intertidal and subtidal zone. They are sedentary species, passing the whole life fixed to a rock or laying on the bottom up to 40 m deepness. The genera *Crassostrea* includes some species that are considered of commercial interest, like *C. gigas,* commonly known as Pacific oyster and *C. virginica*, commonly known as American oyster.

Bivalve molluscs shell morphology presents a large variety among the different species: colour, shape, texture and markings on the shell are characters widely used in species identification.

Crassostrea gigas have a solid, inequivalve, extremely rough, extensively fluted and laminated shell. The valves are hinged together on the dorsal side by a horny ligament that allows valves opening, while a strong adductor muscle allows the valves to close. The left (lower) valve is deeply cupped, its sides are sometimes almost vertical and the right (upper) valve is flat or slightly convex; inequilateral, beaks and umbones are often overgrown. The whole shell tends to be oblong in outline but is often distorted and very irregular. The shape of the shell varies with the environment (Quayle & Newkirk, 1989). The colour is usually whitish with many purple streaks and spots radiating away from the umbo. The interior of the shell is pearl white, with a single muscle scar that is sometimes dark, but never purple or black. The shell has several functions: it acts as a skeleton for the attachment of muscles, it protects against predators and helps to keep mud and sand away from the mantle cavity and it allows the animal fixation to a substrate before larval metamorphosis. Yoon et al. (2003) found that oysters' shell is composed almost completely by calcium carbonate (96%) and other minerals in trivial amount (SiO_2, MgO, Al_2O_3, SrO, P_2O_5, Na_2O, SO_3). The valves develop from the umbo and growing is evident on the shell thanks to

growth lines. Three layers make up the shell: a outer periostracum of horny conchiolin, a middle prismatic layer of aragonite and an inner calcareous layer. The outer mantle fold secretes the calcium carbonate extracted from the water and a complex protein, the conchiolin. This substance creates a matrix where aragonite crystals are entrapped in, to form the periostracum. The middle layer, also secreted by the outer mantle fold, is made of calcite crystals perpendicularly oriented to the shell surface. The inner layer is secreted by the general mantle surface and is in contact with the mantle; it is also made of calcite crystals but in this case they are parallel to the shell surface, so that its texture is very smooth. The energy require for shell growth is not an insignificant portion of oysters total energy budget (Hawkins & Bayne, 1992).

The mantle consists of two lobes of tissue which completely enclose the animal within the shell. Between the mantle and the internal organs is a capacious mantle cavity. The mantle consists of connective tissue with haemolymph vessels, nerves and muscles that are particularly developed near the mantle margins. Cilia on the inner surface of the mantle play an important role directing particles onto the gills and in deflecting heavier material along rejection tracts towards the inhalant opening, the entry point on the mantle for incoming water. The mantle is attached to the shell by muscle fibres. The line of attachment, the pallial line, runs in a semicircle at a short distance from the edge of the shell. In oysters the inhalant opening is very large mirroring the gills, while the exhalant opening is smaller. The mantle margins are thrown into three folds: the outer one secretes the shell, the middle one has a sensory function and the inner one, called velum, controls water flow in the mantle cavity. The mantle plays a role in the storing of energetic reserves and in the bioaccumulation of metal and organic contaminants.

Oysters, as all lamellibranchs, feed by using the incoming current as a source of food. Generally in bivalve molluscs the gills follow the curvature of the shell margin with the maximum possible surface exposed to the inhalant water flow. Each gill is made up of numerous W-shaped filaments: each filament is composed by two V-shaped filament called demibranch and each arm of it is known as lamella. Each demibranch is made up of a descending and an ascending lamella and in the space between them is the exhalant chamber connected with the exhalant area of the mantle edge. In some bivalves, including oysters, the surface area of the gills is

greatly increased by folding and cilia, allowing a specific filtration for large and fine particles. In oysters, cilia are very small, so that they can sort little particles of 5-6 µm (Dillon, 2000). Lateral cilia of the filaments mantain a flow of water through the mantle cavity and the gills. The latero-frontal cilia strain the particles from the water and throw them onto the frontal surfaces of the filaments which are rich of mucocytes. The particles are trapped in a fine mucus layer an then transported towards the ventral ciliated particle grooves and then towards the labial palps (Beninger & St-Jean, 1997).

In addition to feeding role, gills have a respiratory function: their large surface area and rich haemolymph supply make them well suited for gas exchange. Deoxygenated haemolymph is carried from the kidneys to the gills by way of the afferent gill vein. Each filament receives a small branch of this vein. The filaments are essentially hollow tubes within which the haemolymph circulates. Gas exchange takes place across the thin walls of the filaments. The oxygenated haemolymph from each demibranch is collected into the efferent gill vein that goes to the kidney and on to the heart.

The bivalve digestive system consists of a mouth, oesophagus, stomach, digestive gland, intestine and anus. Once the particles trapped in the mucus reach the labial palps, they are ready to enter in the mouth. The mouth is ciliated and leads into a narrow ciliated oesophagus. Both mouth and oesophagus are well supplied with mucocytes secreting acids and neutral mucopolysaccharides, even if the animal is not feeding (Beninger & Le Pennec, 1991). Ciliary movement helps to propel food into the stomach. The stomach is large and oval-shaped and lies completely embedded in the digestive gland that opens into it trough several ducts. Extending from the duct openings are ciliary tracts that traverse the floor of the stomach to the intestinal groove. A semi-transparent gelatinous rod, the crystalline style, originates in the style sac at the posterior end of the stomach and rotates against the gastric shield, a thickened area of the stomach. The rotative movement is caused by the co-ordinated ciliary beats of the stomach and intestinal epithelia. As it does so, it is abraded and dissolved releasing in the process digestive enzymes, such as amylases. Ingested particles are mixed with the released digestive enzymes from the crystalline style. In intertidal bivalves, such as oysters, the style is not a permanent structure: it dissolves at low tide, when the animal is not feeding and is reformed at high tide when the

animal restart feeding (Langton & Gabbott, 1974). During the mixing and extracellular digestion process the stomach content comes under the ciliary tracts that cover the stomach walls. These tracts have fine ridges and grooves and act as sorting areas: finer particles and digested matter are kept in suspension by cilia at the crests of the ridges and are continuously swept towards the digestive gland; larger particles, such as sand grains, are channelled into the intestine along a deep rejection groove on the floor of the stomach. Enzymes reported to be present in bivalves stomach by Bayne et al. (1976) and Mathers (1973) are esterases (that are important in lipid digestion), acid and alkaline phosphateses (playing a role in absorption of material from the stomach) and endopeptidases (such as trypsin), that brake down proteins; enzymes with a carbohydrate splitting activity (such as α-amylase α- and β-glucosidase, β-galactosidase, maltase, chitinase and cellulose) have also been reported.

The intracellular digestion occurs into the digestive gland. The digestive gland consists of blind-ending tubules connecting the stomach by several ciliated ducts. Within these ducts there is a continuous two-way flow: material entering the gland for intracellular digestion and wastes living the gland to return towards the stomach and then to the intestine. The tubules are of two cell types, digestive cells and secretory cells. Digestive cells, responsible for intracellular digestion of food, are columnar and vacuolated. They take up particulate matter by pinocytosis and digest it within large vesicles called lysosomes, containing hydrolytic enzymes. The end products of the digestion are released directly into the haemolymph.

The intestine receives the rejected particles coming from the stomach. These particles are not necessary lost, since there are haemocytes that can freely migrate in the gut and may phagocytose them, digest and transport them round the body. These cells have an important role in intracellular digestion. Mathers (1973) reported the following enzymes being present in the mid-gut wall (first part of the intestine) of *Ostrea edulis* and *Crassostrea angulata*: α-amylase, maltase, trehalase, cellobiase and various glucosidases.

The intestine terminates in an anus and faeces in form of faecal pellets are swept away through the exalant opening.

1.1 Feeding behaviour

Oysters, like all bivalve mollusc, are filter-feeders which regulate the quantity of food taken in by adapting the rhythm of filtration to the concentration of available food (Foster-Smith, 1975; Bayne, 1991). Oysters are suspension-feeders, because they feed on the particles suspended in the surrounding water. The source of food for oysters consist of particle elements and dissolved organic substances. Particle elements are phytoplankton and bacteria. Phytoplankton, mostly diatoms and dinoflagellates, can be present as living organisms, containing chlorophyll, or as deteriorated matter, containing phaeopigments. Bacteria have an important role in supplying oysters metabolism requirements: oysters can supply organic molecules and vitamins by ingesting bacteria directly or can take advantage of the amino acids and exoenzymes (like lipase and protease), that bacteria release in the water. These and other organic substances (like glucose and humic and fulvic acids) are present in the water in different concentration around the year, depending on the season as well as on the tidal action.

Feeding behaviour of bivalve is still a very discussed point. Many researchers proposed bivalve feeding models with the aim to evaluate the potential of shellfish growing areas.

Bougrier et al. (1997) recorded differences in particle selection between the oyster *Crassostrea gigas* and the mussel *Mytilus edulis*. Further, they suggested that pre-ingestive selection may vary according to the relative proportions of different planktonic algal species within the seston. This capability to select is not dependent only upon size. Tamburri & Zimmer-Faust (1996) observed that chemical stimuli were needed to induce oysters (*Crassostrea virginica,* Gmelin) to ingest large particles (0.200 mm). Baldwin (1995) and Baldwin & Newell (1995) recorded variable feeding patterns in oyster larvae, where ingestion is dependent not only on particle size but also on its ‘‘chemical quality’’. Flow cytometry has been considered a powerful technique to analyze pre-ingestive suspension-feeding behaviour on natural assemblages of particles (Bayne, 1998).

Application of flow cytometry techniques to the nutrition of marine organisms has allowed the study of feeding behaviour in animals fed different diets (Cucci et al., 1989).

It is well established that molluscs are able to sort particles using their labial palps, resulting in the preferential rejection of inorganic material in pseudofaeces (Newell et al., 1989). Further, such differential rejection may be influenced by the composition of each particle (Deslous-Paoli et al., 1992). Working with *Crassostrea gigas*, Deslous-Paoli et al. (1992) showed an enrichment of protein and lipid in the ingested food, whereas the concentration of sugar in pseudofaeces increased.

A study performed by Bougrier et al. (1997) on *C. gigas* and *M. edulis* feeding behavior underlined how strong is the preingestive selection activity by the two species. They found that feeding simultaneously the bivalves upon a diatom (*S. costatum*) and two flagellates (*P. lutheri* and *T. suecica*), *M. edulis* carried out a preingestive selection, filtering more *T. suecica* than either other algal species, while *C. gigas* filtered preferentially *S. costatum*. They also observed that oysters selected algae independently of the size, volume or carbon content of each algal species. They may be selected depending on organisms' shape and flexibility.

Saito & Marty (2010) compared Pacific oysters farmed in France and in Japan. They investigated about the relationship occurring between the natural diet of the two farming sites and its effect on the composition of the fatty acid profile, focusing on PUFA n-3, EPA and DHA content. They found that the composition of the fatty acid profile of the oysters did not differ in a significant way between oysters reared in Japan and in France, may be because Pacific oysters are able to filter only a limited type of phytoplankton, according to Bougrier et al. (1997).

2 Environmental factors affecting bivalve and oysters growth

Sedentary bivalve molluscs are able to tolerate the wide range of water conditions which commonly occur in their natural estuarine or coastal habitats. However, their growth rates and flesh condition are strongly influenced by these fluctuations in environmental conditions. Temperature, salinity, particulate matter, food availability, current speed and water depth have been found to exert varying degrees of influence upon their growth and condition (Calow, 1977, Fernandez-Reiriz et al., 1996; Sarà & Mazzola, 1997).

Studies performed on different bivalve molluscs species indicated that substantial variability in bivalve growth rates can occur even within a single estuary or

embayment and that the relationship between growth or flesh condition and environmental parameters is complex. This reflects the wide range and rapid fluctuation that can occur in environmental variables within an area.

In most of the sites studied, environmental data indicate that increased temperature and food availability may have the strongest positive influence on bivalve growth and condition (Hickman et al., 1991; Thorarinsdottir, 1994; Sarà & Mazzola, 1997). Prolonged exposure to low salinity may restrict shell and tissue growth, even though high nutrient levels in these water conditions promote phytoplankton growth (Brown & Hartwick, 1988a, 1988b). While shell growth is slow, flesh condition may remain unaffected by low salinity (Hickman et al., 1991). Adequate vertical mixing and current flow can promote food availability, nutrient and water exchange rates (Heasman et al., 1998; Boyd & Heasman, 1998) while correct farming density and absence of fouling organisms on farming trays is very important in maintaining food availability and therefore growth rates of mussels suspended in rafts (Boyd & Heasman, 1998). In view of that is clear how site selection is a critical point for a successful, profitable and sustainable farming. The existence of a number of factors that can affect shellfish as well as oysters culture site suitability is widely recognized and traditionally they have been grouped as physical, water quality, biological, and socioeconomic factors (Buitrago et al., 2005).

2.1 Biological factors

Food supply has consistently been shown to be the most important factor affecting bivalve growth and it is positively correlated with phytoplankton abundance (Smaal & Van Stralen, 1990). Primary productivity is the rate at which new algae cells are produced in the water, and it is dependent on various environmental factors, including nutrient availability, light (turbidity) and temperature. It has been estimated that when bivalves are grown under similar conditions at different sites, up to 85% of any difference in growth observed between sites can be attributed to water temperature and primary productivity (Laing & Spencer, 2006).

Since the photosynthetic pigment chlorophyll-*a* is present in all autotrophs, levels of phytoplankton at bivalve culture sites can be directly correlated with the amount of chlorophyll-*a* extracted from site water samples. Chlorophyll-*a* concentration is a

measure of the amount of phytoplankton in the water. Phytoplankton cells are the principal food source for sedentary bivalves. However, the chlorophyll-*a* content of water alone may not be sufficient to indicate site suitability for bivalve farming.

Researchers have found that high phytoplankton levels are a stimulant to improving growth and meat condition of bivalves (Brown & Hartwick, 1988a; 1988b; Sarà & Mazzola, 1997; Heasman et al., 1998). The composition of the phytoplankton influences the chemical composition of the farmed oysters. In particular it is reflected by the fatty acids profile: Saito & Marty (2010) investigated about the relationship occurring between the natural diet composition of two farming sites, France and Japan, and the composition of the fatty acid profile, focusing on PUFA n-3, EPA and DHA content. They found that oysters farmed in France had high EPA and low DHA levels, while Japanese oysters had higher DHA levels They attributed this differences to the higher abundance of diatoms in French water and of dinoflagellates in Japanese water, concluding that dinoflagellates are the most important source of EPA for Pacific oysters.

To satisfy their energy requirements bivalves also exploit non-phytoplanktonic carbon in form of re-suspended sediment resulting by the mixture of benthic microflora, fine organic detritus and inorganic material (Hickman et al., 1991; Perez-Camacho et al., 1995). Results from laboratory and field experiments have shown that in appropriate concentration sediment suspension enhances growth (Gosling, 2003).

Food supply in the water is influenced by factors such as temperature, aerial exposure, water depth and population density.

Biofouling is the attachment of marine organisms, either plant or animal, to the object of interest. It is a diffused problem in bivalve molluscs farms, especially on longline systems and where the water is rich of nutrients. The biofouling consists of sessile organisms, mainly filter-feeders affecting both the structure of the sea culture system and the breeding species (Enright, 1993; Lodeiros & Himmelman, 1996; Ross et al., 2004). If the abundance of these organisms becomes too high, there is competition for space and food between the cultivated shellfish and the biofouling species, which can reduce the growth of the oysters. Moreover, the weight of biofouling could increase the weight of the shellfish culture system, which can cause damage and loss of product and increase costs for maintenance of the underwater

structures. Fouling organisms also affect the final product value: tunicates as ascidians, barnacles and other epibiont organisms fix on the shells surface and are difficult to remove, making the final product less valuable on the market.

After the prohibition of use of antifouling paints, Sala & Lucchetti (2008) designed a low-cost tool, called "*Wave Brush*", useful to limit the problem of biofouling. It consists of PVC ropes applied on baskets used for oysters culture: thanks to the movement of the water the ropes constantly brush the outer surface of the container, thereby preventing the fixation of the fouling organisms, especially mussels. The authors observed that seven months after the placement of the structures only 4% of the external surface of the experimental baskets was covered by fouling, while the control baskets had a biofouling coverage of 64%.

2.2 Physical factors

Sea water temperature has a major effect on the seasonal growth of bivalves and may be largely responsible for any differences in growth between sites. It is influenced by factors such as latitude, water depth, shelter, and tidal exchange. It also varies between years.

Water temperature is an important factor that is directly correlated to animals metabolism and reproduction activity and also to the composition and abundance of food. Abad et al. (1995), studying the seasonal variation of lipid composition in *Ostrea edulis*, found a positive correlation between n-3 PUFA content and the peaks of water temperature during the year, probably due to the higher number of diatoms in the water. Spawning and larval surviving are also highly dependent on water temperature. A recent study conducted by Dutertre et al. (2010) on the invasion of European Atlantic coasts by *C. gigas* underlines how warmer water temperatures during the summer, up to 20 °C, create the ideal environment for spawning and for high larval surviving, causing a coastal invasion of the bivalve. It seems that elevated water temperature together with other stressors can be also associated to summer mortality outbreaks occurring in *C. gigas* (Samain & McCombie, 2008; Sauvage et al., 2009).

Pacific oysters tolerate low winter temperatures as well as high summer temperatures: the minimum requirement for growth is of 8-9 °C, while the maximum tolerated temperature is of 29-30 °C (Laing & Spencer, 2006).

Salinity is highly relevant to growth, maturation, distribution food intake, energy activation, and metabolic activities of many types of bivalve, including Pacific oyster (Jo & Choi, 2008). Fluctuations in salinity induce several osmotic responses in bivalves aimed at preserving cellular volume, such as controlling the accumulation of organic compounds known as osmolytes (Somero & Bowlus, 1983). Bivalves generally respond to changes in external salinity by closing the shell valves and by adjusting the intracellular concentration of ions, amino acids and other small molecules to maintain relatively constant cell volume. Initially rates of feeding and respiration are depressed, but gradually recover as osmotic equilibration is reached.

In *C. gigas* two tipes of hemocytes exist, which are differentiated by their morphology: hyalinocytes and granulocytes (Cheng, 1981). The results of a study performed by Jo et al. (2008) on osmoregulation of *C. gigas* suggested that osmolality and Na^+, Cl^-, and Ca^{2+} concentrations were stabilized by the Heat Shock Protein 68 and Glucose Regulated Protein 78, and indicate that these two stress-induced proteins play an important role in regulating the metabolism and protecting the cells of the Pacific oysters exposed to salinity changes (Jo et al., 2007).

Changes in salinity do not affect the growth of Pacific oysters as much as variation in temperature. Pacific oysters have their optimum salinity levels between 20 and 30‰, conditions typical of many estuaries and inshore waters.

Water depth and flow can affect bivalve and oysters growing performances. Water depth is inversely related to light and oxygen availability and consequently to food supply, but also with the presence of fouling organisms, that are generally suspension feeders too (Gosling, 2003). Water flow can stimulate the filtration rate of bivalves. This is important when a farming site have to be chosen: higher water flow can compensate a non-optimal food supply, while a low water flow can create food limitation if the stoking density is high (Wildish & Kristmanson, 1985).

Oyster culture has been traditionally developed in coastal areas (Burbridge et al., 2001), but recently offshore sites are being considered for aquaculture plants.

A feasibility study by Buck (2002) on offshore aquaculture in the North Sea identified *C. gigas* and *O. edulis* among the most suitable species for this farming technique. In this case is important to analyze the hydrodynamic characteristics of the water column. Pogoda et al. (2011) performed experiments on the combinated effect of season, farming site and farmed species on oysters growing performances in order

to try to move farming plants from coastal areas to offshore areas in the North Sea. They found that offshore sites are the most suitable ones to obtain good growth performances from spat to commercial size and that the higher current speed, compared to a coastal zone, can compensate the lower nutrient supply because of the stimulating action of the currents on filtration activity of the oysters. They also observed that an excessive current strength can inhibit filtration rate, causing stress to the bivalves and consequently worsened growing performances, according to Gosling (2003).

3 Farmed species and their distribution

The family Ostraeidae consists in more than hundred species, but only few of them are considered for farming.

The genus *Ostrea* (Linné1758) is distributed in Europe with the species *Ostrea edulis,* also known as European oyster. It is the only one endemic species of European and Mediterranean coasts: it occurs from the coast of Norway to the waters near Morocco, through the Mediterranean Sea, and into the Black Sea (Héral, 1989). It is mainly cultured in France, Spain, Ireland, UK, Norway, Netherlands, Greece and Morocco and, out of Europe and Mediterranean area, in USA and South Africa.

O. edulis exists as a series of physiological strains, and genetic differentiation has been demonstrated along the European coastline. One of the lowest temperature strain occurs in Spain where 12-13 °C is required for spawning while 25 °C is the spawning temperature required in Norwegian fjords. In France, gametogenesis occurs at 10 °C and spawning between 14 and 16 °C (Launey & Hedgecock, 2001).

European oyster has been part of the human diet for many centuries. The Romans built ponds to stock and sort oysters. European oyster always continued to be appreciated and during the Renaissance was increasingly appreciated, especially by the French nobility. High abundance and easiness of harvest led to an over-exploitation of the natural banks causing the irreversible destruction of the natural resource. This situation led to the development of many culture techniques, from the seed collection to the growing and fattening. In 1920 a massive mortality widely struck European flat oyster populations, so it was partially substituted with *C. gigas* coming from Japan. Between 1970 and 1980 two diseases (*Martelia refrigens* and *Bonamia*

ostreae) decimated the remaining population of *O. edulis*. As *C. gigas* presented resistance to these diseases, it substituted the endemic species in European farming. Despite new management practices, and intensive repletion programmes, the production of *O. edulis* has remained low since that time.

The genus *Crassostrea* (Sacco, 1897) consists in many species of economic interest. Among them two species are particularly interesting because of their economic interest and distribution: *Crassostrea gigas* and *Crassostrea virginica*.

Crassostrea gigas is also known as Pacific cupped oyster or simply Pacific oyster; it is originated from the west Indo-Pacific region and was introduced in Europe from Japan in the early 1900. Its diffusion had never stopped, so that today it is farmed worldwide, thanks to its adaptability, diseases resistance and fast growing. Pacific oyster is indeed the most important commercial species, representing 40% of the total bivalves production (FAO, 2012).

The Pacific oyster is an estuarine species, preferring firm bottom substrates where it leads a sedentary existence attached to rocks, debris and shells from the lower intertidal zone to depths of 40 m. However, these oysters can also be found on mud and sand-mud bottoms. Optimal salinity range is between 20 and 25‰ although the species can occur at salinities until 10‰ and will survive salinities in excess of 35‰ (Korringa, 1952; Spencer, 1990).

Crassostrea virginica (Gmelin, 1791), also known as American cupped oyster, ranges from Canada's Gulf of St. Lawrence to the Gulf of Mexico, the Caribbean, and the coasts of Brazil and Argentina. It has been introduced to British Columbia, Canada, the west coast of the United States, Hawaii, Australia, Japan, and the United Kingdom, but has not established self-sustaining commercial populations. It is cultured throughout its eastern North American range and in places on the North American west coast, and almost nowhere else. *C. virginica* has a wide range of tolerance both to temperature and salinity, with an optimal range for larval development between 17.5 and 32.2 °C. Kohen & Shumway (1982) found that the respiratory physiology of *C. virginica* is highly adapted to life in a fluctuating environment.

In eastern North America, culture efforts began a century ago to rehabilitate over-harvested oyster grounds and improve harvests.

In western North American in the late 1800s, pollution and overfishing depleted the beds of the native Olympia oyster *Ostrea conchaphila*, so *Crassostrea virginica* was imported from the east coast. Western summer temperatures were too cool for dependable reproduction to occur in *C. virginica*, so small seed oysters (~2.5 cm) were imported annually and held in coastal bays to reach commercial size.

Eventually the bays became polluted, so west coast governments and entrepreneurs turned to the Pacific oyster as the basis for a thriving aquaculture industry after 1929.

Another species that have to be mentioned for its commercial importance is *Saccostrea glomerata* (Gould, 1850), also known as Sydney rock oyster. Its cultivation began in New South Wales (NSW), Australia, around 1870. Around 1888, spat of oyster from New Zealand were imported into NSW to replenish depleted oyster stocks. The mudworm, *Polydora* sp., appeared in NSW concurrently with the introduction of New Zealand oysters, and farmers had to develop intertidal farming methods using sticks and trays (Smith, 1982). This was the dominant growing system from the 1950s till the 1990s and provided some protection against mudworm infestation. *S. glomerata* is now diffused in Australia, from southern Queensland to eastern Victoria and New Zealand. It grows and survives best in intertidal estuarine habitats such as rocks, mangroves, and man-made structures, but it also occurs subtidally on natural dredge beds. It has a high adaptability to wide ranges of temperature and salinity.

4 Oysters as a commodity

Aquaculture is indeed a growing economic activity in the World. Seafood demand continuously grows worldwide, both in developing and also in developed countries, where seafood is considered a functional food due to its important role in human health. It represents the largest stock available of n-3 polyunsaturated fatty acids, especially eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids (Heidmann Soccol & Oetterer, 2003), both taking part in many biochemical processes. It is also accepted that aquaculture has a great potential as food source, since the human population grows very fast, the overexploitation of the land and sea is already a global

problem and that a relative small share of our marine resources has been used for food production.

While feed is generally perceived to be a major constraint to aquaculture development, one-third of all farmed seafood production is represented by oysters, mussels, clams, scallops and other bivalve species, that are grown with food materials that occur naturally in their culture environment in the sea and lagoons (FAO, 2012). Figure 1 shows the trend of the world aquaculture production of non-fed and fed species from 1980 to 2010.

World fisheries and aquaculture production in 2010 supplied 148,000,000 t of seafood, 40% of which came from aquaculture production. Among the aquaculture production 30.5% of the product came from marine environments. Marine-water aquaculture production (18.3 million tonnes) consists of marine molluscs (75.5%, 13.9 million tonnes), finfishes (18.7%, 3.4 million tonnes), marine crustaceans (3.8%) and other aquatic animals (2.1%) (FAO, 2012).

Aquaculture represents the fastest and largest growing sector in food industry (Costa-Pierce, 2002), since the growth rate in farmed food fish production from 1980 to 2010 far outpaced that for the world population (1.5%), resulting in average annual per capita consumption of farmed fish rising by almost seven times, from 1.1 kg in 1980 to 8.7 kg in 2010, at an average rate of 7.1% per year.

Figure 2 shows the relative contribution of aquaculture and capture fisheries to food fish consumption from 1970 until 2010.

Figure 1. Aquaculture production of non-fed and fed species.

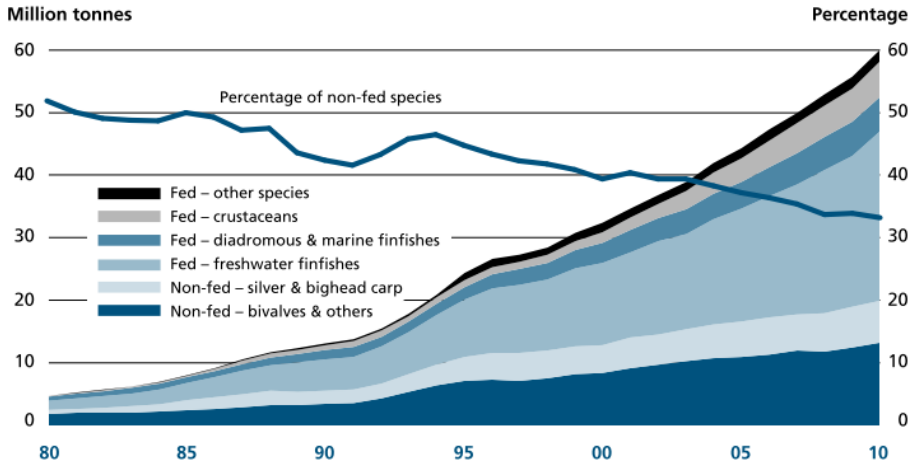
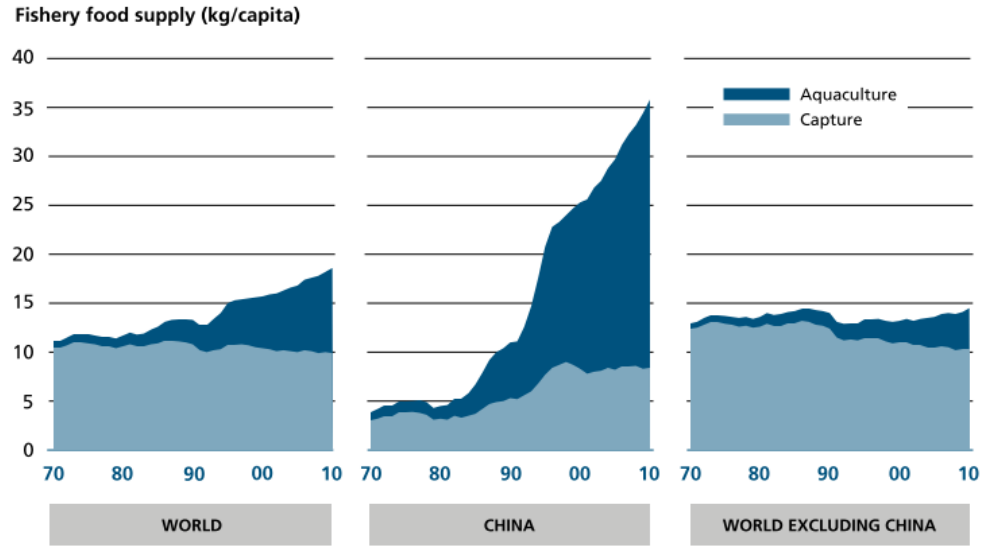


Figure 2. Relative contribution of aquaculture and capture fisheries to food fish consumption from 1970 until 2010 (FAO, 2012).



Of the 18.4 kg of fish per capita available for consumption in 2009, about 74% came from finfish. Shellfish supplied 26% (or about 4.5 kg per capita, subdivided into 1.7 kg of crustaceans, 0.5 kg of cephalopods and 2.3 kg of other molluscs).

Regarding molluscs, aquaculture production of clams and cockles has increased much faster than that of other species groups. In 1990, clam and cockle production was half that of oysters, but by 2008 it exceeded oysters and became the most-produced species group of molluscs.

A significant part of the global production of marine molluscs, particularly in Europe and America, relies on the widely introduced Japanese carpet shell (*Ruditapes philippinarum*, also known as Manila clam) and Pacific oyster (*Crassostrea gigas*).

In many countries, seafood represents a fundamental component of the human diet. In general, despite the demand for finfish is predominant, the request for molluscs becomes increasingly important, in particular for Bivalves. It should be highlighted, however, that the preponderance of Bivalve production results from harvesting on wild populations in the natural environment, thus contributing to deplete the wild stocks. A plausible solution to the overexploitation of resources, but simultaneously to supply the increasing demand from the Bivalve industry, is the hatchery culture, in particular with regard to clams and oysters.

The most important continent for seafood industry coming from aquaculture is Asia, where 89% of the global output were produced in 2010, followed by Americas and Europe producing respectively 4.3 and 4.2% of the total production and then by Africa and Oceania with 2.15 and 0.3%, respectively (FAO, 2012).

In 2010 Spain was the main European producer of fisheries product, that amounted to 1,221,000 tonnes, of which 80% came from captures and 20% from aquaculture. France resulted the 2nd fisheries producer with 651,000 tonnes, of which 65.5% came from captures and 34.5% from aquaculture. Italy followed with a global national fish production of 387,600 tonnes representing 15.4% of the European production, at a value of about 360 million €. Italian production is represented for 60% by fishing activities and the remaining 40% by aquaculture. Molluscs production in Italy represents the 26% of the total aquaculture production. In 2010 Italy was also the 3rd producer of molluscs in Europe after Spain and France, with a total production from aquaculture of 101,000 tonnes of which 35% is represented by *Ruditapes philippinarum* and 64% by *Mytilus galloprovincialis*. Unlike the other two countries, in

Italy the oyster production is negligible, even if this shellfish fetches a high price on the market (Table 1).

If we consider the Italian bivalve production and that Italy in 2009 was the 1st exporter of clams in the world with 3,600 tonnes, it means that bivalve culture in Italy is an important part of aquaculture production, and that farming sites are potentially exploitable for other species as the oysters. This bivalve species is widely appreciated by Italian consumers, since Italy is the 2nd importer of oysters after Spain with 6,000 tonnes. Many authors agree on the fact that oyster rearing in Italy could contribute significantly to the diversification of Italian aquaculture, since this shellfish has a great potential on the market (Maffei et al., 1996; Mattei & Pellizzato, 1997; Roncarati et al., 1998, 2009; Turolla & Rossi, 2004; Prioli, 2008).

Table 1. Prices of some species of bivalve molluscs on Rome market (ISMEA, 2011).

Species	Origin	Minimum price (€/kg)	Maximum price (€/kg)
Razor clam	Italy	8.50	12.50
Scallop	France	9.00	12.00
Smooth clam	Italy	7.00	9.00
Mussel	Italy	1.20	1.80
Pacific oyster	France	7.50	13.50
Flat oyster	France	13.00	18.50
Clam	Italy	4.00	7.00

5 Oysters farming techniques

Oyster seed can be obtained from natural beds by placing artificial collectors into the water. In case a natural seed collection is impossible because of spatfall lack or a disease, it can be obtained by hatcheries specialized in seed production (Goulletquer & Héral, 1997).

Several methods are used to grow-out oysters, depending on the area.

We can distinguish the following types of breeding oyster systems:

- on-bottom culture
- rack culture
- suspended culture.

On-bottom culture is practised intertidally or in deep water, in areas where the sea floor is stable enough to support some kind of farming structures and where silting-up is flat. This method is very old, born in Hong Kong's Bay at least 150 years ago (Bromhall, 1958; Morton, 1975), and practised in Thailand (Bromanonda, 1978), Mexico (Lizarraga, 1974), Philippines (Young & Serna, 1982) and Brazil (Akaboshi & Bastos, 1977). Following 1-2 years of pregrowing, spat are scraped from the collectors, sorted by weight, and then put back on the bottoms for further growth (Goulletquer & Héral, 1997). Oysters can be placed on the bottom inside PVC mesh or still attached to the spat collectors. In this way, oysters are harvested after 4-6 years depending on market demand. Harvest is performed with low tide conditions, then oysters are placed in trays to continue the production cycle. This technique has been almost completely mechanized, so that production costs have been significantly reduced. The mean density for *C. gigas* of 5 and 7 kg/m^2 generally adopted during the pregrowing and growing stages, respectively (Bâcher, 1984), allows to obtain 20 tonnes of final product from 1 ton of seed.

Rack culture is a widely diffused techniques in North Atlantic region, where tidal excursion is high. The seed is sorted and deployed in bags that are 1 m long and 0.5 m wide and whose mesh size depends on the oyster size. The bags are fixed on racks located 0.5 m off-bottom. Oysters stay on the racks for 1-3 years, until commercial size is reached. Although more efficient than on-bottom culture, this method can lead to overcrowding of oysters in bags and siltation underneath the tables by biodeposition. It therefore requires stricter management regulations. Oyster bags weigh around 5 kg initially and 15-20 kg when the oysters reach commercial size (Bâcher, 1984). Rows of racks are placed parallel to each other, depending on the tidal current pattern and direction. The advantages of this culture system are the good growth rate of oysters, good quality of the final product, low mortality rates and the easiness to reach the farming structures, which are periodically exposed to air during low tide, thus limiting fouling organisms. The harvest can be performed manually or with the help of a ship or tractor, depending on the tide conditions.

Suspended culture is nowadays the most used method. It relies on either a raft or longline system (with buoys and lines) floating on the sea surface from which the cultured oysters are suspended, usually into appropriate containers. The raft system was born in Japan and soon the technique was adopted in many other countries, due

to the high growth performances obtained (12-18 months are generally enough to reach commercial size). It consists of frames and floats of different material and is anchored at depths of about 6-10 m (Watters & Martinez, 1976; Ng, 1979; Kamara, 1982). Also the long-line method was born in Japan, with the aim to extend the oyster farming to more open waters, given the growing pollution and overcrowding near the shoreline (Imai, 1971). It is characterized by cheap construction materials and it is easy to maintain. A sequence of buoys are secured together by synthetic ropes, positioned parallel to the dominant winds, and anchored by concrete blocks on the bottom. The suspension culture requires a management activity, like periodical cleaning of the submerged structures from the fouling organisms and also a periodical control of the stability of the structure, depending on the currents energy power.

6 Oysters finishing

Oysters finishing is a technique used on commercial sized oysters, that is performed just before selling with the aim to give to the final product some characteristics as particular taste or colour. Generally, the finishing techniques application give to the final product a higher market value.

In France there is a consolidated tradition in finishing oysters. The technique consists in completing the fattening phase of oysters into special ponds, called *claires*. These ponds are only 0.4 m deep and are characterized by a high phytoplanktonic productivity. The presence of the diatom *Haslea ostrearia* is fundamental for the product refining, since it can give to the oysters a special flavour and colour, very appreciated on the French market. After its death the diatom releases a green pigment absorbed by the oysters trough the gills, that become deep-green coloured.

Piveteau (1999) found that after finishing in *claires*, the oysters had a significantly higher condition index, as a consequence of a higher glycogen content. He also observed that, even if the total content of lipids has not changed significantly, the fatty acids profile presented a higher content of n-3 PUFA. The taste also resulted significantly different, as a result of the glycogen abundance and also of the increase of volatile compounds resulting by the long chain n-3PUFA oxidation.

Baud et al. (1995) found that the flesh of finished oysters was more refined yellow, the mantle edge was black, it was less fat and more translucent appearance. Moreover there was a higher rate of filling intervalvar volume, the taste was more intense due to the increase of total sugars and glycogen content and finally, the mouthfeel of fat oysters was more refined and paste.

Oysters finishing is generally performed during the Autumn-Winter period for 1-2 months. It can be extended up to 4 months with a density reduction up to 5 oysters/m^2, in order to increase the nutrient quantity available for each oyster and to obtain a final product with higher condition index and richer taste. Oysters finished for up to 2 months into the *claires* are known as *fines de claires*, while the oysters that remained into the *claires* for 4 months are known as *poussés en claires.*

Finishing technique application can lead to environmental problems. Trying to standardize this technique, it was tried to increase the microalgae content into the water of the *claires* by using fertilizers (Robert, 1982; Turpin, 1999). The fertilization with phosphates in order to cause eutrophization of the water, did not give satisfactory results. On the other hand, the introduction of pure cultures of *Haslea ostrearia* into the *claires* of about 380,000 cells/oyster/day resulted to be the best technique and it is still used to guarantee oysters greening (Soletchnik et al*.,* 2001).

In France 40% of the produced oysters is finished in the *claires*, since this kind of product has a great demand on the French market.

7 Bivalve molluscs contaminants

Dealing with bivalve molluscs is also necessary to deal with health security. Bivalves are filter-feeders, thus concentrating in their body some viruses, biotoxins, trace elements like metals, or polluting substances coming from anthropic activities, until 100-200 times compared to the water (Dillon, 2000).

Bivalve molluscs, unlike other products, are commercialized alive and vital, following the European Community Reg. 853/2004 (European Commission, 2004) and they are usually consumed raw.

Depending on the species, dimensions and relaying temperatures, bivalve molluscs are able to filter varying quantities of water: a mussel at 14 °C can filter up to

14 L per hour, European oyster 12 L per hour at 15 °C, while American oyster can filter more than 18 L per hour at 20 °C (Richards, 1988).

The role that bivalve molluscs play in transmitting some disease to humans is well known. Even if since the 1950s the water depuration techniques improved and the disease incidence decreased significantly, bivalve molluscs consumption never stopped to represent a problem for human health.

According to data from the Centers for Disease Control and prevention (CDC), for example, in the decade 1978-1987 occurred, only in the United States, a total of 128 outbreaks of various types of gastroenteritis and 3,747 cases associated with consumption of shellfish (National Advisory Committee on Microbiological Criteria for Foods, 1992). It is reasonable to assume, however, that the available epidemiological data are underestimated and in many cases the consumption of shellfish causes only mild gastrointestinal symptoms that do not result in any recourse to the health system.

In order to avoid bivalve molluscs contamination with substances harmful for human health, the EC Regulation n. 853/2004 and the D.LGS 530/1992 sets out the measures to be taken for the market input, hygiene rules to apply to the process of production and harvesting of live bivalve molluscs, requirements of purification centres, health standards concerning marine biotoxins thresholds in the edible parts, wrapping, packaging and labeling.

7.1 Chemical contaminants

Of the food substrate used by bivalve molluscs, phytoplankton represents the most important one.

Of the 5,000 phytoplankton species known to date, under specific circumstances about 300 of them have a high proliferation rate, resulting in high density algae clouds called blooms. Blooms are sometimes beneficial for aquaculture and marine biology (Benemann, 1992). However, of the 300 phytoplankton species mentioned above, more than 40 species belonging to the classes of dinoflagellates and diatoms are known to produce phycotoxins (marine toxins) (Daranas et al., 2001). It has been suggested that certain phytoplankton species produce toxins to compete for space with other phytoplankton species (Botana et al., 1996).

Phycotoxins represent a serious problem to bivalve molluscs production, since once they are accumulated by the animal it is not possible to reclaim the mollusc.

Bivalve molluscs represent the main phycotoxins carrier to humans. Every year in the world occur about 60,000 cases of intoxication with a mortality rate of about 1,5% (Van Dolah & Ramsdell, 2001). In Europe an estimated annual loss of 720 million € for the recreation and tourism industry and 166 million € for the shellfish industry is due to the occurrence of algae blooms (Morgan et al., 2009; Hoagland & Scatasta, 2006).

Based on their chemical properties, marine shellfish toxins can be divided in two different classes: hydrophilic and lipophilic toxins. Toxins associated with the syndromes amnesic shellfish poisoning (ASP) and paralytic shellfish poisoning (PSP) are hydrophilic and have a molecular weight below 500 Da. Toxins responsible for neurologic shellfish poisoning (NSP), diarrhetic shellfish poisoning (DSP), azaspiracid shellfish poisoning (AZP) and other toxins such as pectenotoxins, yessotoxins and cyclic imines all have as common denominator a molecular weight above 600 Da (up to 2,000 Da). These toxins have strong lipophilic properties. Therefore, these toxins are generally called lipophilic marine toxins.

Amnesic shellfish poisoning (ASP)

The diatom *Pseudo-nitzschia pungens* is one of the most important species of the more than 10 known producers of domoic acid, the toxin responsible for ASP. In addition, a number of toxic domoic acid isomers have been described in the literature (Clayden et al., 2005). The primary action of domoic acid is on the hippocampus, which is involved in processing memory and visceral functions (Adams et al., 2009). Domoic acid is a neurotoxin that binds with a high affinity to glutamate receptors. This binding leads to opening of the membrane channels (permeable to sodium); this, in turn, leads to an increased sodium influx and membrane depolarization. The adverse effects reported are gastrointestinal disorders, nausea, vomiting, abdominal cramps and diarrhea. Furthermore, also headache, dizziness and loss of the short-term memory can occur (Kumar et al., 2009; Jeffrey et al., 2004).

Domoic acid occurrence in shellfish is a global issue. In recent years shellfish containing domoic acid have been reported in the USA, Canada, France, United Kingdom (UK), Spain, Ireland and Portugal (Bill et al., 2006; Campbell et al., 2001;

Blanco et al., 2006; James et al., 2005; Vale & Sampayo, 2001). The European Union (EU) has established a permitted level of 20 mg domoic acid /kg shellfish. In 2009, the European Food Safety Authority (EFSA) published an opinion on domoic acid (Alexander et al., 2009a). In this opinion the panel recommended that it is safe to consume shellfish which contain less than 4.5 mg domoic acid /kg shellfish in order to not exceed the acute reference dose.

Paralytic shellfish poisoning (PSP)

Dinoflagellates of the *Alexandrium* genus are the producers of saxitoxins, the group of toxins responsible for PSP. Within the saxitoxin, group around 30 different analogues have been detected (Dell'Aversano et al., 2008). Not every analogue exhibits the same toxicity and nowadays for the most prominent analogues, toxic equivalent factors have been established (Alexander et al., 2009b). Saxitoxin causes inhibition of the voltage-gated sodium channel resulting in a reduced action potential (Lagos & Andrinolo, 2000). Adverse effects of intoxication with saxitoxins start with tingling or numbness around the lips. These effects spread to the neck and face. In a progressed state, prickly sensation of fingertips, headache, dizziness, nausea, vomiting and diarrhea can occur. Even temporary blindness has been reported (Alexander et al., 2009b). When high levels of saxitoxins are consumed also the motor nerves are affected, resulting in respiratory difficulties and other muscular paralytic effects. Eventually, this may lead to death (Azanza, 2006).

Until the 1970s PSP toxins were only detected in European, North American and Japanese waters. Nowadays, saxitoxins have been reported in Chile, South-Africa, Australia and other countries as well (Pitcher et al., 2007; Krock et al., 2007). In most countries monitoring programs have been established to protect the consumer. The EU has established a permitted level of 800 µg saxitoxin 2-HCl equivalents/kg shellfish. The EFSA in 2009 published an opinion on the saxitoxin group (Alexander et al., 2009b). In this opinion it is recommended a safe level as low as 75 µg saxitoxin 2-HCl equivalents/kg in order to avoid exceeding the acute reference dose (Alexander et al., 2009b).

Neurologic shellfish poisoning (NSP)

NSP is caused by brevetoxins. These are produced by the algae species *Karenia* ssp. (Landsberg et al., 2009; Watkins et al., 2008). Brevetoxins cause opening of the voltage-gated sodium channels, leading to an influx of sodium in the cells and to a complete blockade of the neuronal excitability (Al-Sabi et al., 2006). Adverse effects observed are diarrhea, vomiting, cramps, rapid reduction of the respiratory rate and cardiac conduction disturbances which can lead to a coma and eventually to death (Watkins et al., 2008). In addition to consumption of brevetoxin-contaminated shellfish, intoxication can occur due to inhalation of aerosols produced by breaking waves at the shoreline (Pierce et al., 2005; Kirkpatrick et al., 2006). Inhalation of brevetoxin aerosols may result in respiratory problems and eye and nasal membrane irritation. Until now NSP intoxications have been limited to the USA (Gulf of Mexico and Florida) and New Zealand (Heil, 2009). As these toxins have not been found in Europe no legislation has been set for these toxins and no monitoring programs have been established. In the USA, legislation has been set by the Food and Drug Administration (FDA); the current regulatory limit is 800 µg brevetoxin-2 equivalents/kg shellfish (FDA, 2001).

Diarrhetic shellfish poisoning (DSP)

Okadaic acid as well as their esterified forms are produced by the *Dinophysis* genus. Toxins of the okadaic acid group inhibit the serine and threonine phosphatases (Honkanen et al., 1994). This inhibition leads to hyperphosphorylation of proteins involved in the cytoskeletal junctions that regulate the permeability of the cell, resulting in a loss of cellular fluids (Vale et al., 2008). Consumption of shellfish contaminated with high levels of okadaic acid-type toxins will result in adverse effects such as gastrointestinal disorder, diarrhea, abdominal cramps, nausea and vomiting (Garcia et al., 2005).

Nowadays, high levels of okadaic acid group toxins are repeatedly reported in shellfish or algae along the coasts of Europe (UK, Ireland, Denmark, Sweden, Norway, France, Spain, Italy, Portugal, The Netherlands and Belgium), Canada, South America (Chile), Japan, Australia and Africa (Morocco). Toxic equivalent factors values for okadaic acid have been established (Alexander et al., 2008a). Within Europe the permitted level for the total amount of okadaic acid in shellfish has

been set at 160 µg okadaic acid-equivalents/kg shellfish. In 2008, the EFSA panel in the opinion on okadaic acid and analogues concluded that the level should not exceed 45 µg okadaic acid equivalents/kg shellfish in order to not exceed the acute reference dose.

Yessotoxins (YTXs) are produced by the dinoflagellates *Proceratium reticulatum* and *Lingulodinium polyedrum* (Loader et al., 2007). Until now up to 90 YTX analogues have been identified (Miles et al., 2005). EFSA has suggested that a consumer is protected when shellfish do not exceed a concentration of 3.75 mg YTX-equivalents/kg shellfish.

Azaspiracid shellfish poisoning (AZP)

Azaspiracids are produced by a minute dinoflagellates, *Azadinium spinosum.* It is smaller (12–16 µm) than any of the other toxin-producing dinoflagellates known so far. Until now, 24 different AZAs have been described (Rehmann et al., 2008). The mechanism of action is not yet fully understood, but in-vitro experiments in mammalian cell lines showed alterations in the cytoskeletal structure, and an effect on the E-cadherin system, which is responsible for the cell-cell interactions. This could explain the toxic effects such as gastrointestinal disorder, diarrhea and abdominal cramps that are observed during AZP intoxication (James et al., 2004; 2002). AZP outbreaks occurred in Ireland and by now Azaspiracids have been detected in Ireland, UK, Norway, France, Portugal, Northern Africa (Morocco), South America (Chile) and the USA. According to current EU legislation the total amount of Azaspiracids should not exceed 160 µg/kg Azaspiracid-equivalents. Recently, EFSA reviewed all available toxicity data and suggested that a safe level of Azaspiracid toxins in shellfish is below the acute reference dose of 30 µg Azaspiracid equivalents/kg shellfish. Furthermore, EFSA suggested toxic equivalent factors for three most important Azaspiracids (Alexander et al., 2008b).

Other chemical contaminant are represented by heavy metals. They are generally introduced into the environment by anthropic activities.

Metallic compounds can be intaked by the bivalves through the dissolved fraction in the water or the suspended particulate matter. Generally the soluble metals and the free metal ions are more bioavailable and more toxic than the insoluble forms of the

same metals, even though increased hydrophobicity often translates into a greater tendency of the compound to persist in the tissues of the organism (Widdows & Donkin, 1992). From studies performed on the effects of heavy metals on mussels resulted the alterations of the expression of tissue-specific genes (Zorita et al., 2007). The toxicity on different larval stages (Prato & Biandolino, 2007) and some specific protein induction (Kaloyianni et al., 2006). From studies performed on *M. galloprovincialis* resulted that mercury and copper cause a deregulation of the homeostasis of the cytosolic calcium. Moreover, they are able to modify the activity of the membrane calcium dependent ATPase (Burlando et al., 2004).

The heavy metals that are most commonly found in shellfish are copper, zinc, lead, mercury, cadmium, chromium, nickel and arsenic. Some of these are essential for the same molluscs and, in small doses, even for humans, like copper that participate in oxygen transport in the bivalve haemolymph, or zinc, which is a constituent of many metal-enzymes.

Table 2. Daily bivalve intake to avoid problems deriving from the ingestion of some metals.

Metal	References	Product	Daily threshold (g/person/day)
Arsenic	USFDA (1993a)	Bivalve molluscs	340
		Crustaceans	102
Cadmium	USFDA (1993b)	Bivalve molluscs	28
		Crustaceans	110
Chrome	USFDA (1993c)	Bivalve molluscs	500
		Crustaceans	667
Lead	USFDA (1993d)	Bivalve molluscs	83

On humans, high concentrations of metals exceeding the minimum required dose, can cause serious health problems. Copper, even though it is an essential element, can give necrosis of the liver and hypertension; zinc, which is also an essential element, at high concentrations can be toxic; lead causes damage to the nervous system disorder in heme synthesis, chronic damage in the kidney and has teratogenic effects; mercury can damage the nervous system; cadmium is toxic for the kidneys and has mutagenic effects; chrome is an essential element, but at high

concentrations probably causes lung cancer and dermatitis; nickel can cause lung cancer and dermatitis, especially in women; arsenic can be responsible for poisoning episodes.

To this purpose, the Food and Drug Administration (FDA) recommends to not exceed a daily bivalve intake to avoid problems deriving from the ingestion of some metals (Table 2).

7.2 Bacterial contaminants

Bacteria that are normally present in marine waters are Gram-negative. The prevalent species belong to the genus *Vibrio* and *Pseudomonas*. Vibrios are particularly abundant in coastal waters and resulted to be abundant in the digestive system of bivalve molluscs as well as crustaceans and fish. The genus *Vibrio* consists in 30 species of which only a few are considered harmful for human health. Bacteria can accumulate in the bivalves flesh, and in the intervalvar liquid. Since some of these bacteria are toxic, the consume of the raw bivalves can be a risk, because can lead to intestinal infections caused by *Vibrio cholera*e and *Vibrio parahaemolyticus,* but also to extraintestinal vibriosis by *Vibrio vulnificus*.

Bivalve molluscs harvest is allowed depending on the origin of the product, based on the belonging class of each site classified according to faecal contamination (*E. coli* and *Salmonella* spp.), as required by the Regulation EC 854/2004 and by the Regulation 2073/2005 as shown in Tables 3 and 4.

Table 3. Water classification according to *E. coli* contamination. MPN = Most Probable Number.

Classification	DESTINATION	*E. coli* threshold (liquid and flesh)
A	Direct consume	230 MPN/100g
B	Consume after purification before sale	≤ 4600 MPN/100g
C	Relaying over a long time before sale	≤ 46000 MPN/100g

Bacterial contamination in bivalve molluscs is also connected with stocking prior to selling and consume: during storage, bacteria that are normally present in bivalve tissues, meet a favourable environment to develop.

During storage Bivalve molluscs undergo alterations mainly of enzymatic origin. As the storage period goes on, bacteria microflora continues to grow, leading the bivalve to a complete decline of quality. The transition between the enzymatic and bacterial quality decline is not clear yet, since bacteria multiplication need enzymatic action, while some lysis processes occurring on the flesh are directly caused by the presence of microorganisms in the bivalve molluscs (Jackson et al., 1997).

Table 4. Bacteria thresholds according to the EC Regulation 2073/2005 for bivalve molluscs.

Bacteria	Thresholds
Faecal coliforms	< 300 in 100g of liquid and flesh
E. coli	< 230 in 100g of liquid and flesh
Salmonellas	Absent in 25g of flesh

Chemical composition strongly affects oysters and mussels flesh contamination by microorganisms. Carbohydrates are particularly abundant in bivalves (3.4% in clams and 5.6% in oysters) and it is represented mainly by glycogen, that is the reserve substance of Bivalve molluscs. The high glycogen content causes a high saccharolytic activity due to enzymes and bacteria (Jay, 1992). Molecules resulting by saccharolytic activity are easy to assimilate by the microflora living inside the bivalve molluscs: this activity causes both the bacteria proliferation and the pH decrease compared to the values at the moment of harvesting (Linton et al., 2003).

Another characteristic of bivalve molluscs flesh is the abundance of free amino acids, as arginine, aspartic acid and glutamic acid.

As the spoilage carries on, different kind of alteration on the product can occur. Low molecular weight substances containing nitrogen like trimethylamine oxide, taurine, betaine, anserine, creatine, and histidine (Linton et al., 2003) as well as sugars are transformed by bacteria in off-odour substances. Bacteria action can be verified by checking the presence of total volatile base nitrogen (TVBN) and trimethylamine (TMA) (Jay, 1992).

One important aspect for human health is the histamine production by histidine decarboxylation caused by bacteria. This biogenic amine is responsible for some allergic reactions affecting many people that can also lead to anaphylaxis.

In fish and shellfish products a kind of alteration involving amino acids can occur, with the final production of sulphur compounds (H_2S): they are produced by microorganisms attacking amino acids like methionine and cysteine.

Bacterial microflora composition of mussels and oysters is generally composed by the species belonging to the genera *Bacillus*, *Serratia*, *Proteus*, *Clostridium*, *Moraxella/Acinetobacter* and *Pseudomonas* (Linton et al., 2003). The genera *Serratia*, *Pseudomonas*, *Clostridium*, *Bacillus*, *Escherichia*, *Shewanella*, *Lactobacillus*, *Flavobacterium*, *Micrococcus* and the family of *Enterobacteriacee* in general are typical of the early phases of bacterial contamination.

Microorganisms found in mussels and oysters reflect the health status of the water and phytoplankton. As the spoilage goes on during the refrigerated storage (4-6 °C) the bacterial microflora evolves with species adapted to low temperatures. The domination of the genus *Pseudomonas*, for example, can be explained with its attitude to grow rapidly at refrigeration temperatures and with its ability to use many of the flesh compounds of mussels and oysters, in particular non-protein nitrogenous substances (Volonterio, 1999).

The dominant bacterial genera in this phase are *Pseudomonas* and *Acitenobacter-Moraxella*.

During the last phases of the spoilage, when the product is no more edible, other bacterial genera prevail, such as *Enterobacteriaceae*, *Lactobacillus* and yeasts.

Among the many genera of microorganisms contained in bivalve molluscs flesh, some bacteria and virus that are harmful for human health can be found. The most important species that can be found in bivalve molluscs are *Vibrio parahaemolyticus*, *Vibrio* spp., *Salmonella*, *Clostridium*, *Escherichia coli*, *Campylobacter*, *Aeromonas*, *Listeria monocytogenes* (Linton et al., 2003). *Listeria monocytogenes* presence is not connected to the living environments of the bivalve molluscs, but can be the result of post-harvest handling contamination (Linton et al., 2003). The determination of the changes of pH, TVBN and TMA has been proposed to monitor microbiological quality of bivalve molluscs, since the three parameters are connected with microbiological contamination of the final product during storage. Cook (1991) stated that oysters are

classified as being of good quality if their pH is 6.0 or higher and Colby et al. (1995) stated that fresh and good-quality bivalves exhibit pH values varying from 6 to 7. Aaraas et al. (2004) found that muscle pH in all live oysters ranged from 5.6 to 6.3, dead oysters had a tissue pH of 5.2 to 5.4 and the moribund specimen had a tissue pH of 5.5.

The pH monitoring is a fast and reliable technique that allows a first analytical evaluation of oysters quality. TVBN and TMA, as well as sensory analysis, are fast but not completely reliable, since they don't change in a constant way during bacterial contamination (Beacham, 1946). This can be explained with the action of the enzymes that are present in the oyster and mussel flesh.

These products are very susceptible to bacterial contamination and a light cooking is not enough to restore them. Only the control on water and on farmed or fished animals can avoid human pathologies.

8 Quality

Quality is a characteristic that modern consumers believes to be necessary in food. The regulation ISO 8402-1986 defines quality in its generic sense as "the totality of features and characteristics of a product or service that bears its ability to satisfy stated or implied needs".

For seafood and for bivalve molluscs in particular, the "total quality" has to be considered, since they are products that have to satisfy the food security principles defined by the law, the organoleptic quality and the nutritional and nutraceutical properties.

Organoleptic quality together with nutraceutical properties are the reason why consumers choose to buy seafood.

Growth rate together with nutraceutical quality of farmed products like bivalve molluscs depends on the farming environments and conditions like temperature, salinity, pH, food composition and genetics. In particular seasonal changes strongly affect bivalves physiological status and consequently gonads growth and development and edible part composition (Orban et al., 2004).

In the study performed by Orban et al. (2004) on oysters farmed in a suspended culture in the Valle Dogà they found that seasonal variations significantly reflected on

gross chemical composition of the edible part of the molluscs. In the same study also the fatty acids profile has been considered and the authors found that seasonal changes is strictly connected with fatty acids composition, with a higher content of PUFA during the winter, according to the findings of Pazos et al. (1996) and Abad et al. (1995).

Henderson & Tocher (1987) ascribed oysters adaptation to low temperatures to the need of a higher fluidity of the membranes; Beninger & Stephan (1985) stated that the seasonal variations are due to the different composition of the food, while Chu et al. (1990) ascribed the phenomenon to the reproductive cycle.

Seafood as a whole food is highly nutritious. Benefits to human health associated with the consumption of seafood are noted for multiple bodily organs and physiological functions. Seafood compares favorably with other protein sources as it offers superior macronutrients (fats and proteins) in the ideal form of lean proteins combined with healthy omega-3 long chain polyunsaturated fatty acids (n-3 LC-PUFAs), and a wide array of highly bioavailable micronutrients (minerals and vitamins).

Bivalve molluscs have a very low lipid content compared to other seafood. For example mussels and oysters contain about 2% of lipids on their wet weight (8-10% on dry weight) (FDA, 2005), while among fish the fat content can pass the value of 10% on the wet weight, depending on the species and the origin (wild or farmed).

Despite their low lipid content bivalve molluscs and oysters have a very peculiar fatty acids composition. PUFA were found to be the most abundant group of fatty acids in oysters by many authors (Abad et al., 1995; Pazos et al., 1996; Piveteau et al., 2000; Orban et al., 2004; Saito & Marthy, 2010) and in particular the group of n-3 PUFA was found the most abundant one by Piveteau (1999), Soudant et al. (1999), Dridi et al. (2007), Saito & Marty (2010). Among n-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the most abundant: these two fatty acids are the most important of their group, since they play functional and structural functions in the human body. DHA mainly plays a structural function: it is present in cerebral phospholipids, in the retina and in intramembrane sodium channels. It therefore plays an important role in cerebral and reproductive systems and retinal tissue development and maturation. EPA is the main precursor of prostaglandins

belonging to the series 3, that plays an important role in the process of aggregation and disaggregation of platelets (Ciaccio, 2001).

As EPA and DHA are converted at a very low rate from plant sources of α-linolenic acid (ALA) (Goyens et al., 2006), consumption of preformed EPA and DHA from marine food sources is the most efficacious method to increase physiological status of EPA and DHA for the purpose of achieving the documented health benefits associated with these n-3 PUFAs (McManus & Newton, 2011).

The high content of PUFA in the edible part of bivalve molluscs is expressed by the low values of atherogenic and thrombogenic indexes (AI and TI, respectively) described by Ulbricht & Southgate (1991) that are lower if compared to fish species. For example Valfrè et al. (2003), analyzing the health benefits of seafood on human health, found that sea bass presented a value of 0.25 for TI and 0.45 for AI, while the mussel resulted to have 0.12 and 0.40 for TI and AI, respectively. Another important index of the healthiness of the fatty acids component of the lipidic fraction is the PUFA n-3/PUFA n-6 ratio. The ratio value recommended in the human diet is about 1/5 (S.I.N.U., 2011) while in bivalve molluscs is generally about 1-3/1 or even higher and then better.

Glycogen is a multibranched polysaccharide that serves as a form of energy storage in animals. It is a highly digestible for humans. This macronutrient is one of the most characterizing compounds of bivalve molluscs and can represent the 3-7% of the wet weight of the edible part, while in fish species it does not exceed 0.5%.

The high concentration of this compound is due to the fact that bivalve molluscs use glycogen as reserve substance, unlike other animals that use lipids for the same function. For this reason glycogen content in bivalve molluscs changes with seasons and physiologic status variations of the animals. In fact, during the reproductive period, molluscs mobilize a high quantity of glycogen to face gonads development and spawning. After that they return in conditions of physiological rest so that glycogen concentration increases again.

Bivalve molluscs contain many mineral elements that they absorb by the surrounding water and phytoplankton. They accumulate minerals in their body to use them to built the shell.

Bivalve molluscs are rich in iodine, selenium and sodium. Iodine is important in human diet because it is part of the thyroid hormones acting on growth and

morphogenesis of various organs. Selenium is important for its anti-oxidant action on the cellular membranes. Bivalve molluscs also present an optimal calcium/phosphorous ratio and a high iron content, even though it is less assimilable if compared to the meat of terrestrial animals.

8.1 Measuring quality

There are many different techniques useful to measure the quality of bivalve molluscs.

A parameter of ecophysiological and economic relevance, especially in view of the industrial processing, is represented by the Condition Index, a measure of the apparent health and commercial quality of Bivalves (Orban et al., 2002). The Condition Index should be regularly monitored for successful bivalves culturing activities.

A close relationship has been reported between the gametogenic cycle, condition index and the storage–consumption cycle of reserves, particularly glycogen, and meat quality in bivalve molluscs (Gabbott, 1975). Condition index has been used for nearly half a century for biological and commercial purposes as indicator of seasonal tissue storage cycle in mussels (Baird, 1958). This can be considered a measure of fatness and marketability of commercially exploited species of bivalve molluscs and, together with approximate biochemical composition, probably the most practical and simplest method of monitoring gametogenic activity (Okumus & Stirling, 1998).

Condition index has been used by authors to compare the effect of different farming systems on clams growth (Boscolo et al., 2003), or to deepen the knowledge about summer mortality of oysters occurring every year in Marennes–Oléron bay (Patrick et al., 2006), or to study the effects of the *Polydora* spp. infestation on oysters ability to accumulate reserve substances (Wargo & Ford, 1993).

In a study performed by Dittman et al. (2001) decreases in oyster condition index after spawning were always coincident with increase in seasonal temperatures and disease outbreaks.

Li et al. (2009) used the condition index proposed by Crosby & Gale (1990) to detect correlations between the changes of the farming conditions and the physiological status along a year on farmed Pacific oysters.

One of the formulas more frequently utilized for the Condition index (CI) calculation is the following:

*CI = flesh dry matter weight * 100/ (total weight-shell weight)*

For the oyster industry, shell morphology is also considered an important trait since it can dramatically influence product value when animals are commercialized live or half shell (Ward et al., 2005). Brake et al. (2003) showed that length and depth indexes as well as the index of shell shape (which combines the information of the previous two indexes) were useful to describe oyster shells with a desirable shape. The same authors observed that length index was not as important as depth index to describe a desirable shell shape. Although it is known that the relative depth of oyster shells is extremely influenced by environmental conditions and husbandry procedures (Galtsoff, 1964; Quayle, 1988), genetic variation has been also observed for these traits (Wada, 1994; Ward et al., 2005). However, Ward et al. (2005) observed that for *C. gigas* the genetic gains for depth indexes were low, and hence genetic selection was not a powerful tool to manipulate this trait. Brake et al. (2003) proposed an index based on linear measurements to characterize the shell shape of the oysters, with the aim to have a useful, rapid and easy to use tool for industrial quality evaluation. The formula is the following:

Shape Index = shell depth / shell length.

The authors also calculated the threshold values indicating good and bad shaped oysters: an average shape index of 0.316 indicated good shaped oysters, while an average shape index of 0.219 characterized bad shaped oysters.

Another characteristic that strongly affects seafood and bivalve molluscs value on the market is freshness. Ólafsdóttir et al. (1997) stated that sensory evaluation is the most important method to evaluate the freshness in seafoods.

Sensory assessment is a time-consuming procedure that requires the mobilization of trained panelists (Chang et al., 1998), but it is considered by some authors as the most accurate quality predictor (Khan et al., 2005).

Sensory evaluation is defined as the scientific discipline used to evoke, measure, analyse and interpret characteristics of food as perceived by the senses of sight, smell, taste, touch and hearing. Sensory tests can be divided into three groups:

discriminative tests, which indicate whether there is a difference between samples; descriptive tests; and affective tests. Discriminative and descriptive tests are objective analytical tests in which a trained panel is used. Affective tests are subjective consumer tests that are based on a measure of preference or acceptance. The choice of method depends on the purpose of the application of the sensory evaluation and whether it is used in product development, quality control, consumer studies or research. The most commonly used descriptive tests are structured scaling for quality assessment and profiling for a detailed description of one or more attributes (Ólafsdóttir et al., 1997). Usually, for oyster two to three sensory criteria are assessed, such as appearance and odour (Aaraas et al., 2004), but flavour and texture have also been evaluated (Boyd et al., 1980). Sensory changes occur in the appearance, odour, taste and texture of bivalve molluscs when they deteriorate. Spoilage bacteria and tissue degradation are strongly correlated with flavour changes (Boyd et al., 1980; Gram & Huss, 1996; Aaraas et al., 2004), and end-product quality can be evaluated through sensory assessment to determine the maximum storage time compatible with food safety for consumers (Buzin et al., 2011).

Sensory evaluation of bivalves has been not standardized yet, so that authors performing this kind of analysis have to draw up a descriptors scheme in order to eliminate the subjectivity from the analysis. Aaraas et al. (2004) evaluated oysters quality using descriptors for smell, appearance, texture and taste; Cao et al. (2009) drew up a descriptors scheme for odour, body colour, fluid and texture**,** while Buzin et al. (2011) considered odour, appearance, texture and flavour.

Even though sensory test is considered a reliable tool in freshness evaluation (Reineccius, 1990), it is not able to detect specific spoilage-causing microorganisms, so it is often associated with biochemical and microbiological analysis, in order to have a complete quality evaluation of the product (Ryder et al., 1993).

As aromatic perception is a crucial criterion that motivates the consumers to eat bivalves, in the last years the sensory analyses have been often associated with the analysis of volatile organic compounds (VOCs) that are responsible for the typical odour and flavour characterizing the different species.

Therefore, VOCs play a fundamental role in the acceptance of shellfish by the consumers (Pennarun et al., 2002). Moreover, the modern consumer has a high consciousness about food safety and quality issues, being the attention threshold

higher on shellfish than on other foodstuffs since shellfish are very susceptible to deterioration. Either enzymatic or auto-oxidative reactions of PUFA produce VOCs responsible for both marine fresh flavors and off-flavors. The study of the flavor profile can be used to check the organoleptic quality acceptance of seafood and to identify the distinctive VOCs of fresh, deteriorated and transformed shellfish (Cruz-Romero et al., 2004; Yasuhara, 1987). VOCs profile is an important quality index taking into consideration its relationship with geographical origin (Fuentes et al., 2009), rearing or harvesting water quality, season (Fernández-Reiriz et al., 1989), freshness (Ólafsdóttir et al., 1997) and the species analyzed (Josephson, 1991).

Different extraction methods have been used to analyze VOCs in food, such as, steam distillation (SD), “purge and trap” (P&T) (Grigorakis et al., 2009), simultaneous distillation extraction (SDE) (Caprino et al., 2008), dynamic headspace (DHS), vacuum distillation (VD) (Pennarun et al., 2002) and solid phase microextraction (SPME) (Zhang et al., 1994). Methods based on distillation as SD and SDE are time consuming and require the use of organic solvents for extracting VOCs. Some methods involving high temperatures or longer times for volatile extraction could direct a deep modification of thermally unstable compounds present in the volatile fraction. Josephson et al. (1985) investigated VOCs of two different oysters species by using P&T technique and GC–MS, while Pennarun et al. (2002) compared the reliability of dynamic head space and VD to analyze oysters' VOCs. SPME is a fast, sensitive, solventless and economical method for sample preparation before gas chromatography analysis in comparison to well established techniques for analyzing volatiles in food. It was developed by Arthur & Pawliszyn (1990) and combines sampling and sample preparation in one step. In the last years it has been successfully used for determining the volatile flavor profiles of different fish species (Iglesias et al., 2010).

9. REFERENCES

Aaraas, R., Hernar, J.I., Vorre, A., Lunestad, B.T., Skeie, S., Slinde, E., & Mortensen, S. (2004). Sensory histological and bacteriological changes in flat oysters, *Ostrea edulis*, L., during different storage conditions. *Journal of Food Science, 69*(6), 205-210.

Abad, M., Ruiz, C., Martinez, D., Mosquera, G., & Sanchez, J.L. (1995). Seasonal variations of lipid classes and fatty acids in flat oyster, *Ostrea edulis*, from San Cibran (Galicia, Spain). *Comp Biochem Physiol 110C*, 109–118.

Adams, A.L., Doucette, T.A., James, R., & Ryan, C.L. (2009). Persistent changes in learning and memory in rats following neonatal treatment with domoic acid. *Physiol. Behav.* 96, 505–512.

Akaboshi, S., & Bastos, A.A. (1977). El cultivo de la ostra *Crassostrea brasiliana* en la region lagunar de Cananéia, São Paulo, Brasil. *Paper presented at*: Simposio sobre aquicultura en America Latina, November 26, 1977, Montevideo, Uruguai.

Alexander, J., Audunsson, G.A., Benford, D., Cockburn, A., Cradevi, J.-P., Dogliotti, E., Domenico, A.D., Fernandez-Cruz, M.L., Fink-Gremmels, J., Furst, P., Galli, C., Grandjean, P., Gzyl, J., Heinemeyer, G., Johansson, N., Mutti, A., Schlatter, J., van Leeuwen, R., van Peteghem, C., & Verger, P. (2008a). Marine biotoxins in shellfish - okadaic acid and analogues. *EFSA Journal* 589, 1–62.

Alexander, J., Benford, D, Boobis, A., Ceccatelli, S., Cravedi, J.-P., Domenico, A.D., Doerge, D., Dogliotti, E., Edler, L., Farmer, P., Filipic, M., Fink-Gremmels, J., Furst, P., Guerin, T., Knutsen, H.K., Livesey, C., Machala, M., Mutti, A., Schlatter, J., van Leeuwen, R., & Verger, P. (2009a). Marine biotoxins in shellfish—Domoic acid. *EFSA Journal* 1181, 1–61.

Alexander, J., Benford, D., Cockburn, A., Cradevi, J.-P., Dogliotti, E., Domenico, A.D., Fernandez-Cruz, M.L., Fink-Gremmels, J., Furst, P., Galli, C., Grandjean, P., Gzyl, J., Heinemeyer, G., Johansson, N., Mutti, A., Schlatter, J., van Leeuwen, R., van Peteghem, C., & Verger, P. (2008b). Marine biotoxins in shellfish—azaspiracid group *EFSA Journal* 723, 1–52.

Alexander, J., Benford, D., Cockburn, A., Cradevi, J.-P., Dogliotti, E., Domenico, A.D., Fernandez-Cruz, M.L., Fink-Gremmels, J., Furst, P., Galli, C., Grandjean, P., Gzyl, J., Heinemeyer, G., Johansson, N., Mutti, A., Schlatter, J., van Leeuwen, R., van

Peteghem, C., & Verger, P. (2009b). Marine biotoxins in shellfish - saxitoxin group. *EFSA Journal* 1019*,* 1–76.

Al-Sabi, A., McArthur, J., Ostroumov, V., & French, R.J.(2006). Marine toxins that target voltage-gated sodium channels. *Mar. Drug* 4, 157–192.

Arthur, C.L., & Pawliszyn, J. (1990). Solid-phase microextraction with thermal desorption using fused silica optical fibres. *Analytical Chemistry* 62, 2145–2148.

Azanza, M.P.V. (2006). Philippine foodborne-disease outbreaks (1995–2004). *Journal of Food Safety, 26,* 92–102.

Bâcher, C. (1984). Echantillonnage du stock d'huîtres du bassin de Marennes-Oléron. Oyster stock assessment in the Bay of Marennes-Oléron. Rapp. DEA, Univ. Paris, 38 p.

Baird, R.H. (1958). Measurement of condition in mussels and oysters. *J. Cons. Perm. Inter. Explor. Mer.* 23, 249–257.

Baldwin, B.S. (1995). Selective particle ingestion by oyster larvae (*Crassostrea virginica*) feeding on natural seston and cultured algae. *Mar. Biol.* 123, 95–107.

Baldwin, B.S., & Newell, R.I.E. (1995). Feeding rate responses of oyster larvae (*Crassostrea virginica*) to seston quantity and composition. *J. Exp. Mar. Biol. Ecol.* 189, 77–91.

Baud, J.-P., Brisset, E., & Cardinal, M. (1995). Affinage controle en bassin de l'huitre creuse *Crassostrea gigas*. *IFREMER, Nantes.* Pp. 47.

Bayne, B.L. (1991). Introduction of "The Biology and Cultivation of Mussels". *Aquaculture* 94*,* 121-278.

Bayne, B.L. (1998). The physiology of suspension feeding by bivalve molluscs: an introduction to the Plymouth "TROPHEE" workshop. *Journal of Experimental Marine Biology and Ecology* 219(1-2), 1-19.

Bayne, B.L., Thompson, R.J., & Widdows, J. (1976). Physiology: I. In: Marine mussels: their ecology and physiology (ed. B. L. Bayne), pp. 121-206. Cambridge University Press, Cambridge.

Beacham, L.M. (1946). A study of decomposition in canned oysters and clams. *J. Assoc. Offic. Agric. Chem.* 29, 89-92.

Benemann, J.R. (1992). Microalgae aquaculture feeds. *J. Appl. Phycol*. 4, 233–245.

Beninger, P., & Stephan G. (1985). Seasonal variations in the fatty acids of the triacylglycerols and phospholipids of two populations of adult clam (*Tapes*

decussatus L and *T. philippinarum*) reared in a common habitat. *Comp. Biochem. Physiol.* 81B, 591–601.

Beninger, P.G., & Le Pennec, M. (1991). Functional anatomy of scallops. In: Scallops: Biology, Ecology and Aquaculture (Ed. S.E. Shumway), Elsevier Science Publisher B.V. Amsterdam, pp. 133-223.

Beninger, P.G., & St-Jean, S.D. (1997). The role of mucus in particle processing by suspension-feeding marine bivalves: unifying principles. *Marine Biology* 129,389-397.

Bill, B.D., Cox, F.H., Horner, R.A., Borchert, J.A., & Trainer, V.L. (2006). The first closure of shellfish harvesting due to domoic acid in Puget Sound, Washington, USA. *Afr. J. Mar. Sci.* 28, 435–440.

Blanco, J., Cano, J., Marino, M.D.C., & Campos, M.J. (2006). Effect of phytoplankton containing paralytic shellfish and amnesic shellfish toxins on the culture of the king scallop *Pecten maximus* in Malaga (SE Spain). *Aquatic Living Resources* 19, 267–273.

Boscolo, R., Cornello, M., & Giovanardi, O. (2003). Condition index and air survival time to compare three kinds of Manila clam *Tapes philippinarum* (Adams & Reeve) farming systems. *Aquaculture International* 11, 243-254.

Botana, A.M., Rodriguez Veijtes, M., Alfonso, A., & Louzao, M. (1996). Phycotoxins: paralytic shellfish poisoning and diarrhetic shellfish poisoning. In: Handbook of Food Analysis—Residues and Other Food Component Analysis (Nollet, L.M.L. Ed.), Dekker: New York, NY, USA, pp. 1147–1169.

Bougrier, S., Hawkins, A.J.S., & Heral, M. (1997). Preingestive selection of different microalgal mixtures by *Crassostrea gigas* and *Mytilus edulis*, analysed by flow cytometry. *Aquaculture* 150(1-2), 123-134.

Boyd, A.J., & Heasman, K.G. (1998). Shellfish mariculture in the Banguela system: water flow patterns within a mussel farm in Saldanha Bay, South Africa. *Journal of Shellfish Research* 17, 25-32.

Boyd, N.S., Wilson, N.D.C., & Hall, B.I. (1980). Storage of live Pacific oysters out of water. *New Zealand Journal of Science* 23, 171–176.

Brake, J., Evans, F., & Langdon, C. (2003). Is beauty in the eye of the beholder? Development of a simple method to describe desirable shell shape for the Pacific oyster industry. *Journal of Shellfish Research* 22(3), 767-771.

Bromanonda, P. (1978). Study on some biological aspects and culture of oyster. *Thai Fisheries Gazette* 31(3), 202-228.

Bromhall, J.D. (1958). On the biology and culture of the native oyster of Deep Bay, Hong Kong, *Crassostrea* sp.. *Hong Kong University Fisheries Journal* 2, 93-107.

Brown, J.R., & Hartwick, E.B. (1988a). Influences of temperature, salinity and available food upon suspended culture of the Pacific oyster, *Crassostrea gigas* I. Absolute and allometric growth. *Aquaculture* 70, 231-251.

Brown, J.R., & Hartwick, E.B. (1988b). Influences of temperature, salinity and available food upon suspended culture of the Pacific oyster, *Crassostrea gigas* II. Condition index and survival. *Aquaculture* 70, 253-267.

Buck, B.H. (2002). Open ocean aquaculture und Offshore-Windparks: Eine Machbarkeitsstudie über die multifunktionale Nutzung von Offshore-Windparks und Offshore Marikultur im Raum Nordsee. Reports on Polar and Marine Research, Bremerhaven 412, 1–252.

Buitrago, J., Rada, M., Hernandez, H., & Buitrago, E. (2005). A single-use site selection technique, using GIS, for aquaculture planning : choosing locations for mangrove oyster raft culture in Margarita Island, Venezuela. *Environmental Management* 35(5), 544-556.

Burbridge, P., Hendrick, V., Roth, E., & Rosenthal, H. (2001). Social and economic policy issues relevant to marine aquaculture. *Journal of Applied Ichthyology* 17, 194–206.

Burlando, B., Bonomo, M., Caprì, F., Mancinelli, G., Pons, G., & Viarengo, A. (2004). Different effects of Hg^{2+} and Cu^{2+} on mussel (*Mytilus galloprovincialis*) plasma membrane Ca^{2+}-ATPase: Hg^{2+} induction of protein expression. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* 139(4), 201-207.

Buzin, F., Baudon, V., Cardinal, M., & Barille, L. (2011). Cold storage of Pacific oysters out of water : biometry, intervalval water and sensory assessment. *International Journal of Food Science and Technology* 46, 1775-1782.

Calow, P. (1977). The joint effect of temperature and starvation on the metabolism of triclads. *Oikos* 29, 87-92.

Campbell, D.A., Kelly, M.S., Busman, M., Bolch, C.J., Wiggins, E., Moeller, P.D.R., Morton, S.L., Hess, P., & Shumway, S.E. (2001). Amnesic shellfish poisoning in

the king scallop, *Pecten maximus*, from the west coast of Scotland. *Journal of Shellfish Research* 20, 75–84.

Cao, R., Xue, C.-Hu, Liu, Q., & Xue, Y. (2009). Microbiological, chemical, and sensory assessment of Pacific oysters (*Crassostrea gigas*) stored at different temperatures. *Czech Journal of Food Science* 27(2), 102-108.

Caprino, F., Moretti, V.M., Bellagamba, F., Turchini, G., Busetto, M.L., & Giani, I. (2008). Fatty acid composition and volatile compounds of caviar from farmed white sturgeon (*Acipenser transmontanus*). *Analytica Chimica Acta* 617, 139-147.

Chang, K.L.B.J., Chang, J., Shiau, C.-Y.**,** & Pan, B.S. (1998). Biochemical, microbiological, and sensory changes of sea bass (*Lateolabrax japonicus*) under partial freezing and refrigerated storage. *Journal of Agricultural and Food Chemistry* 46, 682–686.

Cheng, T.C. (1981). Bivalves. In: Invertebrate blood cells I (N.A. Ratcliffe, A.F. Rowley, Eds.). Academic Press. London, pp. 233–299.

Chu, F.L.E., Webb K.L., & Chen, J. (1990). Seasonal changes of lipids and fatty acids in oyster tissues (*Crassostrea virginica*) and estuarine particulate matter. *Comp. Biochem. Physiol.* 95A, 385-391.

Ciaccio, M.D. (2001). Gli acidi grassi ω-3 ed ω-6: dalla biochimica all'applicazione clinica. www.sinu.it.

Clayden, J., Read, B., & Hebditch, K.R. (2005). Chemistry of domoic acid, isodomoic acids, and their analogues. *Tetrahedron* 61, 5713–5724.

Colby, J.-W., Enriquez-Ibara, L.G., & Flick, G.J. (1995). Shelf life of fish and shellfish. In: Shelf life studies of foods and beverages, chemical, biological, physical and nutritional aspects (G. Charalambous, Ed.). New York, NY: Elsevier. Pp. 85–143.

Cook, D.W. (1991). Microbiology of bivalve molluscan shellfish. In: Microbiology of marine food products (D.R. Ward & C. Hackney, Ed.). New York: Van Norstrand Rheinhold. Pp. 301–330.

Costa-Pierce, B. (2002). Ecology as the paradigm for the future of aquaculture. In: Ecological Aquaculture: The Evolution of the Blue Revolution (B. Costa-Pierce, Ed.). Blackwell Science, Oxford, UK. Pp. 339-372.

Crosby, M.P., & Gale, L.D. (1990). A review and evaluation of bivalve condition index methodologies with a suggested standard method. *Journal of Shellfish Research* 9, 233–237.

Cruz-Romero, M., Smiddy, M., Hill, C., Kerry, J.P., & Kelly, A.L. (2004). Effects of high pressure treatment on physicochemical characteristics of fresh oysters (*Crassostrea gigas*). *Innovative Food Science & Emerging Technologies* 5, 161-169.

Cucci, T.L., Shumway, S.E., Brown, W.S., & Newell, C.R. (1989). Using phytoplankton and flow cytometry to analyze grazing by marine organisms. *Citometry* 10, 659-669.

Daranas, A.H., Norte, M., & Fernandez, J.J. (2001). Toxic marine microalgae. *Toxicon* 39, 1101–1132.

Dell'Aversano, C., Walter, J.A., Burton, I.W., Stirling, D.J., Fattorusso, E., & Quilliam, M.A. (2008). Isolation and structure elucidation of new and unusual saxitoxin analogues from mussels. *J. Nat. Prod.* 71, 1518–1523.

Deslous-Paoli, J.M., Lannou, A.M., Geairon, P., Bougrier, S., Railard, O., & Héral, M. (1992). Effect of the feeding behaviour of *Crassostrea gigas* (Bivalve Molluscs) on biosedimentation of natural particulate matter. *Hydrobiologia* 231, 85-91.

Dillon, R.T. (2000). The ecology of freshwater molluscs. *Cambridge University Press*, 8-14.

Dittman, D.E., Ford, S.E., & Padilla, D.K. (2001). Effects of *Perkinsus marinus* on reproduction and condition of the eastern oyster, *Crassostrea virginica*, depend on timing. *Journal of Shellfish Research* 20, 1025–1034.

Dridi, S., Salah, M., & Elcafsi, M. (2007). Seasonal variation in weight and biochemical composition of the Pacific oyster, *Crassostrea gigas***,** in relation to the gametogenic cycle and environmental conditions of the Bizert Lagoon, Tunisia. *Aquaculture* 263, 238-248.

Dutertre, M., Beninger, P.G., Barillé, L., Papin, M., & Haure, J. (2010). Rising water temperatures , reproduction and recruitment of an invasive oyster, *Crassostrea gigas*, on the French Atlantic coast. *Marine Environmental Research* 69, 1-9.

Enright, C. (1993). Control of fouling in bivalve aquaculture. *World Aquaculture* 24, 44–46.

European Commission (2004). Commission Decision of 29 April 2004 concerning laying down specific hygiene rules for on the hygiene of foodstuffs, 04/853/EC. In: Official Journal, L 139, 30/04/2004.

FAO (2012). Fishery and Aquaculture Statistics Yearbook.

FDA (2001). Chapter 6: Natural Toxins (A chemical Hazard). Fish and fisheries products hazards and control guidance. Food and Drug Administration: Washington, DC, USA.

Fernández-Reiriz, M.J., Pérez-Camacho, A., Ferreiro, M.J., Blanco, J., Planas, M., Campos, M.J., & Labarta, U. (1989). Biomass production and variation in the biochemical profile (total protein, carbohydrates, RNA, lipids and fatty acids) of seven species of marine microalgae. *Aquaculture* 83, 17–37.

Fernández-Reiriz, M.J., Labarta, U., & Babarro, J.M.F. (1996). Comparative allometries in growth and chemical composition of mussel (*Mytilus galloprovincialis* Lmk) cultured in two zones in the Ría Sada (Galicia, NW Spain). *Journal of Shellfish Research* 15, 349–353.

Foster-Smith, R.L. (1975). The effect of concentration of suspension on the filtration for the rates and pseudofecal production for *Mytilus edulis* (CL.), *Cerastoderma edule* (L.) and *Venerupsis pullastra* (Montagu). *J. Exp. Mar. Biol. Ecol.* 17, 1-22.

Fuentes, A., Fernandez-Segovia, I., Escriche, I., & Serra, A.J. (2009). Comparison of physico-chemical parameters and composition of mussels (*Mytilus galloprovincialis* Lmk.) from different Spanish origins. *Food Chemistry* 112, 295-302.

Gabbott, P.A. (1975). Storage cycles in marine bivalve molluscs: a hypothesis concerning the relationship between glycogen metabolism and gametogenesis. In: Barnes, H. Ed., Proc. 9th Europ. Mar. Biol. Symp., Aberdeen Univ. Press, pp. 191–211.

Galtsoff, P.S. (1964). The American oyster *Crassostrea virginica* Gmelin. *Fish. Bull. US* 64, 1-480.

Garcia, C., Truan, D., Lagos, M., Santelices, J.P., Diaz, J.C., & Lagos, N. (2005). Metabolic transformation of dinophysistoxin-3 into dinophysistoxin-1 causes human intoxication by consumption of O-acyl-derivatives dinophysistoxins contaminated shellfish. *J. Toxicol. Sci.* 30, 287–296.

Gosling, E. (2003). Bivalve molluscs. biology, ecology and culture. Fishing News Books. Pp. 443.

Goulletquer, P., & Héral, M. (1997). Marine molluscan production trends in France: from fisheries to aquaculture. In: The history, present condition, and future of the molluscan fisheries of North and Central America and Europe. *US Department of Commerce, NOAA Technical Report* 129(3), 137-164.

Gram, L., & Huss, H.H. (1996). Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology* 33, 121–137.

Grigorakis, K., Fountoulaki, E., Giogios, I., & Alexis, M.N. (2009). Volatile compounds and organoleptic qualities of gilthead sea bream (*Sparus aurata*) fed commercial diets containing different lipid sources. *Aquaculture* 290, 116-121.

Hawkins, A.J.S., & Bayne, B.L. (1992). Physiological processes, and the regulation of production. In: The Mussel *Mytilus*: ecology, physiology, genetics and culture (E. Gosling, Ed.). Elsevier Science Publishers, Amsterdam, pp. 171–222.

Heasman, K.G., Pitcher, G.C., McQuaid, C.D., & Hecht, T. (1998). Shellfish mariculture in the Banguela system: raft culture of *Mytilus galloprovincialis* and the effect of rope spacing on food extraction, growth rate, production and condition of mussels. *Journal of Shellfish Research* 17, 33-39.

Heidmann Soccol, M.C., & Oetterer, M. (2003). Seafood as functional food. *Brazilian Archives of Biology and Technology* 46(3), 443-454.

Heil, D.C. (2009). *Karenia brevis* monitoring, management, and mitigation for Florida molluscan shellfish harvesting areas. *Harmful Algae* 8, 608–610.

Henderson, R., & Tocher, D. (1987). The lipid composition and biochemistry of freshwater fish. *Progr. Lipid Res.* 26, 281–347.

Héral, M. (1989). L'ostréiculture française traditionnelle. In: Aquaculture (G. Bérnabé, Ed.), Vol. I, pp. 342-387. TEC et DOC Edition, Paris. Pp. 565.

Hickman, R.W., Waite, R.P., Illingworth, J., Meredyth-Young, J.L., & Payne, G. (1991). The relationship between farmed mussels, *Perna canaliculus*, and available food in Pelorus-Kenepuru Sound, New Zealand. *Aquaculture* 99, 49-68.

Hoagland, P., & Scatasta, S. (2006). The economic effect of harmful algal blooms. In: ecology on harmful algae (E. Graneli, T. Turner, Eds.). Springer: Berlin, Germany; 189, pp. 391–402.

Honkanen, R.E., Codispoti, B.A., Tse, K., Boynton, A.L., & Honkanan, R.E. (1994). Characterization of natural toxins with inhibitory activity against serine/threonine protein phosphatases. *Toxicon* 32, 339–350.

Iglesias, J., Gallardo, J.M., & Medina, I. (2010). Determination of carbonyl compounds in fish species samples with solid-phase microextraction with on-fibre derivatization. *Food Chemistry* 123(3), 771–778.

Imai, T. (1971). Aquaculture in shallow seas: progress in shallow sea culture. National Technical Information Service, PB 280 493-T.

Jackson, T.C., Acuff**,** G.R., & Dickson, J.S. (1997). Meat, shellfish and seafood. In: Food microbiology, fundamentals and frontiers (M.P.Doyle, L.R. Beauchat, T.J. Montville, Ed.). Washington, D.C.: ASM Press. 90 pp.

James, K.J., Gillman, M., Amandi, M.F., Lopez-Rivera, A., Puente, P.F., Lehane, M., Mitrovic, S., & Furey, A. (2005). Amnesic shellfish poisoning toxins in bivalve molluscs in Ireland. *Toxicon* 46, 852–858.

James, K.J., Lehane, M., Moroney, C., Fernandez-Puente, P., Satake, M., Yasumoto, T., & Furey, A. (2002). Azaspiracid shellfish poisoning: Unusual toxin dynamics in shellfish and the increased risk of acute human intoxications. *Food Addit. Contam. A* 19, 555–561.

James, K.J., Saez, M.J.F., Furey, A., & Lehane, M. (2004). Azaspiracid poisoning, the food-borne illness associated with shellfish consumption. *Food Addit. Contam. A* 21, 879–892.

Jay, J.M. (1992). Modern food microbiology. Fifth Edition. Ed. An Avi Book, Published by Van Nostrand Reinhold, New York.

Jeffrey, B., Barlow, T., Moizer, K., Paul, S., & Boyle, C. (2004). Amnesic shellfish poison. *Food Chem. Toxicol. 42*, 545–557.

Jo, P.G., An, K.W., Park, M.S., & Choi, C.Y. (2008). mRNA expression of HSP90 and SOD, and physiological responses to thermal and osmotic stress in the Pacific oyster, *Crassostrea gigas*. *Molluscan Research* 28, 158–164.

Jo, P.G. & Choi, C.Y. (2008). Osmoregulation and mRNA expression of calcitonin-related receptor in the Pacific oyster *Crassostrea gigas. Molluscan Research* 28(2), 137-141.

Josephson, D.B. (1991). Seafood. In: Volatile compounds in foods and beverages (H. Maarse, Ed.). 1st ed. New York: Marcel Dekker Inc. Pp.179-202.

Josephson, D.B., Lindsay, R.C., & Stuiber, D.A. (1985). Volatile compounds characterizing the aroma of fresh Atlantic (*Crassostrea virginica*) and Pacific (*Crassostrea gigas*) oysters, *Journal of Food Science* 50, 5-9.

Kaloyianni, M., Ragia, V., Tzeranaki, I., & Dailianis, S. (2006). The influence of Zn on signaling pathways and attachment of *Mytilus galloprovincialis* haemocytes to

extracellular matrix proteins. *Comp*. *Biochem. Physiol. C Toxicol. Pharmacol.* 144(1), 93-100.

Kamara, A.B. (1982). Oyster culture in Sierra Leone. In: Westview special studies in agriculture and aquaculture science and policy (A.B. Smith, K.J. Peterson, Eds.), Westview Press, Boulder, Colorado. Pp. 91-107.

Khan, M.A., Parrish, C.C., & Shahidi, F. (2005). Enumeration of total heterotrophic and psychrotrophic bacteria using different types of agar to evaluate the microbial quality of blue mussels (*Mytilus edulis*) and sea scallops (*Placopecten magellanicus*). *Food Research International* 38, 751-758.

Kirkpatrick, B., Fleming, L.E., Backer, L.C., Bean, J.A., Tamer, R., Kirkpatrick, G., Kane, T., Wanner, A., Dalpra, D., Reich, A., & Baden, D.G. (2006). Environmental exposures to Florida red tides: effects on emergency room respiratory diagnoses admissions. *Harmful Algae* 5, 526–533.

Koehn, R.K., & Shumway, S.E. (1982). A genetic/physiological explanation for differential growth rate among individuals of the American oyster, *Crassostrea virginica* (Gmelin). *Mar. Biol. Lett.* 3**,** 35-42.

Korringa, P. (1952). Recent advances in oyster biology. *The Quarterly Review of Biology* 27(3), 266-308.

Krock, B., Seguel, C.G., & Cembella, A.D. (2007). Toxin profile of *Alexandrium catenella* from the Chilean coast as determined by liquid chromatography with fluorescence detection and liquid chromatography coupled with tandem mass spectrometry. *Harmful Algae* 6, 734–744.

Kumar, K.P., Kumar, S.P., & Nair, G.A. (2009). Risk assessment of the amnesic shellfish poison, domoic acid, on animals and humans. *Journal of Environmental Biology* 30, 319–325.

Lagos, N., & Andrinolo, D. (2000). Paralytic shellfish poisoning (PSP): toxicology and kinetics. In: Seafood and freshwater toxins: pharmacology, physiology and detection (L.M. Botana, Ed.). Marcel Dekker: New York, NY, USA. Pp. 203–215.

Laing, I., & Spencer, B.E. (2006). Bivalve cultivation : criteria for selecting a site. *Technical Report no. 136*, 1-34. Weymouth.

Landsberg, J.H., Flewelling, L.J., & Naar, J. (2009). *Karenia brevis* red tides, brevetoxins in the food web, and impacts on natural resources: decadal advancements. *Harmful Algae* 8, 598–607.

Langton, R.W., & Gabbott, P.A. (1974). The tidal rhythm of extracellular digestion and the response to feeding in *Ostrea edulis*. *Marine Biology* 24, 181-187.

Launey, S., & Hedgecock, D. (2001). High genetic load in the Pacific oyster *Crassostrea gigas*. *Genetics* 159, 255–265.

Li, Y., Qin, J., Li, X., & Benkendorff, K. (2009). Monthly variation of condition index, energy reserves and antibacterial activity in Pacific oysters, *Crassostrea gigas,* in Stansbury (South Australia). *Aquaculture* 286(1-2), 64-71.

Linton, M., Mac Clements, J.M.J., & Patterson, M.F. (2003). Changes in the microbiological quality of shellfish, brought about by treatment with high hydrostatic pressure. *International Journal of Food Science and Technology* 38, 713-726.

Lizarraga, M. (1974). Tecnicas aplicadas en el cultivo de moluscos en America Latina. In: La acuicultura en America Latina. Food and Agriculture Organization, Infide Pesca, 152(2), 96-105.

Loader, J.I., Hawkes, A.D., Beuzenberg, V., Jensen, D.J., Cooney, J.M., Wilkins, A.L., Fitzgerald, J.M., Briggs, L.R., & Miles, C.O. (2007). Convenient large-scale purification of yessotoxin from *Protoceratium reticulatum* culture and isolation of a novel furanoyessotoxin. *Journal of Agriculture and Food Chemistry* 55, 11093–11100.

Lodeiros, C.J.M., & Himmelman, J.H. (1996). Influence of fouling on the growth and survival of the tropical scallop *Euvola* (*Pecten*) *ziczac* (L. 1758) in suspended culture. *Aquaculture Research* 27, 749–756.

Maffei, M., Piva, P., & Prioli, G. (1996). Allevamento sperimentale di *Crassostrea gigas* (Thunberg, 1793) in mare aperto su filari flottanti semi-sommersi. *Biol. Mar. Medit.* 3, 252–255.

Mathers, N.F. (1973). A comparative histochemical survey of enzymes associated with the process of digestion in *Ostrea edulis* and *Crassostrea angulata* (Mollusca: Bivalvia). *Journal Zool. Lond.* 169, 169-179.

Mattei, N., & Pellizzato, M. (1997) Mollusk fisheries and aquaculture in Italy. *NOAA Technical Report NMFS* 129, 201–216.

McManus, A., & Newton, W. (2011). Seafood, nutrition and human health: A synopsis of the nutritional benefits of consuming seafood. Centre of Excellence Science, Seafood & Health, Curtin Health Innovation Research Institute, Curtin University of Technology, Perth. Pp. 1-10.

Miles, C.O., Samdal, I.A., Aasen, J.A.B., Jensen, D.J., Quilliam, M.A., Petersen, D., Briggs, L.M., Wilkins, A.L., Rise, F., Cooney, J.M., & MacKenzie, A.L. (2005). Evidence for numerous analogs of yessotoxin in *Protoceratium reticulatum*. *Harmful Algae* 4, 1075–1091.

Morgan, K.L., Larkin, S.L., & Adams, C.M. (2009). Firm-level economic effects of HABS: a tool for business loss assessment. *Harmful Algae* 8, 212–218.

Morton, B. (1975). Pollution of Hong Kong's commercial oyster beds. *Marine Pollution Bulletin* 6(8), 117-122.

National Advisory Committee on Microbiological Criteria for Foods (NACMCF) (1992). Hazard analysis and critical control point system. *International Journal of Food Microbiology* 16, 1-23.

Newell, C.R., Shumway, S.E., Cucci, T.L., & Selvin, R. (1989). The effects of natural seston particule size and type on feeding rates, feeding selectivity and food resource availability for the mussel *Mytilus edulis* Linnaeus, 1758 at bottom culture sites in Maine. *Journal of Shellfish Research* 8, 187-196.

Ng, F.O. (1979). Experimental culture of flat oyster (*Ostrea folium*) in Malaysian waters. *Malaysian Agricultural Journal* 52(2), 103-113.

Okumus, I., & Stirling, H.P. (1998). Seasonal variations in the meat weight, condition index and biochemical composition of mussels *Mytilus edulis* L. in suspended culture in two Scottish sea lochs. *Aquaculture* 159, 249-261.

Ólafsdóttir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., Mackie, I.M., Henehan, G., Nielsen, J., & Nilsen, H. (1997). Methods to evaluate fish freshness in research and industry. *Trends in Food Science & Technology* 8, 258-265.

Orban, E., Di Lena, G., Masci, M., Nevigato, T., Casini, I., Caproni, R., Gambelli, L., & Pellizzato, M. (2004). Growth, nutritional quality and safety of oysters(*Crassostrea gigas*) cultured in the lagoon of Venice (Italy). *Journal of the Science of Food and Agriculture* 84(14), 1929-1938.

Orban, E., Di Lena, G., Nevigato, T., Casini, I., Santaroni, G., Marzetti, A., & Caproni, R. (2002). Quality characteristics of Sea Bass intensively reared and from lagoon as affected by growth. *Journal of Food Science* 67(2), 542-546.

Patrick, S., Faury, N., & Goulletquer, P. (2006). Seasonal changes in carbohydrate metabolism and its relationship with summer mortality of Pacific oyster *Crassostrea*

gigas (Thunberg) in Marennes–Oléron bay (France). *Aquaculture* 252(2-4), 328-338.

Pazos, A.J., Ruiz, C., Garcia-Martin, O., Abad, M., & Sanchez, J.L. (1996). Seasonal variations of the lipid content and fatty acid composition of *Crassostrea gigas* cultured in El Grove, Galicia, NW Spain. *Comp. Biochem. Physiol.* 114B, 171–179.

Pennarun, A.L., Prost, C., & Demaimay, M. (2002). Aroma extracts from oyster *Crassostrea gigas*: comparison of two extraction methods. *Journal of Agricultural and Food Chemistry* 50, 299-304.

Pérez Camacho, A., Labarta, U., & Beiras, R. (1995). Growth of mussels (*Mytilus edulis galloprovincialis*) on cultivation rafts: influence of seed source, cultivation site and phytoplankton availability. *Aquaculture* 138, 349-362.

Pierce, R.H., Henry, M.S., Blum, P.C., Hamel, S.L., Kirkpatrick, B., Cheng, Y.S., Zhou, Y., Irvin, C.M., Naar, J., Weidner, A., Fleming, L.E., Backer, L.C., & Baden, D.G. (2005). Brevetoxin composition in water and marine aerosol along a Florida beach: assessing potential human exposure to marine biotoxins. *Harmful Algae* 4, 965–972.

Pitcher, G.C., Cembella, A.D., Joyce, L.B., Larsen, J., Probyn, T.A., & Sebastian, C.R. (2007). The dinoflagellate *Alexandrium minutum* in Cape Town harbour (South Africa): Bloom characteristics, phylogenetic analysis and toxin composition. *Harmful Algae* 6, 823–836.

Piveteau, F. (1999). Étude des arômes de l'huître creuse *Crassostrea gigas*: conséquences d'un affinage à l'aide des microalgues *Skeletonema costatum* et *Haslea ostrearia.*Thèse doctorale, Université de Nantes, Nantes, France. Pp. 213.

Piveteau, F., Le Guen, S., Gandemer, G., Baud, J.P., Prost, C., & Demaimay, M. (2000). Aroma of fresh oysters *Crassostrea gigas*: composition and aroma notes. *Journal of Agricultural and Food Chemistry* 48(1964), 4851-4857.

Pogoda, B., Buck, B.H., & Hagen, W. (2011). Growth performance and condition of oysters (*Crassostrea gigas* and *Ostrea edulis*) farmed in an offshore environment (North Sea, Germany). *Aquaculture* 319(3-4), 484-492.

Prato, E, & Biandolino, F. (2007). Combined toxicity of mercury, copper and cadmium on embryogenesis and early larval stages of the *Mytilus galloprovincialis*. *Environ. Technol*. 28(8), 915-920.

Prioli, G. (2008). La Molluschicoltura in Italia. In: Estado actual del cultivo y manejo de moluscos bivalvos y su proyección futura: factores que afectan su sustentabilidad en América Latina (A. Lovatelli, A. Farías, I. Uriarte, Eds). Taller Técnico Regional de la FAO. 20–24 de agosto de 2007, Puerto Montt, Chile. Actas de Pesca y Acuicultura. No. 12. Roma, FAO. Pp. 159-176.

Quayle, D.B. (1988). Pacific oyster culture in British Columbia. *Can. Bull. Fish. Aquat. Sci.* 218-241.

Quayle, D.B., & Newkirk, G.F. (1989). Farming bivalve molluscs: methods for study and development (P.A. Sandifer, Ed.). Processing, 1, 1-307). Louisiana State University, Baton Rouge, LA 70803: International Development Research Centre, Canada.

Rehmann, N., Hess, P., & Quilliam, M.A. (2008). Discovery of new analogs of the marine biotoxin azaspiracid in blue mussels (*Mytilus edulis*) by ultra-performance liquid chromatography/tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 22, 549–558.

Reineccius, G. (1990). Off-flavors in foods. *Critical Reviews in Food Science and Nutrition,* 29, 381–402.

Richards, G.P. (1988). Microbial purification of shellfish. *J. Food Prot.* 51, 218-251.

Robert, J.M. (1982). Fertilité des eaux des claires ostréicoles et verdissement: utilisation de l'azote par les diatomées dominantes. Thèse de doctorat d'Etat, Université de Nantes, 281 pp.

Roncarati, A., Felici, A., Dees, A., Forlini, L., & Melotti, P. (2009). Trials on Pacific oyster (*Crassostrea gigas* Thunberg) rearing in the middle Adriatic Sea by means of different trays. *Aquaculture International* 18(1), 35-43.

Roncarati, A., Mordenti, O., Benedetti, S., & Melotti, P. (1998). Growth performance of diploid and triploid oysters (*Crassostrea gigas* Thunberg, 1793) reared in the middle Adriatic sea. In: Proc. XXXIII International Symposium on New Species for Mediterranean Aquaculture. Alghero, April 22–24.

Ross, K.A., Thorpe, J.P., & Brand, A.R. (2004). Biological control of fouling in suspended scallop cultivation. *Aquaculture* 229(1–4), 99–116.

Ryder, J.M., Fletcher, G.C., Stec, M.G., & Seelye, R.J. (1993). Sensory, microbiological and chemical changes in hoki stored in ice. *International Journal of Food Science and Technology* 28, 169–180.

Saito, H., & Marty, Y. (2010). High **l**evels of icosapentaenoic acid in the lipids of oyster *Crassostrea gigas* ranging over both Japan and France. *Journal of Oleo Science* 59(6), 281-292.

Sala, A., & Lucchetti, A. (2008). Low-cost tool to reduce biofouling in oyster longline culture. *Aquacultural Engineering* 39(1), 53-58.

Samain, J.F., & McCombie, H. (2008). Summer mortality of Pacific oyster *Crassostrea gigas*, The Morest project. Eds. Quae, Versailles, France.

Sarà, G., & Mazzola, A. (1997). Effects of trophic and environmental conditions on the growth of *Crassostrea gigas* in culture. *Aquaculture* 153, 81-91.

Sauvage, C., Pépin, J.F., Lapègue, S., Boudry, P., & Renault, T. (2009). Ostreid herpes virus 1 infection in families of the Pacific oyster, *Crassostrea gigas*, during a summer mortality outbreak: differences in viral DNA detection and quantification using real-time PCR. *Virus Research* 142, 181-187.

S.I.N.U. (2011). L.A.R.N. www.sinu.it.

Smaal, A.C., & Van Stralen, M.R. (1990). Average annual growth and condition of mussels as a function of food source. *Hydrobiologia* 195, 179-188.

Smith, V.M. (1982). The nitrogen and phosphorus dependence of algal biomass in lakes. An empirical and theoretical analysis. *Limnol. Oceanogr.* 19, 889–901.

Soletchnik, P., Le Moine, O., Goulletquer, P., Géairon, P., Razet, D., Faury, N., Fouche, D., & Robert, S. (2001). Optimisation of the traditional Pacific cupped oyster (*Crassostrea gigas* Thunberg) culture on the French Atlantic coastline: autumnal fattening in semi- closed ponds. *Aquaculture* 199, 73-91.

Somero, G.N., & Bowlus, R.D. (1983). Osmolyte and metabolic end products of molluscs: the design of compatible solute systems. In: Mollusca, Vol. 2 (P.W. Hochachka, Ed). Academic Press, New York, 77–100.

Soudant, P., Ryckeghem, K.V., Marty, Y., & Moal, J. (1999). Comparison of the lipid class and fatty acid composition between a reproductive cycle in nature and a standard hatchery conditioning of the Pacific Oyster *Crassostrea gigas*. *Comparative Biochemistry and Physiology Part B* 123, 209-222.

Spencer, B.E. (1990). Cultivation of Pacific oysters. Lab. Lesfl., MAFF Direct. Fish. Res., Lowesoft, 63, 1-47.

Tamburri, M.N., & Zimmer-Faust, R.K. (1996). Suspension feeding: basic mechanisms controlling recognition and ingestion of larvae. *Limnol. Oceanogr.* 41, 1188–1197.

Thorarinsdottir, G.G. (1994). The Iceland scallop, *Chlamys islandica* (O.F. Mueller), in Breidafjoerdur, west Iceland. 3. Growth in suspended culture*. Aquaculture* 120, 295-303.

Turolla, E., & Rossi, R. (2004). Esperienze di allevamento dell'ostrica concava (*Crassostrea gigas*) in Alto Adriatico. Ed. C.Ri.M., Centro Ricerche sui Molluschi, Goro (Fe). Pp. 31.

Turpin, V. (1999). Etude des evénements physico-chimiques et biologiques présidant à la proliferation d'*Haslea ostrearia* Simonsen dans les claires ostréicoles de la région de Marennes-Oléron: implication dans la maıtrise du verdissement. Thèse de l'Université de Nantes, pp. 208.

Ulbricht, T.L.V., & Southgate, D.A.T. (1991). Coronary heart disease: seven dietary factors. *The Lancet* 338, 985-992.

USFDA (United States Food and Drug Administration), (1993a). Guidance document for arsenic in shellfish. Center for Food Safety and Applied Nutrition, USFDA, Washington, DC.

USFDA (United States Food and Drug Administration), (1993b). Guidance document for cadmium in shellfish. Center for Food Safety and Applied Nutrition, USFDA, Washington, DC.

USFDA (United States Food and Drug Administration), (1993c). Guidance document for chrome in shellfish. Center for Food Safety and Applied Nutrition, USFDA, Washington, DC.

USFDA (United States Food and Drug Administration), (1993d). Guidance document for lead in shellfish. Center for Food Safety and Applied Nutrition, USFDA, Washington, DC.

Vale, C., & Botana, L.M. (2008). Marine toxins and the cytoskeleton: okadaic acid and dinophysistoxins. *FEBS J.* 275, 6060–6066.

Vale, P., & Sampayo, M.A. (2001). Domoic acid in Portuguese shellfish and fish. *Toxicon* 39, 893–904.

Valfré, F., Caprino, F., & Turchini, G.M. (2003). The health benefit of seafood. *Veterinary Research Communication* 27(1), 507-512.

Van Dolah, F.M., & Ramsdell, J.S. (2001). Review and assessment of in vitro detection methods for algal toxins. *J. AOAC Int*. 84, 1617–1625.

Volonterio, A.G. (1999). Microbiologia degli alimenti. Ed. Paravia Scriptorium. Torino.

Wada, K.T. (1994). Genetics of pearl oyster in relation to aquaculture. *Jap. Agric. Res. Quart.* 28, 276-282.

Ward, R.D., Thompson, P.A., Appleyard, S.A., Swan, A.A., & Kube, P.D. (2005). Sustainable genetic improvement of Pacific oysters in Tasmania and South Australia. CSIRO, Fisheries Research Development.

Wargo, R.N., & Ford, S.E. (1993). The effect of shell infestation by *Polydora* sp. and infection by *Haplosporidium nelsoni* (MSX) on the tissue condition of oysters*, Crassostrea virginica*. *Estuaries* 16(2), 229-234.

Watkins, S.M., Reich, A., Fleming, L.E., & Hammond, R. (2008). Neurotoxic shellfish poisoning. *Mar. Drugs* 6, 431–455.

Watters, K.W., & Martinez, P.A. (1976). A method for the cultivation of the mangrove oyster in Puerto Rico. Department of Agriculture. Commonwealth of Puerto Rico.

Widdows, J., & Donkin P. (1992). Mussel and environmental contaminants: bioaccumulation and physiological aspects. In: The mussel *Mytilus*: ecology, physiology, genetics and culture (E.M. Gosling, Ed.). Developments in Aquaculture and Fisheries Science, 25, 383-417.

Wildish, D.J., & Kristmanson, D.D. (1985). Control of suspension feeding bivalve production by current speed. *Helgo. Wiss. Meeresunters.* 39, 237-243.

Yasuhara, A. (1987). Comparison of volatile components between fresh and rotten mussels by gas chromatography-mass spectrometry. *Journal of Chromatography* 409, 251-258.

Yoon, G.L., Kim, B.T., Kim, B.O., & Han, S.H. (2003). Chemical–mechanical characteristics of crushed oyster-shell. *Waste Manage* 23, 825–834.

Young, A., & Serna, E. (1982). Philippines. In: Bivalve culture in Asia and the Pacific (F.B. Davy, M. Graham, Eds.). Workshop Proceedings, Singapore. International Development Research Centre, Ottawa, Canada. Pp. 55-68.

Zhang, Z., Yang, M.J., & Pawliszyn, J. (1994). Solid-Phase Microextraction. *Analytical Chemistry* 66 (17), 844-853.

Zorita, I., Bilbao, E., Schad, A., Cancio, I.S., & Cajaraville, M.P. (2007). *Toxicol. Appl. Pharmacol.* 220(2), 186-96.

EXPERIMENTAL SECTION

1. AIM OF THE RESEARCH

The presented research was developed with the general purpose of evaluating the possibility to rear oysters (*Crassostrea gigas*) in Tuscany coast. Considering that oysters production in Italy is negligible even though the product is widely appreciated and requested, an Italian production of oysters could be of economic interest.

In detail, this research was performed with the following specific purposes:

- developing a proper experimental protocol to analyze quality and shelf life of marketable oysters and to characterize the marketable product;
- developing an appropriate analytical procedure able to determine the volatile component of oysters, considered one of the most important aspects for characterizing their quality
- comparing growth performances, chemical and commercial characteristics of oysters reared in two different environments of the Tuscany coast, Orbetello Lagoon (Grosseto, Italy) and offshore Porto Ercole (Grosseto, Italy);
- comparing the effect of 3 different containers (*poches*, baskets, lanterns) on growing performances and on commercial characteristics on offshore reared oysters;
- assessing the effect on marketable characteristics, quality and shelf life during refrigerated storage out of water of a finishing period in the offshore site (using two different containers, lanterns and baskets) on oysters grown in the lagoon.

The experimentation was composed by three main trials, one preliminary (Trial 1) and the other two (Trial 2 and Trial 3) performed in the two different environments of the Tuscany coast, Orbetello Lagoon and offshore near Porto Ercole. The sites were chosen due to their diversities, to test the effect of each rearing site on oyster characteristics, singularly and in combination.

The Trial 1 was a preliminary experiment on oysters quality and shelf life: oyster of commercial size, coming from two different Italian rearing sites, Orbetello Lagoon and offshore near Manfredonia (Foggia, Italy), were used to develop proper experimental protocols and analysis methods to assess oyster quality and shelf life during refrigerated storage, analyzing commercial, morphological, chemical, microbiological characteristics and the shelf life evolution.

During this trial a procedure for the analysis of the volatile organic compounds was developed, by the selection of the fibre, the time and the temperature most appropriate for the detection of the molecules responsible of the odour of shellfish. The effectiveness of the method was tested on 6 different species of shellfish in order to verify its sensitivity. The method was also tested on samples of oysters subjected to 10 days of storage at low positive temperatures

The Trial 2 (farming experiment) had the purpose to evaluate the potentiality of the two experimental sites (Orbetello Lagoon and offshore Porto Ercole) and the aptitude of three oyster containers to the growth of the oysters from juvenile to adult stage, evaluating the morphological, commercial and chemical characteristics during the whole experimental period.

The Trial 3 (finishing experiment) was performed to evaluate the effects on oyster quality and shelf life characteristics of a finishing period in the open sea, after growing in the lagoon. The sense of this trial stays in the differences existing between the two environments: Orbetello Lagoon presents high nutritional potentialities for oysters farming, throughout the year, but it is also subject to periodic algal blooms. It also presents a series of hazards linked to the microbiological quality of the water and to toxins of algal origin, harmful for human health, that can accumulate in oysters tissues, so that Orbetello Lagoon water is classified as “B”, according to the D. lgs. 530/92. The open sea presents a wide fluctuation of nutrient availability in the water during the year, so that in the periods corresponding to the negative peaks, a lack of nutrients can occur, but its water quality is classified as A. This means that the two environments can be exploited integrating their characteristics, and a finishing period in the open sea after growing in the lagoon could be a solution to the disadvantages of both sites and on opportunity to exploit the advantages of the two sites.

2. MATERIALS AND METHODS

Organization of the experiments

2.1 Preliminary trial (Trial 1)

To perform the preliminary experiment, carried out in November 2009, marketable oysters farmed in the Orbetello Lagoon (Grosseto) and offshore in the Adriatic sea near Manfredonia (Foggia), were used to perform analyses on biometric, commercial and chemical characteristics and the analyses to test the performance of the two groups of oysters during 10 days of 4 °C refrigerated storage, from the point of view of shelf life characteristics. For this trial, oysters coming from Orbetello Lagoon were in total 75, while the oyster coming from Manfredonia were 91. The analysis sessions during the refrigerated storage were scheduled at the 1^{st}, 3^{rd}, 7^{th}, and 10^{th} day after harvesting.

At the beginning of each sampling session measures on the whole oyster were performed in order to collect informations about the following morphological characteristics:

- maximum length (maximum antero-posterior axis)
- maximum width (maximum back-ventral axis)
- maximum thickness (maximum transversal axis)

  using a precision digital calliper (CEDWP15, Borletti, Italy)
- total weight

  by a precision electronic balance (Mettler-Toledo S.p.A., Novate Milanese, Milano, Italy).

The oysters were then opened the colour of gills and mantle was measured on ten oysters. The measurement was performed using a Dr. Lange Spectro-Color® (Düsseldorf, Germany) portable colorimeter, d/8° with a 10 mm measuring aperture and SPECTRAL-QC 3.6 software colorimeter, for data processing. The measurement was performed in triplicate on each measuring point and the mean value was considered for the data analysis. Colour measurement was expressed following the CIELAB system (CIE, 1976) as lightness (*L**), red index (*a**), yellow index (*b**), colour saturation (Chroma: $[C^*_{ab}=(a^{*2}+b^{*2})^{1/2}]$) and hue angle (Hue: $H°_{ab}=\tan^{-1}b^*/a^*$).

Then the oysters were dissected. The edible parts of each oyster, before further measurements and analyses, were arranged on a sieve, washed with distilled water and left for 10 minutes for dripping, while the shells were placed on a tray.

Both the edible parts and the shells were arranged in order to maintain the individuality of the collected data. The measurements performed on the edible parts and on the shells were the following:

- weight of each edible part
- weight of the shell
  by a precision electronic balance

- economic condition index (Imai & Sakai, 1961)
  expressed by the formula:
  ECI=shell thickness / 0.5(shell length + width)*

- condition index (I) (Booth, 1983)
  expressed by the formula:
  CI (I) = flesh weight / total individual weight

- condition index (II) by the Walne (1976) modified formula:
  CI (II) = dry flesh weight/shell weight

- shape index (Brake et al., 2003)
  expressed by the formula:
  SI= shell depth/ shell length.

After weighting the edible parts were pooled together respecting the belonging group. The use of pooled tissues to analyse invertebrate edible part composition is recommended by Giese (1966) and Giese et al. (1967).Each pool was frozen and then lyophilized and stored into airtight plastic bags at the temperature of -20 °C. On the lyophilized samples the following chemical parameters were analyzed.

- moisture (method 950.46; AOAC, 2000)
- crude protein (method 976.05; AOAC, 2000)
- ash (method 920.153; AOAC, 2000)

- total lipids (Folch et al., 1957)
- qualitative and quantitative analysis of fatty acid profile (Morrison & Smith, 1964) by gas-chromatography and mass-spectrometry.

Selected samples were used to perform the analyses on microbiological loads, performed on the fresh edible part of the oysters. The analyses carried out were related to the loads of:

- *Escherichia coli*
- total coliforms
- total aerobic bacteria (Total Viable Count)
- *Pseudomonas* spp. (Total Viable Count)
- Micrococcaceae (Total Viable Count).

2.2 Experimental trial

2.2.1 Rearing environments and plants

The rearing plants were located in the Orbetello Lagoon and in open sea, as shown in Figure 1.

Figure 1. The sites chosen for the experimental trials on oysters.

Orbetello Lagoon is located on the southern Tuscany coast. It is composed by two connecting ponds, called “Laguna di Ponente” and “Laguna di Levante”, extending respectively for 15.25 km^2 and 10.00 km^2 (Travaglia & Lorenzini, 1985) and with an average depth of 1.00 m.

Orbetello Lagoon, as many others lagoons, can suffer of eutrophia and hypertrophia. These phenomena are caused by the scarce exchange of water occurring between the lagoon and the sea and also by the high inputs of nutrients of anthropogenic origin, especially since the 1960s, when agriculture, aquaculture and tourism activities increased dramatically along this area of the Tuscany coast.

The rearing plant placed in Orbetello Lagoon, was located in Laguna di Ponente (west side of the lagoon). The area, which is managed under concession by the Soc. Coop. GIGAS, is placed near the Nassa channel mouth and is delimited by the following coordinates: 42° 26' 10'' N - 11° 11' 03'' E; 42° 26' 05'' N - 11° 10' 3 7'' E; 42° 26' 06'' N - 11° 10' 35'' E; 42° 26' 14'' N - 11° 10' 59'' E.

Lagoon's water was classified as "B" according to the Italian D.lgs. 530/92.

The sea rearing site was placed offshore and was located in an area of the Tyrrhenian Sea, managed under concession by the Soc. Il Vigneto, Ansedonia (Grosseto), and placed at 2000 m offshore the coast of Porto Ercole, with the following coordinates: 42° 23' 176'' N, 11° 14' 393' E. The plant is a long line support consisting in a 144 m long line.

The sea water quality of this site was classified as "A" class according to the Italian D.lgs. 530/92.

2.2.2 Water characteristics: chlorophyll pigments and phytoplanktonic biomass

For analysing water characteristics of the two sites, from March to October 2010 eight water samples were withdrawn to determine chlorophyll pigments and phytoplankton biomass content. To determine the chlorophyll pigments concentration, 2 L of superficial water was collected, from the Orbetello Lagoon as from the open sea site offshore Porto Ercole. In the laboratory the samples were filtered with a filter equipped with glass fibre Whatman GF/F. The filter was introduced into a tube with 5 mL of neutralized acetone 90% and frozen for the determination of the concentration of chlorophyll-*a* (Chl*a*). At the moment of the analysis the samples were defrosted, the glass fibre filter were crumbled and homogenized with a muller for 2 minutes, washing the muller with cold acetone 90% neutralized with anhydrous sodium carbonate. The mix was transferred in centrifuge tubes, where acetone 90% was added to reach the volume of 10 mL. The tubes were maintained for 24 hours at 5 °C in the dark. The samples were then centrifuged at 4000 r/min for 5 minutes, and then

the supernatant was transferred in a quartz cuvette and analyzed with a molecular absorption spectrophotometer (Lambda, Perkin Elmer). The absorbance of the sample was determined at 664 and 750 nm. Chlorophyll-*a* concentration was calculated as follows:

$$Chla = \frac{[(As664 - Aw664) - (As750 - Aw750) * v * 10^6]}{\varepsilon * CO * V}$$

where

As664 = sample absorbance at 664 nm;

As750 = sample absorbance at 750 nm;

Aw664 = “white” absorbance at 664 nm;

Aw750 = “white” absorbance at 750 nm;

ε = absorbance coefficient specific for chlorophyll suspended in acetone al 90% (89.67L/cm xg);

CO = cell optical path (cm);

v = extract volume (cm^3);

V = filtered sample volume (cm^3).

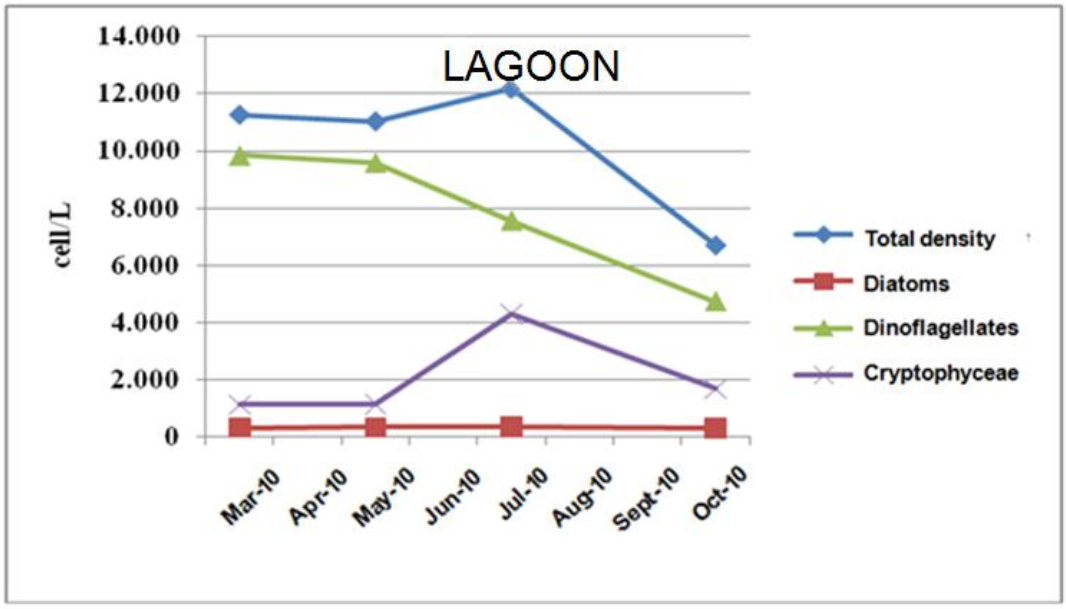
To analyze the phytoplankton biomass, 500 mL of superficial water were withdrawn from the lagoon as from the sea. In the laboratory each sample was fixed with 2-4% of 40% formalin, neutralized with sodium carbonate. The sample was centrifuged until sedimentation and then analyzed with an optical microscopy with a final zoom of 300x. Phytoplankton density was calculated by counting and expressed as cells per litre (cell/L).

The deep differences existing between the two sites are confirmed by the results of the analyses on the trophic characteristics of the water shown in Figures 2, 3 and 4.

The samples collected during March and May 2010 in the sea presented high concentration of phytoplankton cells (between 14000 and 17000 cells/L), while the lagoon water in the same period presented a concentration of about 11000 cells/L. This means that during the spring the water in the sea had a high nutrient capability, even higher than the lagoon, that is generally eutrophic (Lenzi et al., 2003). From the analysis of the water performed in July 2010 resulted that the two environments had

opposite trends: in the water of the sea occurred the minimum peak of phytoplankton concentration of about 3000 cells/L, while in the lagoon there was the maximum phytoplankton level registered of about 12000 cells/L; moreover, from July until October 2010 the concentration of phytoplankton tended to increase in the sea, while decreased in the lagoon.

Figure 2. Phytoplankton composition of the Orbetello Lagoon water analyzed during the growing trial.



These results suggest an opposite nutrient content trend in the two farming sites, reinforcing the idea that the lagoon environment can be exploited together with sea environment in an integrated way for oysters growing and finishing. Figure 4 showing the Chl*a* concentration in the sea and in the lagoon, underlines the higher ability of the lagoon to sustain the nutritional needs of an oyster culture all over the year.

Figure 3. Phytoplankton composition of the open sea water near Porto Ercole analyzed during the growing trial.

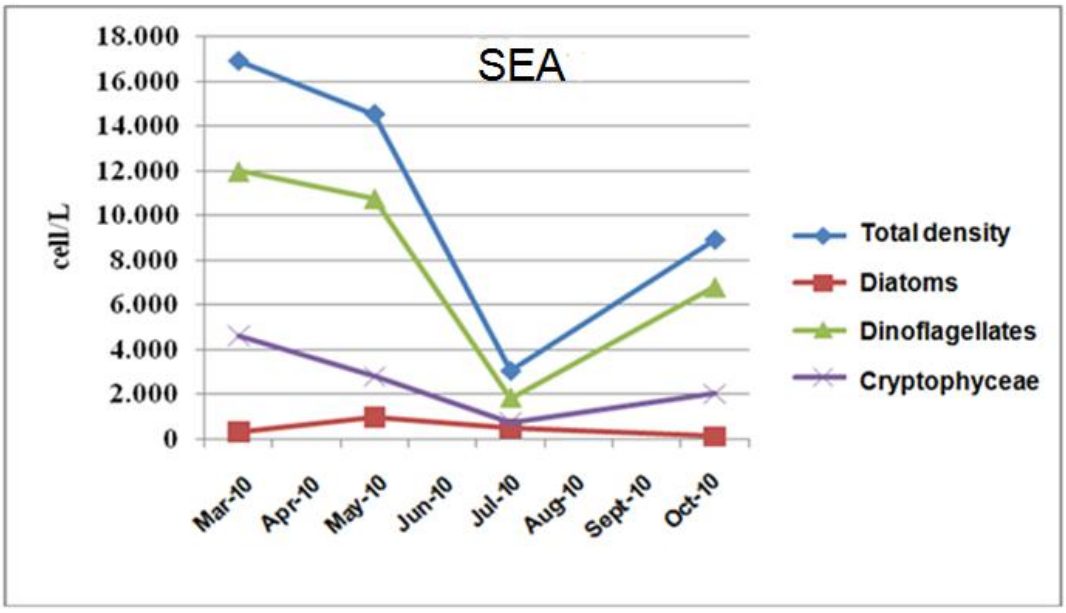
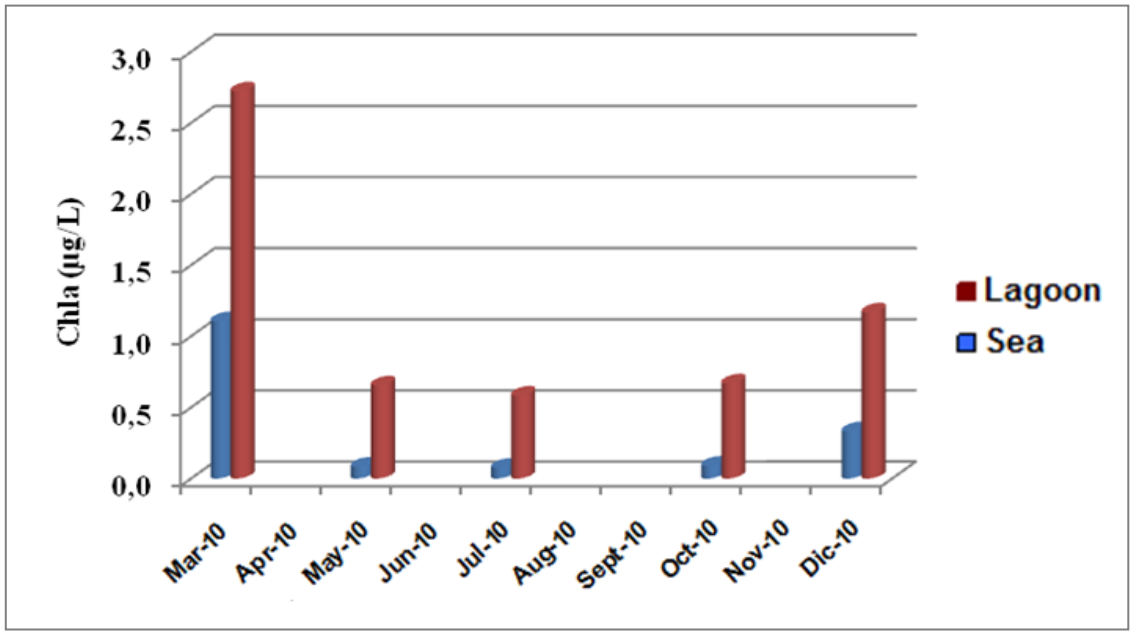


Figure 4. Chlorophyll-*a* content in the superficial water of the Orbetello Lagoon and the open sea near Porto Ercole analyzed during the growing trial.



2.2.3 Farming containers

During the growing experiment performed in the open sea site near Porto Ercole three different kind of containers were used: *poches*, baskets and lanterns (Figure 5). *Poches* were also used in the lagoon, where no other kind of containers is suitable for oysters growing, due to the little depth of water (1 m).

Figure 5. The containers used in the experimental trial: *poches* (left), baskets (middle) and lanterns (right).

Poches is the container widely used in oyster farming in France and in general diffused in tidal zones, where the *poches* are periodically exposed to air. In these zones, *poches* are commonly accommodated horizontally on racks made of wood or steel. This kind of lay has a good area/volume ratio, allowing an optimal water circulation. *Poches* have rectangular shape and in our experiment they measured 60x40x5 cm and consisted in PVC net, wrapping two supports arranged on the long side of the *poches* and fixed with plastic ties to form a big pocket. Inside a *poches* there is no partition of the space.

Baskets are cylindrical, rigid modular containers made of PVC. Each module, consisting in a cylinder with a diameter of 58 cm and a height of 10 cm, is partitioned inside in two or four parts, giving a higher stability to the structure. The modules were assembled one upon another by means of a central rod connecting the parts between the two tips, formed by a cylindrical groove perpendicular to the plane of the containers and the modules were maintained together with plastic ties. Our baskets were composed by three overlapped modules subdivided into two sections. This kind

of container presents a poor resistance to currents; the partitioning of each module allows the correct circulation of the water inside the container. The whole structure is easily demountable, easy to clean and reusable, so it allows to save money for transport and stocking, but also to reduce wastes.

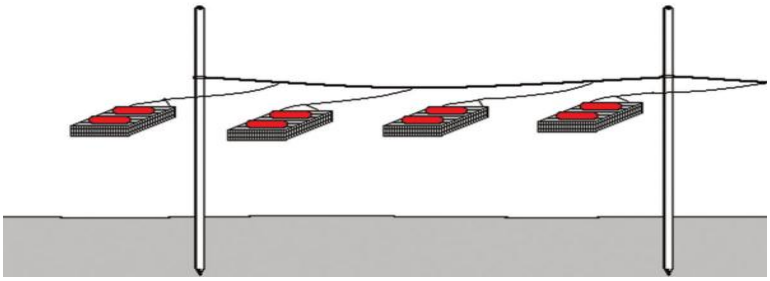
Lanterns are cylindrical, soft containers made with tubular net. They are divided into five sections with a diameter of 49 cm and 18 cm high. The structure is maintained by five rigid rings and the lateral vertical slot is closed by plastic ties.

2.2.4 Growing experimental trial (Trial 2)

Oyster juveniles were bought from the British hatchery Seasalter Shellfish (Whitstable) Ltd. (Herne Bay, Kent, UK). A total number of 40,000 juveniles corresponding to a total weight of 60 kg were employed for the growing trial.

In lagoon, the plant consisted in poles driven bottom supporting a rope, where 16 *poches* were hanged to the floating supporting line (Figure 6) and divided into 4 sections (A, B, C, D). Each *poche* was marked with the belonging section name and with a progressive number (A1, A2, A3, etc.).

Figure 6. Scheme of the Orbetello Lagoon plant.



For the sea plant it was chosen a long line support consisting in 4 sections (A, B, C, D) with 4 *poches*, 4 lanterns and 4 baskets each, so that, on the long-line structure 48 containers were hanged, as shown in Figure 7. Each container was marked with the name of the container (P = *poche*; L= lantern; B = basket), the name of the section and a progressive number.

Before seeding, a pool of 98 animals was withdrawn for analyses: length, width and thickness were measured with a precision caliper, individual total weight of the live animals was measured with a precision balance, giving the following mean measures: length 24.76±4.79 mm, width 14.85±1.45 mm, thickness 8.65±1.77 mm, weight 1.83±0.60 g.

Figure 7. Scheme of the offshore plant in the Tyrrhenian Sea near Porto Ercole.

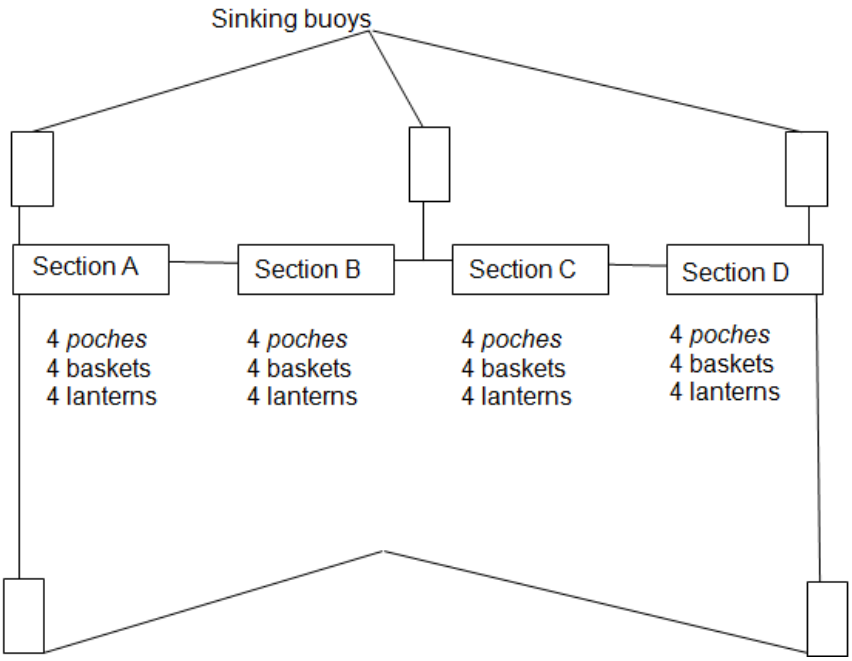


Table 1. Sample size during each sampling session.

Sampling sessions	Days	Oysters analyzed
July 2009 (T0)	0	98
September 2009 (T1)	68	615
January 2010 (T2)	185	668
March 2010 (T3)	242	660
May 2010 (T4)	305	567
July 2010 (T5)	361	453
September 2010 (T6)	438	422

The trial started in July 2009 with seeding operations. In order to evaluate growing performances of the oysters, six sampling sessions were performed at different times. Table 1 shows the sampling sessions and the total number of oysters analyzed during each one.

In order to assess the right number of juveniles to introduce in each container, we referred to the biometric data of Spencer (1990) to estimate the weight of the animals throughout the all growing period.

In the lagoon each *poche* was filled with 350 oysters (0.15 oysters cm^{-2}).

In the sea, whereas the loading capacity of the containers has been limited and the winch of the boat at our disposal can load 30 kg maximum, the juveniles were placed considering the final weight and a mortality of 0%. Then in each *poche*, 100 oysters were placed, 325 oysters (65 per floor; 0.04 oysters cm^{-2}) in each lantern and 360 oysters (120 per floor, 60 per section; 0.05 oysters cm^{-2}) in each basket. For the early phase of the study, in order to avoid losses of animals, the young oysters were introduced into plastic mesh bag (7 mm mesh size).

In each experimental session, between 35 and 45 animals from 2 of each typology of container in lagoon and in the sea were withdrawn. The samples were transported in an icebox from the farming sites (Orbetello and Porto Ercole) to the laboratories of Department of Agricultural Biotechnology – Animal Science Section of the Florence University, where the oysters were stored at 4 °C until the following day, when the oysters were individually analyzed.

During each experimental section were performed analyses on the whole and alive oysters, as described in the preliminary trial. The analyses carried out were the following:

- maximum length
- maximum width
- maximum thickness
- total individual weight.

Analyses on the dissected animals were also performed as described in the preliminary trial. They were the following:

- weight of the edible part
- weight of the shell.

The edible parts of the oysters of each container were pooled and frozen together, then they were lyophilized. The lyophilized samples were crumbled and stored in airtight plastic bags at the temperature of -20 °C.

On the lyophilized samples were performed the analyses of the following chemical parameters:

- moisture
- crude proteins
- ash
- total lipids
- qualitative and quantitative analysis of fatty acid profile by gas-chromatography and mass-spectrometry
- heavy metals and selenium content by atomic absorption.

2.2.5 Finishing experimental trial (Trial 3)

In September 2010 from the oysters grown in the Orbetello Lagoon and used during the Trial 2, a group was selected for size and for translocation to the sea plant located near Porto Ercole to analyze the performance of oyster during 98 days of finishing in comparison with the performance obtained in lagoon by the group of oysters selected for size and left in lagoon, i.e. in the same site where the growing phase was realized. Oysters from lagoon used to perform the experiment were manually selected and presented the following average characteristics: length 24.76±4.79 mm, width 14.85±1.77 mm, thickness 8.65±1.45 mm, total weight 59.29±17.50 g.

In each *poche*, 280 oysters (0.12 oysters cm^{-2}) were placed, 325 oysters (65 per floor; 0.04 oyster cm^{-2}) in each lantern and 300 oysters (100 per floor, 50 per section; 0.04 oysters cm^{-2}) in each basket.

In the finishing experimental trial, the oysters for the sea-finishing were reared only in lanterns and baskets, because, on the basis of the results obtained in Trial 2, the performance of the oysters reared in *poches* resulted the worst. The selected oysters were put in 4 *poches* for the lagoon-finishing (control group) and in 4 lanterns and 4 baskets for the sea-finishing.

In this experiment, three samplings were performed during the finishing period. During the first two samplings, realized in October and in December 2010, we analyzed the growing performances, and commercial characteristics obtained from the oysters finished in the lagoon compared with the oysters finished; we analysed also the effect of container (baskets *vs* lanterns) utilised for the finishing in the sea. In the 3rd sampling, in February 2011, the analyses of morphological, marketable, chemical, and sensory parameters and the changes of the characteristics during refrigerated storage of the oysters finished in the lagoon compared with the oysters finished in the sea were performed.

Following the same methods used for the refrigerated storage experiment performed in the Trial 1, in each experimental session (at 1st, 4th, 7th and 10th day) during the refrigerated storage at 4 °C n. 15 oysters from the lagoon plant and n. 15 oysters from the sea plant were utilised to analyze the biometric, commercial and chemical characteristics, while for microbiological analyses n. 9 oysters from the lagoon plant and n. 9 oysters from the sea plant were utilised.

In this experiment all the methods developed in the preliminary experiment (Trial 1) were applied. In addition, a panel test was performed on the 2nd day of storage in order to evaluate the sensory characteristics of the oysters finished in the two sites. While the identification of the bacteria species was not performed.

2.3 Chemical analyses

2.3.1 Moisture

Edible parts of the oysters were pooled in a calibrated aluminium container. The sample was frozen and then lyophilized. As soon as the lyophilisation was completed, the dry samples were put immediately in a desiccator and weighted on a precision electronic balance.

Moisture (g/100g) was calculated as following:

$$\text{Moisture \%} = 100 - (\text{NWF} \times 100 / \text{NW})$$

where:

NWF = Net weight of freeze dried sample

NW = Net weight of fresh sample.

2.3.2 Crude protein

Analysis of crude proteins was performed using Kjeldahl method (AOAC, 2000), that allows the detection of protein, amino, ammonia and urea nitrogen in the sample.

The first step was the digestion of the sample: 0.5 g of sample were heated with 15 ml of sulphuric acid 99% and 5 g of selenium as catalyst until complete degradation of the organic matter to inorganic occurs (the mix changes completely from very dark to perfectly clear). In this step all the protein nitrogen was converted in ammonium sulphate. The second step was the distillation of the sample. The tube containing the sample was introduced in a distillator and added of a solution of sodium hydroxide 40% to change the pH of the sample, from acid to alkaline: in this step the ammonium sulphate was converted in free ammonia that can be distilled. Distilled ammonia is collected in 60 mL of boric acid and a pH indicator (methyl red). The ammonia amount is determined trough back titration with sulphuric acid 0.2%.

To obtain the value of crude protein (in percentage), the following formula was used:

$$P = T \times 0.28 \times 6.25$$

where:

T: is the volume (mL) of sulphuric acid used for the back titration to determine the ammonia amount

0.28: is the correction factor representing the equivalent of nitrogen of the sulphuric acid used for the back titration

6.25: is the conversion factor of the nitrogen quantity to crude protein content.

2.3.3 Ash

To analyze ash content of the edible part of the oysters, the samples were weighted in calibrated melting pots. After a pre - ashing on a hot plate, the pots were transferred for 6 hours in a muffle furnace. After cooling in a desiccator, the pots were weighted on an analytical precision balance. The ash fraction was obtained by the difference between the initial weight (W1) and final weight (W2) of the samples applying the following formula:

$$\text{Ash (\%)} = 100 \times (W1 - W2)/W1$$

where:

W1 = weight (g) of sample before drying

W2 = weight (g) of sample after drying.

2.3.4 Total lipid

0.5 grams of lyophilized sample were hydrated with 1.5 mL of deionized water to reconstitute approximately 2.00 g of oyster edible part. The reconstituted sample was mixed in a stainless steel tube of a SORVALL Omni-mixer® with 34 mL of a Chloroform:Methanol 2:1 solution with 0.01% BHT as antioxidant. The sample was homogenized putting the cylinder in an ice bath for 5 minutes and then filtered on a filter paper. The tube was washed twice, each time with 2 mL of the Chloroform:Methanol solution, as the filter paper. Another washing with the same solution was performed on the glass funnel supporting the filter paper. The lipidic extract was washed with 10 mL of a KCl 0.88% solution; the tube was closed and shaken vigorously for one minute to make the emulsion occurr. The opening of the tube was washed with 1.5 mL Chloroform:Methanol 2:1 solution and the emulsion was let stand at 4 °C for a night, until the complete separation of the emulsion. The organic phase was recovered and the remaining solvent was removed using a rotary evaporator (Büchi RE111 Rotavapor). The obtained lipid extract was dissolved in 5.00 mL chloroform and then introduced into dark glass hermetic bottles which were stored at the temperature of -20 °C until analysis.

To analyze the total lipids content of the sample, 1 mL of dissolved lipid extract was pipetted into a calibrated hand-made aluminium bowl. The bowl was introduced into a vacuum stove maintained at 40 °C for 1 hour, then it was put into a desiccator to chill without reabsorb the humidity from the air. After that, the aluminium bowl was weighted on a precision balance.

The total lipid content of the sample was obtained with the following formula:

$$TL=(FW-TW)$$

where

FW: is the gross final weight of the aluminium bowl with the lipid extract

TW: is the weight of the tare.

A further amount of 1 mL of chloroform solution was set for fatty acid profile analysis by gas-chromatography, following the method of Morrison and Smith (1964).

2.3.5 Fatty acid composition

To analyze the quantitative and the qualitative fatty acid composition of the edible parts of the oysters, the method developed by Morrison & Smith (1964) was applied. The method consists in two phases: saponification and esterification.

Saponification

In this phase occurs the separation between neutral and polar lipidic phase using potassium hydroxide: 3 mg of lipid extract dissolved in chloroform were withdrawn and transferred into a tube with screw cap. With a rotary evaporator (Büchi RE111 Rotavapor) all the solvent was removed, and the internal standard (IS) C23:0 (0.5 ml of a solution containing 0.1 mg/ml of IS dissolved in benzene) and 5 mL of KOH 0.5 M dissolved in methanol were added. Then the tube was blown with nitrogen and put in a water bath at 80 °C for 40 minutes, shaking every 10 minutes. To check if the saponification occurred, 1 mL of distilled water was added and the pH measured: if the values were higher than 10 the saponification occurred correctly. Subsequently 2.5 mL of HCl 2M were added to obtain free fatty acids. Finally, free fatty acids were extracted with 2.5 mL petroleum ether 40-60 for two times and removing the solvent with the rotary evaporator.

Esterification

Free fatty acids were resuspended in a tube with 1 mL of cyclohexane and 2 mL of BF_3-methanol 14%. Nitrogen was blown into the tube and put in a water bath at 95 °C for 4 minutes.

After chilling, 2.5 mL of distilled water were added, then methyl-esters were extracted with 2.5 mL of petroleum ether (40-60), the extraction was repeated twice. After the solvent was removed, the methyl-esters were dissolved in 1 mL of hexane and transferred in hermetic vials. The chromatographic analysis were performed within 24 hours using the following experimental conditions of the gas-chromatograph (Varian GC-430): Column: Supelco Omegawax™ 320, length 30 m, internal diameter 0.32 mm, thickness of the stable phase 0,25 μm.

Temperature program: 100 °C (hold for 2 minutes), increase of 12 °C/min until 160 °C (hold for 4 minutes), increase of 3 °C/min. 220 °C (hold for 14 minutes). Run duration 45 minutes.

Detector: FID 300 °C

Carriers: He 1.5 mL/min, air 300 mL/min, H_2 30 mL/min.

The resulting chromatograms were analyzed with the software Galaxie Chromatography Data System 1.9.302.952.

The gas chromatograph system was routinely calibrated with standard FAME mixtures (reference standard Supelco 37 Comp. FAME Mix, Supelco, Bellefonte, PA, USA). Identification of sample fatty acids was made by comparing the relative retention times of FAME peaks from samples with those of the standards.

By the quantitative analysis of the fatty acids profile, two indexes of the healthiness quality of lipids were calculated, the atherogenity index (AI) and the trombogenic index (TI) (Ulbricht & Southgate, 1991) as follows, where PUFA are polyunsaturated fatty acids, MUFA monounsaturated fatty acids, and SFA saturated fatty acids:

$$AI = \frac{C12{:}0 + (4 * C14{:}0) + C16{:}0}{PUFA\ n6 + PUFA\ n3 + MUFA}$$

$$TI = \frac{C14{:}0 + C16{:}0 + C18{:}0}{(0.5 * PUFA\ n6) + (3 * PUFA\ n3) + (0.5 * MUFA) + \frac{PUFA\ n3}{PUFA\ n6}}$$

The following ratios were also computed for fatty acids:

- PUFAn-6/PUFAn-3
- PUFA/SFA
- hypocholesterolemic fatty acids/hypercholesterolemic fatty acids (h/H) ratio which considers the effect of fatty acid profile on cholesterol metabolism, according to Santos-Silva et al.(2002):

$$\mathrm{h/H} = \frac{(\Sigma \mathrm{C18{:}1c{-}9{+}C18{:}1c{-}11{+}C18{:}2n{-}6{+}C18{:}3n{-}6{+}C18{:}3n{-}3{+}C20{:}3n{-}6{+}C20{:}4n{-}6{+}C20{:}5n{-}3{+}C22{:}4n{-}6{+}C22{:}5n{-}3{+}C22{:}6n{-}3)}{(\Sigma \mathrm{C14{:}0{+}C16{:}0})]}$$

2.3.6 Heavy metals and selenium content

The heavy metals determined were: cadmium, lead and mercury.

The determination of the content of Cd and Pb in the edible part of the oysters was performed by electrothermal atomic absorption spectrophotometry (GF-AAS) using a Perkin Elmer AAnalyst 700. In order to minimize interference and stabilize the analyte, modifying salts were added. To quantify Cd, 15 g of palladium and 10 g of

magnesium nitrate were added; to quantify Pb, 200 g of ammonium phosphate were added. Hg content was determined by cold vapor generation atomic absorption spectrometry (CV-AAS) technique, using a Perkin-Elmer flow-injection mercury system (FIMS) 400.

The determination of selenium (Se) content was carried out in hydride generation atomic absorption spectrometry (GH-FIAS), using a Perkin-Elmer flow-injection mercury system (FIMS) 400, after pre-concentration of selenium (VI) in tetravalent state. To this purpose, the resulting solutions obtained by mineralization were heated at 90 °C for 20 minutes with HCl 6 mol/L. A calibration curve was obtained adding known and increasing quantities of the element to be determined to equal volume of sample. The resulting calibration curve was used to calculate the concentration of the analyte.

The final results obtained, as the average value of three readings, were expressed in mg kg^{-1} dry weight and then converted to mg kg^{-1} fresh weight. The analytical precision was expressed as variation coefficient (VC%) on three analysis replications for each sample. Accuracy was checked referring to the certified “Oyster Tissue” of the National Institute of Standard & Technology.

2.4 Indicators of changes during shelf life

In the trial 3, the following parameters were determined on each sampling day (1^{st}, 3^{rd}, 7^{th}, and 10^{th}) in refrigerated oysters from two finishing sites sampled in February: intervalvar liquid content, colour, volatile organic compound profile, pH. The microbiological load was determined at 1^{st}, 7^{th} and 10^{th} day of storage, since a technical problem prevented analysis on day 3.

The intervalvar liquid content was determined in all oysters analyzed also for morphological characteristics, with the formula: [100 x (total weight – edible part weight – shell weight) / total weight].

Colour was measured by a Dr. Lange Spectro-color® (Düsseldorf, Germany) portable colorimeter, d/8° with a 10 mm measuring aperture and SPECTRAL-QC 3.6 software. The results are given in the tristimulus colour-coordination system CIE

L*a*b* (CIE, 1976) and Hue ($H°_{ab}=tan^{-1}b*/a*$) and Chroma [$C*_{ab}=(a*^2+b*^2)^{1/2}$] values were also calculated.

The measurements were performed in two different sites of the edible part, the gill and mantle. Determinations were made in duplicate on all oysters analyzed for morphological characteristics, and the mean of the two measurements was used for data analysis.

The measurement of pH was performed with a Jenway Mod. 3100 (Bibby Scientific Limited, Staffordshire, UK) pHmeter, directly inserting the electrode into three different sites of the edible part of the oysters also analyzed for morphological characteristics. The mean values were used for data analysis.

2.5 Volatile organic compounds analysis

Volatile Organic Compounds (VOCs) profiles of the fresh and processed seafood is a useful tool to evaluate flavour and odour changes occurring after handling or different kind of storage, due to the strong relationship between the lipid fraction and the VOCs profile.

Head Space Solid Phase Micro Extraction (HS-SPME) has been recently applied to the analysis of VOCs (Arthur & Pawlszyn, 1990). We chose to optimize this method for our purposes because of its sensibility, accuracy, cheapness, easiness and rapidity of execution.

Analysis of the VOCs was performed:

1. preliminary on *Venerupis pullastra* to develop the method
2. subsequently it was applied to other 5 species of shellfish (*Mytilus galloprovincialis*, *Ostrea edulis*, *Cerastoderma edule*, *Ensis ensis*, and *Pollicipes cornucopia*) in order to test the suitability of the method to analyze different profiles.
3. a preliminary trial was performed on *Ostrea edulis* stored in refrigerated conditions at (4 °C), and setting the analysis sessions on the 1st, 3rd, 7th and 10th day after harvest in order to test the suitability of the method to analyze flavour and odour changes during the refrigerated storage;

4. finally the method was applied during refrigerated storage at 4 °C of the oysters at the end of the finishing experiment (trial 3).

2.5.1 Sample preparation

Sample preparation was the same used during the validation of the method and the experiments.

Before starting with analysis, all the samples were alive and during the preparation they were always maintained at low temperature, in ice. The edible part of the animals was separated from the non edible one, washed with deionized pure water and left on a net for 5 minutes. Then the samples were homogenized all together. The aqueous extract was obtained putting 5 g of sample with 10 ml of ultrapure water saturated in NaCl, according to Zhang & Pawlszyn (1993), into a tube and then homogenizing for 1' with an Ultra-Turrax® device at the speed of 9500 rpm, cooled in ice. The tubes were then put in a centrifuge for 20' at 3750 rpm at 4 °C to obtain separation between solid fibrous phase and liquid phase. The liquid phase was introduced in a vial with 50 µL of 3-methyl-3-buten-1-ol (50 ppm), as internal standard (IS), and the vial was closed with a hermetical stopper. The vials were stored at -80 °C before the analysis.

2.5.2 GC-MS Settings

In order to concentrate the analytes, the fibre was exposed to the gaseous phase of the vial placed in the GC-MS incubator. A Thermo Finnigan ThermoQuest (San Jose, CA, USA) gas chromatograph equipped with a split/splitless injector and coupled with a Trace quadrupole mass detector (Thermo Finnigan ThermoQuest) was used.

Compounds were separated in a capillary column (30 m x 0.250 mm x 1 µm, stationary phase DB-1701) with the following instrumental conditions: hydrogen as gas carrier at the speed flow of 1mL/min, splitless mode, injector temperature of 220 °C, ionization energy 70eV. The GC oven temperature program was: initial temperature of 35 °C held for 3', followed by an increase of 4 °C/min to 140 °C; then an increase of 20 °C/min to 260 °C hold for 7 min.

2.5.3 Fiber preparation and selection

Fused silica fibres with 4 different coating were bought from Supelco (Bellefonte PA, USA), and were the following:

75 µm carboxenTM/polydimethylsiloxane (CAR-PDMS)

65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB)

85 µm polyacrylate

2 cm-50/30 µm DVB/CarboxenTM/PDMS StableFlexTM.

According to technical recommendations, fibers were thermally conditioned to activate them respectively as following: 300 °C for 1h; 250 °C for 0.5h; 300 °C for 2h; 270 °C for 1h.

To test which one of these fibers was the suitable one for shellfish VOC's analysis, preliminary experiments were performed and fresh pullet carpet shell (*Venerupis pullastra)* was used as test sample.

Before each analysis session, each fiber has been introduced in the HS of the GC-MS for 10' without any previous sample incubation to verify if it was clean. The temperature cycle was the same used for the analysis. Then the fiber was introduced in the vial containing the aqueous extract of pullet carpet shell. In this step the fiber did not touch the sample. At the end of the incubation time, the fiber was introduced immediately in the HS for VOCs desorption during 10'. The selection was performed comparing the sensibility of the fiber to each volatile compound detected.

2.5.4 Selection of extraction conditions: experimental design

The influence of incubation temperature and time and vial's extract volume on the sensibility of the analysis was evaluated. To this purpose a factorial design was selected (3^{3-1}) and all the experiments were randomly performed (Table 2).

This experimental design evaluates the linear and quadratic main effects, investigating each factor at three levels: volume (1, 3, 6 mL), time (5, 15, 30 min), temperature (60, 80, 100 ºC). Incubation temperatures were selected in the range 60-100 ºC in order to improve the recovery of long-chain hydrocarbons, which have been previously identified in mussels by using steam distillation procedures (Fuentes et al., 2009). Sampling time was set at 30 min maximum because this time is useful for analysis automatization, since the sample incubation is entirely completed during one

chromatographic run. The order of the 9 runs that completed the design was fully randomized.

To optimize the incubation time and temperature, an additional number of experiments was performed setting the sample volume at 6 mL and varying the temperature and incubation time as shown in detail in Table 2.

Identification of the volatile compounds was based on computer matching with the reference mass spectra of the Wiley 6, Mainlib and Replib libraries. Quantitative determination of volatiles was performed by the method of internal standard using 3-methyl-3-buten-1-ol as standard.

Table 2. Experimental fractional factorial design $3^{(3-1)}$ and experiments performed in addition to the experimental design.

Sample	Incubation time (min)	In vial volume (mL)	Incubation temperature (°C)
Fractional factorial design (3^{3-1})			
3	5	6	80
7	30	1	80
5	15	3	80
6	15	6	60
2	5	3	100
1	5	1	60
8	30	3	60
9	30	6	100
4	15	1	100
Further experiments			
A	5	6	100
B	15	6	80
C	30	6	80

2.5.5 Validation of the method

The method was evaluated considering volatile compounds detected in pullet carpet shell. The calibration curves were built for 11 compounds at four concentration levels by adding mixtures of commercial standards in salt saturated aqueous solutions. The detection limits were calculated for a signal to noise (S/N) of 3. Repeatability and reproducibility of the analysis were evaluated on 23 compounds.

Repeatability was evaluated by analyzing 6 replicates of saline aqueous extract from fresh pullet carpet shell. Reproducibility was determined by analyzing two replicates of fresh pullet carpet shell extracts for 3 consecutive days.

2.5.6 Volatile analysis

Volatile compounds were analyzed by means of the optimized HS-SPME method The CAR-PDMS fiber was exposed to the aqueous extract by incubating at 60 ºC during 30 minutes under magnetic stirring. The fiber was immediately desorbed in the gas chromatograph injector at 300 ºC during 10 min. The analysis was performed in triple.

Once the analytical procedure was defined in the phase previously detailed, the methodology was applied for samples of the trial 3. The volatile organic compounds (VOCs) were extracted by Headspace Solid Phase Microextraction (HS-SPME) and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS). In details, after shucking, the recovered edible parts were washed with deionised pure water and subsequently dewatered on a net for 5 min, maintaining them on ice during the whole sample preparation process. Five grams of each sample (analysed in triplicate) were mixed with 10 mL of NaCl saturated water, according to Zhang and Pawliszyn (1993), and then homogenized on ice for 1 min with an Ultra-Turrax® disperser. Each sample was centrifuged at 3000 rpm (4 °C, 20 min) and the supernatant was introduced with 50 µL of the internal standard 3-methyl-3-buten-1-ol (50 ppm) in a 20 mL vial fitted with a silicone septum. The extract was incubated at 80 °C under magnetic stirring and the CAR-PDMS fiber was exposed to the headspace during 30 minutes. The fiber was immediately desorbed in the gas chromatograph injector at 260 °C during 10 minutes. GC–MS analysis was performed in a Thermo Finnigan ThermoQuest (San Jose, CA, USA) gas chromatograph, equipped with a split/splitless injector and coupled with a Trace quadrupole mass detector. Compounds were separated in a capillary column (30 m x 0.250 mm x 1µm film thickness, fused silica DB-1701). The temperature program was as following: initial temperature 35 °C held for 3 min, from 35 to 140 °C at 4 °C/min, from 140 to 260 °C at 20 °C/min, and final temperature holding for 7 min. Helium was employed as carrier gas, with a constant flow of 1 mL/min. Injector was operated in the split mode and its temperature was set at 260 °C. Transfer line temperature was maintained at 260 °C. The quadrupole mass

spectrometer operated in the electron impact (EI) mode and the source temperature was set at 200 °C. Initially, full scan mode data were acquired to determine appropriate masses for the later acquisition in selected ion monitoring mode (SIM), under the following conditions: mass range 10-200 amu and scan rate 0.220 s/scan. All analyses were performed setting ionization energy at 70 eV, filament emission current at 150 μA and the electron multiplier voltage at 500 V.

The identification of the components was based on computer, matching with the reference mass spectra of the Wiley 6, Mainlib and Replib libraries and by comparison mass spectra and retention times with commercial standards. Quantitative determination of volatiles was performed by the method of internal standard using 3-methyl-3-buten-1-ol.

About chemical and reagents utilized, 2-ethylfuran, 2,3-pentanedione, (E)-2-pentenal, hexanal, (E)-2-hexenal, heptanal, (Z)-4-heptenal, (E,E)-2,4-heptadienal, (E)-2-octen-1-ol and 3-methyl-3-buten-1-ol (used as internal standard) were purchased from Sigma-Aldrich (Steinheim, Germany). 1-Penten-3-ol was obtained from Fluka (New-Ulm, Switzerland). All other chemicals and solvents were of reagent/analytical grade and water was purified using a Milli-Q system (Millipore, Billerica, Massachusetts, USA).

2.6 Microbiological characteristics

2.6.1 Sample preparation

In each analysis session, analyses were performed in triplicate, using three pools composed by three oysters (one small, one medium and one large sized) for each sample group (lagoon and sea). Analysis sessions were performed on the 1st, 3rd, 7th and 10th day after harvesting, on oysters stored out of water at the temperature of 4°C.

During each analysis session the oysters were carefully washed with tap water, dried with paper towels and then opened maintaining sterile conditions. Edible parts and intervalvar liquid were collected separately in calibrated disposable Petri dishes and then weighted. The volume of the intervalvar liquid and then of the edible parts were measured in an autoclaved graduated cylinder. Edible parts and intervalvar liquid were homogenized in a sterile Stomacher plastic bag (Stofilter Kenzo 500) equipped with an inner filter in a stomacher (Seward-Laboratory Blender, Stomacher

400) for 120 seconds at high speed (Sharpe & Jackson, 1972). The filtered mixture was withdrawn with a sterile 10 ml pipette and collected in a sterile tube. Serial decimal dilutions were performed in sterile peptone water 0.1%.

2.6.2 Total aerobic bacteria, Pseudomonas spp. and Micrococcaceae load

Four different kind of culture medium were used: Plate Count Agar (PCA) + 1% NaCl (Table 3) used in trial 1 and Marine Agar (MA) (Table 4) used in trial 3 for total aerobic bacterial count, Pseudomonas Agar Base (Table 5) added with CFC additive selective for *Pseudomonas* spp. (Table 6) and 5% of glycerol, Mannitol Salt Agar (Table 7), selective for *Micrococcaceae*.

Table 3. Composition of Plate Count Agar (pH at 25 °C= 7.0 ± 0.2).

Component	g/L
Yeast extract	2.5
Pancreatic digest of casein	5.0
Glucose	1.0
Agar	15.0

Table 4. Composition of Marine Agar (pH at 25 °C= 7.6±0.2).

Component	g/L	Component	g/L
Sodium Chloride	19.40	Magnesium Chloride	8.80
Bacteriological Peptone	5.00	Sodium Sulfate	3.24
Calcium Chloride	1.80	Yeast Extract	1.00
Potassium Chloride	0.55	Sodium Bicarbonate	0.16
Ferric Chloride	0.10	Potassium Bromide	0.08
Disodium Phosphate	0.008	Boric Acid	0.022
Strontium Chloride	0.034	Sodium Silicate	0.004
Sodium Fluoride	0.0024	Ammonium Nitrate	0.0016
Bacteriological Agar	15.00		

To inoculate the Petri plates containing the culture medium, the spread plate technique was used: 0.1 mL of diluted samples were spreaded on the surface of culture medium with disposable sterile spreaders. For each dilution the analyses were

performed in triplicate. Plates were incubated at 25 °C for 24-72 hours, until bacterial colonies were visible.

Table 5. Composition of *Pseudomonas* Agar Base (pH at 25 °C=7.1 ± 0.2).

Component	g/L
Gelatin peptone	16.0
Casein hydrolysate	10.0
Potassium sulphate	10.0
Magnesium chloride	1.4
Agar	11.0

Table 6. Composition of CFC additive selective for *Pseudomonas*.

Component	mg/vial	mg/L
Cetrimide	5.0	10.0
Fucidin	5.0	10.0
Cephalosporin	25.0	50.0

Table 7. Composition of Mannitol Salt Agar (pH at 25 °C=7.5 ± 0.2).

Component	g/L
`Lab-Lemco' powder	1.0
Peptone	10.0
Mannitol	10.0
Sodium chloride	75.0
Phenol red	0.025
Agar	15.0

2.7 Sensory characteristics

A panel test was performed on the oyster obtained from the experimental trial 3. The panel test was performed in the laboratory of IBIMET-CNR in Bologna. 60 oysters coming from the Orbetello Lagoon experimental farming and 60 oysters coming from the offshore experimental rearing site near Porto Ercole were used. In order to guarantee the safety of the product for the judges, oysters were previously accommodated in a molluscs depurator.

Ten judges (2 women and 8 men) took part to the test. Each judge received the raw edible part of three oysters from each site for each replicate. Two replicates were done.

The two samples to be compared, lagoon (Y) and sea (X), were balanced and randomized according to the following experimental design: YXX, XYX, XXY, XYY, YXY, and YYX. The samples were named with casual, different numbers.

The judges evaluated the oysters using Qualitative and Descriptive Analysis (QDA) (Nollet & Toldrá, 2009) to evaluate the attributes described in Table 8, giving a score from 1 (minimum intensity) to 10 (maximum intensity).

Table 8. Oysters' panel test sensory descriptors.

Colour	Colour level from yellow/light brown to creamy
Odour intensity	Global odour perception before introducing the sample into the mouth
Odour	Odour perception from putrid to fresh alga
Salty	Fundamental taste perceived mainly on the tip of the tongue and on the near lateral areas
Acidity	Fundamental taste perceived mainlyon the lateral area of the tongue
Bitterness	Fundamental taste perceived mainly on the bottom of the tongue
Consistence	Tactile sensation during the first few bites of the force required to compress the sample
Elasticity	Tactile perception during chewing, related to the attitude of the sample to regain its original shape and size after being compressed and deformed between the molars
Persistence	Permanence of taste perception after swallowing
Global opinion	Perception of the global quality

2.8 Statistical analyses

The data of the Trial 1 related to the oysters of commercial size sampled in Orbetello and in Manfredonia was processed by the analysis of variance using the PROC GLM of the SAS® (SAS, 2007) statistical software, including in the model the effects of site (Orbetello, Manfredonia), day of storage (1^{st}, 3^{th}, 7^{th}, 10^{th}) and the interaction site x day.

Data related to the selection of extraction conditions for the VOCs analysis was analyzed with the statistical software Statistica 6.0 (StatSoft Inc., Tulsa, Oklahoma, USA).

The data of the volatile compounds amount in the shellfish analysed by the analytical procedure developed were submitted to the analysis of variance by the PROC GLM of SAS® (SAS, 2007), to test the difference among the species.

The data of the Trial 2, related to the oysters reared in Orbetello and in Porto Ercole sites and sampled at different times during the growing phase, was processed by the analysis of variance using the PROC GLM of the SAS® (SAS, 2007) statistical software, including in the model the effects of site (Orbetello, Porto Ercole), time of sampling (July, September 2009; January, March, May, July and September 2010), kind of container (basket, lantern, *poche*) and the interaction site x time of sampling.

The effect of the kind of container on the performance of the oysters during the growing phase was considered in detail only for the oysters grown in Porto Ercole site.

The data of the Trial 3, related to the oysters submitted to a finishing phase in Orbetello and in Porto Ercole sites, was processed by ANOVA using the GLM procedure of SAS® (2007) statistical software, with a two-way (site, day of storage) model, entering the interaction (site x day of storage).

Differences between means were tested by Student's t-test for $p<0.05$, and the significance level was declared at $p < 0.05$.

3 RESULTS AND DISCUSSION

3.1 Trial 1: Quality and shelf life. Preliminary trials

3.1.1 Oysters biometric, commercial and chemical characteristics

Biometric and commercial characteristics of the oysters coming from the Orbetello Lagoon and from the sea farming site of Manfredonia used in this preliminary experiment are shown in Table 9.

Table 9. Biometric and commercial characteristics of the oysters coming from Orbetello Lagoon and sea site near Manfredonia analyzed during the preliminary trial.

	FARMING SITE		RSD
	Orbetello	Manfredonia	
Biometric characteristics			
Total weight (g)	155.84	149.56	28.36
Flesh weight (g)	13.22	13.39	4.06
Shell weight (g)	110.85	110.97	22.67
Length (mm)	117.78	116.16	11.37
Width (mm)	60.90[a]	57.35[b]	6.18
Depth (mm)	38.39[b]	40.73[a]	4.80
Commercial characteristics			
Flesh weight (%)	8.36	8.96	1.97
Shell weight (%)	71.66	74.53	8.67
Shape index			
ECI	0.44[b]	0.47[a]	0.63
CI (I)	0.08	0.09	0.02
CI (II)	0.02	0.03	0.02

Lowercase superscript letters indicate statistically significant differences ($p < 0.05$); ns: not significant.

The oysters sampled can be considered of commercial size: Pogoda et al. (2011) did not consider the oysters with an average shell length of 55.3 mm as commercial sized, according with Orban et al. (2004) that stated the commercial size for oysters corresponding to a shell length higher than 60 mm. The marketable size indicated for the oysters by Roncarati et al. (2009) (shell length ranging from 98.8 to 103.3 mm, depth from 31.6 to 34.1 mm and width from 61.1 to 70.5) accord with our values. The two groups of oysters, from Orbetello and from Manfredonia, resulted to be quite homogeneous from the point of view of biometric and commercial characteristics but the oysters of Manfredonia showed a lower total weight (even if not significantly different from the value registered in the other group), and at the same time showed a higher shape index. This means that the soft part of the oysters of Manfredonia not only presented a better shell shape, but also had the possibility to grow better due to

the deeper shell cavity in respect to shell length. This is consistent with the higher CI of the oysters of Manfredonia, even if the difference with the Orbetello oysters was not significant. A significantly higher ECI of the oysters of Manfredonia was also registered, indicating large oysters having a higher economic value due to the proportions of their dimensions. This difference is probably due to the different environmental conditions and in particular to stress factors: even if in the water of Orbetello Lagoon there is a high food availability, stressors like rapid variation of temperature and salinity could affect the growing performances of oysters.

Table 10 reports chemical characteristics of the two groups of oysters. Crude protein was the predominant constituent of the edible part of the oysters of both groups. Ash (15.10 vs 11.96% d.m.) and crude protein (49.47 vs 46.17% d.m.) content both resulted significantly higher in sea oysters. Total lipid content resulted to be significantly higher in the lagoon oysters (12.35 vs 10.48 % d.m.) as well as the energy content (4453.50 and 3978.77 cal g^{-1}). Higher energy content is consistent with higher total lipid value of the lagoon oysters, having lipids a high energy content. This is probably due to the different composition of food in the two farming sites that is confirmed by analyzing the fatty acid composition of the soft part of the oysters. Considering the fatty acid (f.a.) groups, the oysters from Manfredonia resulted to be significantly richer in SFA and MUFA (30.82 vs 26.85% of total f.a. and 18.56 vs 15.45%, respectively), while Orbetello oysters resulted to be significantly richer in PUFA (48.77 vs 40.08%). In particular C16:0 was the most abundant fatty acid in both groups of oysters, resulting significantly more abundant in Manfredonia oysters (20.65 vs 18.95%) together with C18:0 (4.80 vs 4.01%).

PUFA were the most abundant group of fatty acids both in lagoon and sea oysters, according to the results obtained by many authors (Abad et al., 1995; Pazos et al., 1996; Piveteau et al., 2000; Orban et al., 2004; Saito & Marthy, 2010) and in particular the group of n-3 PUFA was the most abundant, in agreement with Piveteau et al. (1999), Soudant et al. (1999), Dridi et al. (2007), Saito & Marty (2010), resulting 39.69 and 30.93%, respectively for Orbetello Lagoon and Manfredonia oysters, with a significant difference ($p<0.005$) between the two groups. The major PUFAs were EPA (20:5 n-3) and DHA (22:6 n-3). These two fatty acids were significantly more abundant in Orbetello oysters (10.84 vs 8.26% and 18.14 vs 15.08%, respectively).

This difference reflects the different composition of the phytoplankton in the two

farming sites as it is generally agreed that these PUFAs derive mainly from the microalgae used as food, as bivalves have a limited or total incapability to synthesise them (De Moreno et al., 1976; Waldock & Holland, 1984). EPA and DHA were the most abundant PUFAs in both oysters groups, resulting significantly more abundant in Orbetello Lagoon oysters (EPA: 10.84 vs 8.26%.; DHA: 18.14 vs 15.08%).

Table 10. Proximate composition and fatty acid profile of the oysters coming from Orbetello Lagoon and Manfredonia analyzed during the preliminary trial.

	Orbetello	Manfredonia	RSD
Moisture (%)	82.00	83.27	1.92
Crude protein (% d.m.)	46.17^{b}	49.47^{a}	2.64
Total lipid (% d.m.)	12.35^{a}	10.48^{b}	2.11
Ashes (% d.m.)	11.96^{b}	15.10^{a}	1.72
Energy content (cal g^{-1})	4453.50^{a}	3978.77^{b}	90.68
Fatty Acids (% on total F. A.)			
SFA			
C16:0	18.95^{b}	20.65^{a}	1.37
C18:0	4.01^{b}	4.80^{a}	8.56
MUFA			
C16:1 n-7	1.86^{b}	2.05^{a}	0.17
C18:1 n-7	3.13	3.12	0.25
C18:1 n-9	5.56	5.35	0.70
C20:1 n-11	2.62	2.90	0.56
C20:1n7	2.07	2.13	0.64
PUFA			
C16:4 n-1	2.35	2.40	
C18:3 n-3	3.84^{a}	2.60^{b}	0.74
C18:4 n-3	3.98^{a}	2.85^{b}	0.89
C20:5 n-3	10.84^{a}	8.26^{b}	2.22
C22:6 n-3	18.14^{a}	15.08^{b}	1.79
C18:2 n-6	2.64	2.52	0.01
C20:4 n-6	2.29	2.18	0.27
SFA	26.85^{b}	30.82^{a}	3.08
MUFA	15.45^{b}	18.56^{a}	2.24
PUFA	48.77^{a}	40.08^{b}	4.52
PUFA n-3	39.69^{a}	30.93^{b}	4.63
PUFA n-6	6.72	6.32	0.60
PUFA n-3/PUFA n-6	5.87^{a}	4.87^{b}	0.77
PUFA n-6/PUFA n-3	0.17^{b}	0.22^{a}	0.04
Healthiness indexes			
AI	0.41^{b}	0.54^{a}	0.08
TI	0.06	0.07	0.01

The following fatty acids C12:0, C13:0, C14:0, C15:0, C17:0, C22:0, C24:0, C17:1, C20:1 n-9, C22:1 n-11, C22:1 n-9, C22:1 n-7, C18:4 n-1, C16:2 n-4, C18:2 n-4, C18:3 n-4, C20:3 n-3, C20:4 n-3, C22:5 n-3, C18:3 n-6, C20:2n-6, C20:3 n-6, C22:4 n-6, C22:5 n-6 are not shown in the table but considered in the groups.
Lowercase superscript letters indicate statistically significant differences ($p< 0.05$); ns: not significant.

PUFA n-3 to PUFA n-6 ratio was also higher in Orbetello oysters (5.87 vs 4.87), while AI was higher for Manfredonia oysters (0.54 vs 0.41).

3.1.2 Shelf life during refrigerated storage

Mantle and gills colour

Mantle and gills colour parameters of the oysters harvested in the Orbetello Lagoon and in the Adriatic sea near Manfredonia stored for 10 days at 4 °C, are shown in Table 11.

Table 11. Effect of farming site and days of storage at 4 °C on colour parameters, pH values and intervalvar liquor content on oysters farmed in the Orbetello Lagoon and Manfredonia.

	Farming site (S)		Days of storage (D)				SxD[1]	RSD
	Orbetello	Manfredonia	1st	3rd	7th	10th	P=...	
Colour								
Mantle								
L*	--	46.05	52.67^{a}	--	41.67^{b}	47.67^{ab}	0.0015	11.01
a*	--	-0.61	-0.63	--	-0.81	0.07	n.s.	1.46
b*	--	16.52	15.79^{ab}	--	18.68^{a}	14.19^{b}	n.s.	5.82
Gills								
L*	47.07^{a}	42.27^{b}	47.00	44.32	41.15	46.20	0.0001	8.04
a*	-0.59	-0.52	-0.95	-0.59	-0.82	-0.15	n.s.	1.50
b*	11.62	11.65	11.85^{a}	14.47^{a}	12.28^{a}	7.95^{b}	n.s.	5.17
pH	6.38	6.33	6.10^{c}	6.37^{b}	6.52^{a}	6.43^{ab}	n.s.	0.19
Intervalvar liquor (%)	16.74	16.42	15.36	20.42	14.75	15.80	0.0451	9.00

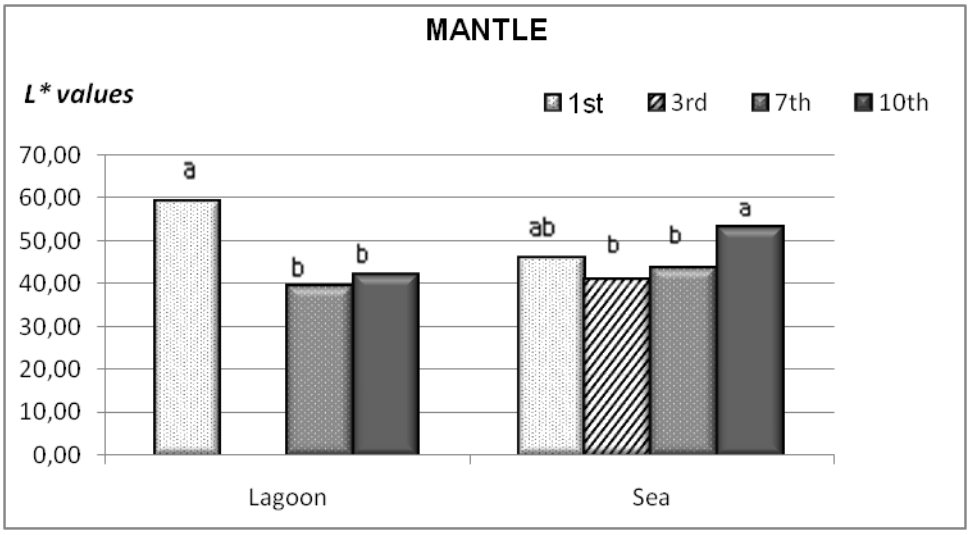
Lowercase superscript letters indicate statistically significant differences ($p< 0.05$); ns: not significant.
[1]SxD = interactions site x day.

Lightness (*L**) values of oysters mantle were significantly influenced by the effect of the time of storage, during wich mantle lightness decreased from the 1st to the 7th day of storage and then slightly increased until the 10th day.The interaction between farming site and days of storage resulted significant for this parameter. Figure 8 shows the lightness values trend presented by the mantle of the oyster of each farming site during the refrigerated storage.

In particular, the mantle lightness decreased during the storage in a significative way for the oysters farmed in the Orbetello Lagoon, while the oysters farmed in the open sea near Manfredonia presented a lower mantle lightness value at the 1st day of storage (59.26 vs 46.07), but during the last day of storage the oysters coming from

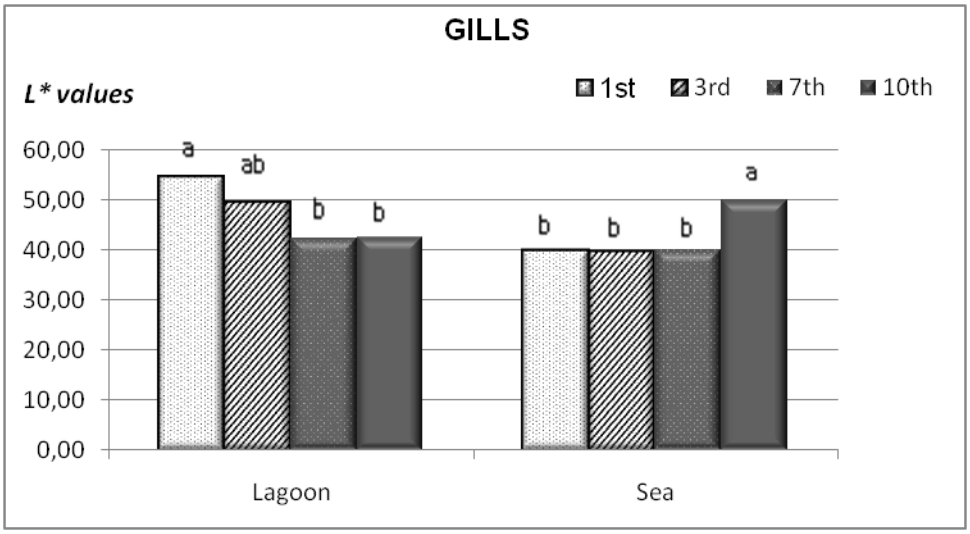
Manfredonia registered the higher lightness value (53.26 vs 42.07). This means that the oysters of Orbetello had a lighter colour and a more transparent aspect during the first day, but the mantle lost this characteristic during the storage period, while Manfredonia oysters maintained their mantle lightness throughout the 10 days of refrigerated storage.

Figure 8. Mantle *L values measured on oysters coming from Orbetello Lagoon and Manfredonia during 10 days of 4°C refrigerated storage.**



Within each group, lowercase letters indicate significant statistical difference ($p<0.05$).

Figure 9. Gills *L values measured on oysters coming from Orbetello Lagoon and Manfredonia during 10 days of 4°C refrigerated storage.**



Within each group, lowercase letters indicate significant statistical difference ($p<0.05$).

The effect of the farming site also influenced the lighness values of the gills, resulting significantly higher for Orbetello oysters (47.07 vs 42.27). Also in this case the interaction between the farming sites and the days of storage resulted significant; the trend registered for gills lightness values was consistent with the trend measured on the mantle, so that the values of Manfredonia oysters kept stable from the 1st to 7th day of storage then increased significantly at the 10th day, while the lightness values measured on Orbetello oysters decreased along the storage period (Figure 9).

Yellowness (*b**) values measured on the mantle was significantly influenced by the time of storage, resulting slightly increasing from the 1st until the 7th day and then significantly decreasing at the 10th day. This is consistent with the yellowness values measured on the gills that were influenced by the effect of the storage time and presented the same trend (Table 11).

pH

pH values measured in three different locations of the edible part of all the oysters analyzed during 10 days of 4 °C refrigerated storage, are also shown in Table 11. No significant differences were recorded between the two groups, but this parameter was significantly influenced by the storage time. From the 1st to the 7th day pH values increased significantly and then slightly decreased from the 7th to the 10th day.

Our results are consistent with the results of other authors that always found pH values higher than 6, immediately after harvest as well as during refrigerated storage (Cruz-Romero et al*.,* 2007). Cook (1991) stated that oysters are classified as being of good quality if their pH is 6.0 or higher and Colby et al. (1995) stated that fresh and good-quality bivalves exhibit pH values varying from 6 to 7. More detailed values are proposed by Aaraas et al. (2004), that found that muscle pH in all live oysters ranged from 5.6 to 6.3, dead oysters had a tissue pH of 5.2 to 5.4 and the moribund specimen had a tissue pH of 5.5. They also stated that stable pH values during storage indicate maintenance of extracellular pH, typical for many intertidal bivalve molluscs (Newell, 1973, 1979) to maintain respiratory gas exchange and thus tolerate emersion during low tide (Delaporte, et al., 2005). The active compensation for a progressive acidosis stops when death occurs and then the pH rapidly falls. pH values measured on the two sample groups ranged from 6.1 to 6.52, so that we can state that oysters were alive throughout the chilled storage period. Moreover, the stability of the pH values along the storage period shows that the oysters activated

compensatory system to mantain the extracellular pH during air exposure in the refrigerate storage phase. This activation was probably facilitate by the low temperature (4 °C) utilised for the storage.

pH values of all the oysters during refrigerated storage showed the trend to slightly increase: this trend is consistent with the findings of Cruz-Romero et al. (2008a), Briones-Labarca et al. (2012); whereas they are inconsistent with the findings of Cao et al. (2009a; 2009b) that recorded a decrease of pH values during refrigerated storage of raw oysters. That inhomogeneous behaviour could be attributed to the different storage conditions (like temperature) applied in the different trials and probably also to the handling techniques practiced, which deeply affect the shelf life of a perishable product like raw oysters are.

pH, a parameter easy to measure, is considered very informative about the health condition in molluscs since it can discriminate the living specimens from the dead ones (Aaraas et al., 2004), these latter having a pH in progressive lowering as a consequence of the loss of capacity to actively compensate the acidosis due to glycolisis.

Microbiological characteristics

The results of the microbiological characteristics of Orbetello and Manfredonia oysters obtained by counting total aerobic bacteria, *Pseudomonas* spp. and Micrococcaceae are shown in Table 12.

Table 12. Bacterial load changes during 10 days of chilled storage at 4°C on oysters farmed in the Orbetello Lagoon and Manfredonia.

Bacteria load	Farming site (S)		Days of storage (D)				SxD	RSD
	Orbetello	Manfredonia	1st	3rd	7th	10th	P=...	
Total aerobic bacteria	5.23[a]	4.98[b]	4.91[bc]	4.55[c]	5.22[b]	5.73[a]	<.0001	0.37
Pseudomonas spp.	4.01	--	3.35[c]	3.76[b]	4.39[a]	--	n.s.	0.31
Micrococcaceae	5.81	5.91	5.05[c]	5.13[c]	6.47[b]	6.79[a]	0.0036	0.26

Lowercase superscript letters indicate statistically significant differences (p< 0.05); ns: not significant.

Total bacteria load resulted to be influenced both by the effect of the farming sites and the storage time. As expected, Orbetello oysters presented a higher total bacteria

load (5.23 vs 4.98 Log CFU g^{-1}) and during the storage the value increased significantly from the 1st to the 10th day (from 4.91 to 5.73 Log CFU g^{-1}).

The interactions between the farming sites and the days of storage resulted significant for total aerobic bacteria load. Figure 10 shows the trend registered for the oysters of the two farming sites during each day of refrigerated storage. As expected Orbetello oysters presented higher values at the beginning and at the end of the storage period, but the trend observed for the two sampling groups was quite different; while in Manfredonia oysters the total bacteria load increased gradually and of only 0.62 Log CFU g^{-1}, in Orbetello oysters the total bacteria load increased of 1.03 Log CFU g^{-1} from the 1st to the 10th day, passing by an initial decrease from the 1st to the 3rd day and then a strong increase until the 10th day.

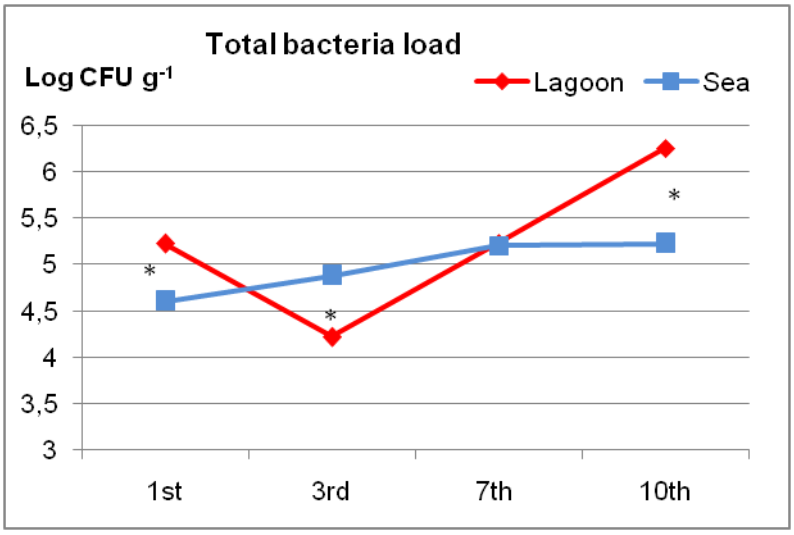
Pseudomonas spp. and Micrococcaceae loads were influenced by the storage time, during which the concentration of both bacteria groups significantly increased.

As regards total aerobic bacteria counts, both sample groups maintained below the value of 5×10^5 CFU/g (5.70 log CFU g^{-1}) indicated by ICMSF (1980) as the upper limit to consider fresh bivalve of good quality. This result is consistent with the findings of other authors (Cruz-Romero et al., 2008a; Cao et al., 2009a). Total bacteria counts recorded during the 10th day of storage in both sample groups was lower than the widely accepted threshold of 7 log CFU g^{-1} (Cao et al., 2009a; Buzin et al., 2011), so they all could be considered acceptable for human consumption from the microbiological point of view.

The microflora of shellfish is closely related to environmental conditions, microbiological quality of the water, water temperature, salt content, natural concurrence of bacteria, ingestion of food, methods of catch and chilling conditions (Felhusen, 2000). The influence of the environment is much more evident in oysters that, like all bivalves, are filter-feeders and concentrate the bacteria present in the water on the gills, mantle and digestive gland. The limit of acceptability in terms of total aerobic bacteria count for untreated oysters was not reached during storage at 4 °C for up to 10 days, perhaps because the immune system was enough efficient to protect live oysters against spoilage: bivalves possess various types of non-specific immune-related humoral defence molecules, including agglutinins, opsonizing lectins, bactericidins, lysozymes and serine proteases (Roch, 1999). In our study the deep difference between the two farming environments reflected on the initial total aerobic

bacteria load, indicating the higher quality of the water of Manfredonia farming site, that is located in open sea, compared to the water of Orbetello Lagoon, characterized by a limited water exchange and high concentration of nutrients, including bacteria.

Figure 10. Total aerobic bacteria load changes during 10 days of chilled storage at 4°C on oysters farmed in the Orbetello Lagoon and Adriatic sea near Manfredonia.



Asterisks indicate significant statistical difference ($p<0.05$) between groups.

The composition of the microflora changes dramatically during storage, but during aerobic chilled storage the microflora of fish and shellfish is comprised almost exclusively of *Pseudomonas* spp., *Shewanella putrefaciens* and *Moraxella acinetobacter* (Ashie et al., 1996; Gram & Huss, 2000; Jay, 2000), due to the competition occurring between bacteric species. Cao et al. (2009a) and Buzin et al. (2011) found that the bacterial population present in raw oyster consisted of 22% *Pseudomonas* spp.; *Pseudomonas* together with *Shewanella* and Vibrionaceae are known to be involved in the spoilage of shellfish (Cao et al., 2009a) and fish products (Gram & Huss, 1996; Gennari et al., 1999). *Pseudomonas* spp. were found in fresh oysters both untreated and high pressure treated as the main bacteria (Cruz-Romero et al., 2008a). Due to their importance and constant presence in refrigerated fish and shellfish, Pseudomonads have been used as indicators of spoilage of iced fish, fish products and oysters (Dalgaard et al., 1993; Gram & Huss 1996; Hoz et al., 2000; Lopez-Caballero et al., 2000; Kyrana & Lougovois, 2002; Mendes et al., 2002).

Micrococcaceae were found in refrigerator stored meat products (Girova & Gochev, 2009), on Atlantic salmon skin and gills (Horseley, 1973) and also in the gut and on the scales of fish grown in polyculture systems (Rice, 1984). Micrococcaceae were also found by Gonzalez-Rodriguez et al. (2002), on cold–smoked, vacuum-packed salmon as the 3rd bacteric group in abundance. Species of the genus *Micrococcus* were found in raw fresh oysters, representing 7% of the total bacteria (Cao et al., 2009a).

Micrococcaceae are considered as a minor gram-negative bacteria group affecting the shelf-life of shellfish (Cao et al., 2009a) and in oysters in particular were found species belonging to the genus *Micrococcus*, responsible for spoilage of oysters even at 0 °C (Colwell & Liston, 1960).

Pseudomonas spp. and some genus of Micrococcaceae (*Streptococcus* and *Micrococcus*) are important responsible of shellfish spoilage because they possess the amino acid decarboxylase producing considerable amounts of biogenic amines (Shalaby, 1996), in particular putrescine, histamine, tyramine (Hu et al., 2007). The consumption of high amount of biogenic amines whith food can result in histamine poisoning and tyramine toxicity (Chong et al., 2011).

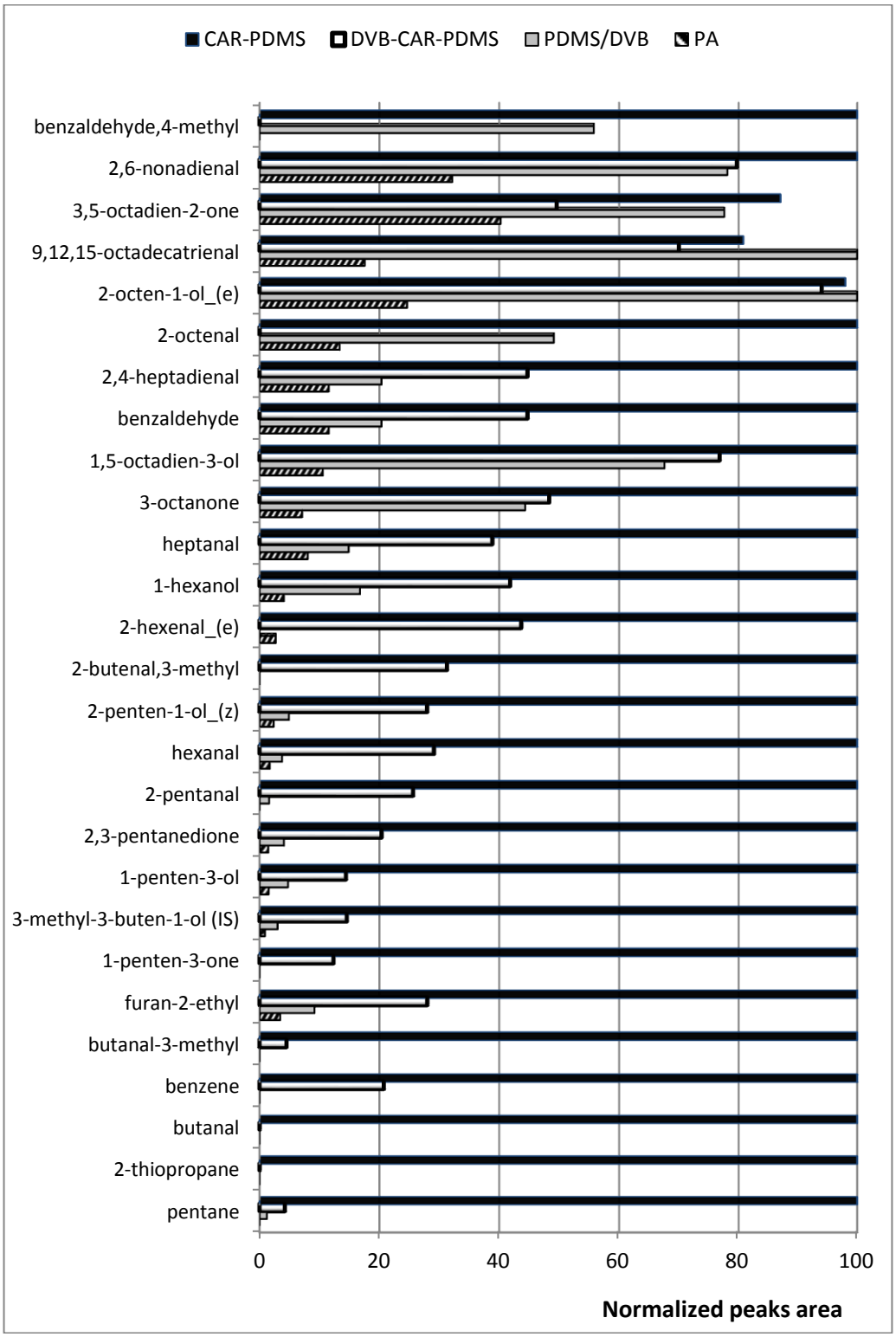
3.1.3 Volatile compounds profile: development of the analysis method

Fiber selection

The influence of the type of SPME fiber on the extraction efficiency of shellfish VOCs was first evaluated. Figure 11 shows the differences in the extraction of VOCs from pullet carpet shells by the different fibres, according with the diversity of polarity and size of the SPME coatings: 75 µm CAR-PDMS is a bipolar fiber designed for gases and low molecular weight compounds, 65 µm PDMS/DVB is for polar semivolatiles and larger volatiles, 85 µm PA for polar semivolatiles and 50/30 µm DVB/ CAR/PDMS is a dual fiber coating of divinylbenzene and carboxen suspended in poly(dimethylsiloxane) for a broad range of volatile and semi volatile compounds (Risticevic et al., 2010).

CAR/PDMS enabled the detection of a wider range of compounds and produced higher signal intensities in the whole range of masses studied (Figure 11). CAR/PDMS was found to be extraordinary efficient to extract analytes with low molecular weights compared to the other SPME coatings.

Figure 11. Evaluation of extraction efficiency of volatile organic compounds from pullet carpet shells by different fiber coatings.



The application of HS-SPME together with CAR/PDMS fiber allowed the detection of low boiling point compounds hardly absorbed using other extraction methods based on distillation and P&T because of their high volatility. For the long-chain VOCs, CAR/PDMS exhibited also the greatest recovery of volatiles, although DVB/CAR/PDMS and PDMS-DVB showed also good performances. This result agrees with Kataoka et al. (2000) that found CAR-PDMS coating more efficient for extracting oxidation products, sulfur-aroma compounds and, more generally, low molecular mass VOCs with mid-low polarity. CAR-PDMS coating has been successfully used to detect spoilage and quality indicators in fish species such as whiting (*Merlangius merlangus*) (Duflos et al., 2005), swordfish (*Xiphias gladius*) and cod (*Gadus morhua*) (Guillen et al., 2006), King salmon (*Oncorhynchus tshawytscha*) (Wierda et al., 2006), and Atlantic horse mackerel (*Trachurus trachurus*) (Iglesias & Medina, 2008). PDMS-DVB fibres have been used for analyzing VOCs of *Crassostrea gigas* edible part (Zhang et al., 2009).

The VOC profile provided by this fibre coating exhibited the absence of most of the aldehydes detected in this work, especially low weighting aldehydes, which are known to be strong contributors to seafood flavour (Durnford & Shahidi, 1998).

Selection of the extraction conditions

The sampling conditions affecting the microextraction process with CAR/PDMS coating were evaluated by a factorial experimental design (Table 2). The variable responses were the peak areas of the different volatiles.

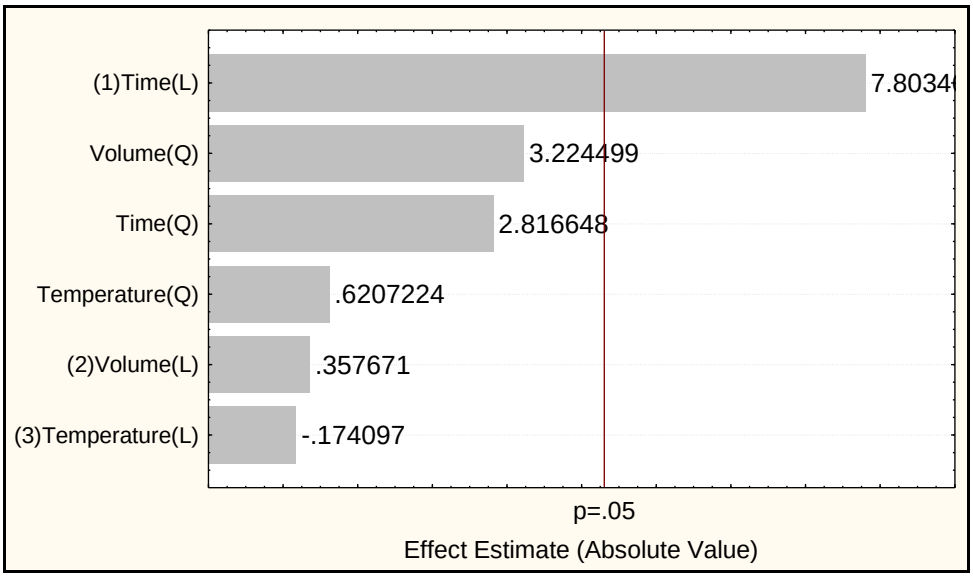
Results put into relevance that sampling time was the most important factor for alcohols and ketones. Figure 12 shows the standardized Pareto chart obtained for the representative alcohol (Z)-2-penten-1-ol.

The main effect of time resulted positive and significant for (Z)-2-penten-1-ol, (Z)-1,5-octadien-3-ol, 3-octanone, and 3,5-octadien-2-one. Similar tendency was observed for 1-penten-3-ol, 1-hexenol, and 2,3-pentanedione, although time did not reach the significance level for these volatiles.

Sample volume had also an important positive effect for most of the alcohols and ketones. However, aldehydes displayed a high dependence from temperature, since it was the most important factor with positive effect for 2-pentanal, hexanal, (E)-2-

hexenal, heptanal, benzaldehyde, 2,4-heptadienal, 2-octanal, 9,12,15-octadecatrienal and 2,6-nonadienal. Among those aldehydes, the main effect of temperature was found to be significant for 2-pentanal, (E)-2-hexenal, 2,4-heptadienal 9,12,15-octadecatrienal. The highest significant effect for temperatures was detected above 80 °C. Temperatures round to 100 °C gave a strong modification of the pattern of aldehydes, leading a major extraction of volatiles resulting from thermal oxidation of fatty acids and the formation of new compounds probably resulting from thermal degradation (Figure 13).

Figure 12: Standardized Pareto chart obtained for (Z)-2-penten-1-ol. Vertical line indicates statistical significance bound for the different effects. L indicates linear effect, Q indicates quadratic effect.



Sampling time was also an important factor with positive effect for most of aldehydes, resulting its effect significant for 2-pentanal and (E)-2-hexenal. Figure 14 shows the Pareto chart of standardized effects for 2-pentanal, as representative of the behaviour observed for most of the aldehydes.

These results indicated that the sampling time has a positive effect on the extraction of most of VOCs, and consequently, a time of 30 min was chosen as optimum. The optimized sample volume was set at 6 mL since it improved the sensibility of many alcohols and ketones, and the temperature of incubation was established at the intermediate level (80 °C). These conditions were confirmed with

an additional number of experiments in which incubation time and temperature were varied and sample volume was set at 6 mL (Table 2 of the Experimental Section).

Therefore, the optimum experimental conditions for the analysis of VOCs in fresh shellfish were fixed on: CAR/PDMS coating, 6 mL of aqueous extract, 80 °C of incubation temperature and 30 min of sampling time.

Figure 13. Influence of the incubation temperature on the VOC profile of *Venerupis pullastra*.

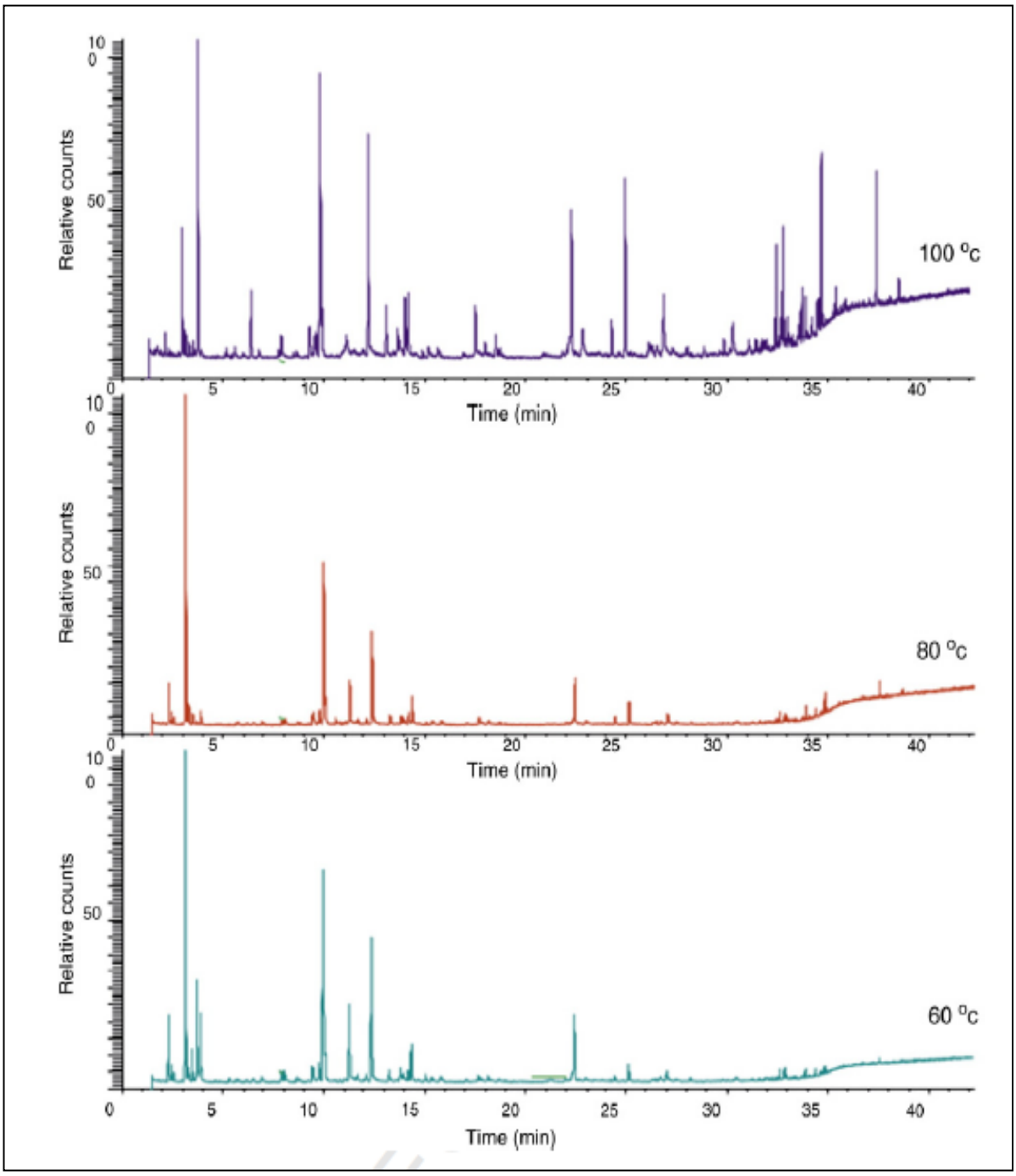
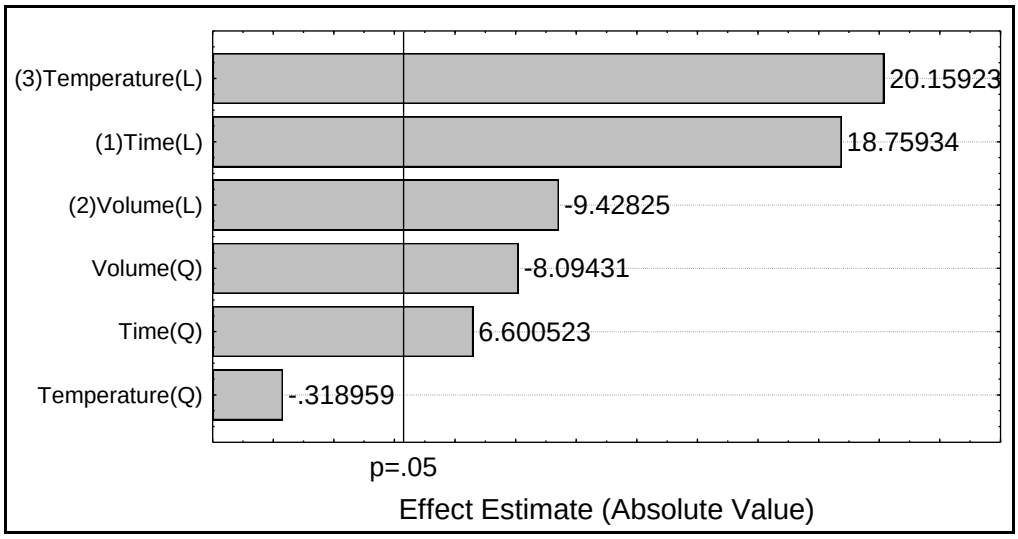


Figure 14: Standardized Pareto chart obtained for 2-pentanal. Vertical line indicates statistical significance bound for the different effect. L indicates linear effect, Q indicates quadratic effect.



Validation of the method

A satisfactory linearity (linear correlation coefficients from 0.976 to 0.998) was obtained for the eleven compounds studied (Table 13).

Table 13. Slope, intercept, correlation coefficient, linear range and detection limits of 11 of the VOCs of *V. pullastra* profile detected with the optimized method.

Compound	Slope	Intercept	Correlation coefficient (R)	Linear range (ppb)	Detection limits (S/N=3) (ppb)
2-Ethylfuran	0.009	-0.0635	0.9984	5-170	0.12 ± 0.02
1-Penten-3-ol	0.0027	0.0208	0.9927	15-320	0.16 ± 0.01
2,3-Pentanodione	0.0025	-0.024	0.9982	10-175	0.20 ± 0.03
(E)-2-Pentenal	0.0054	-0.0544	0.992	5-160	0.24 ± 0.03
Hexanal	0.0128	-0.1548	0.9877	5-160	0.16 ± 0.04
(E)-2-Hexenal	0.0052	-0.0702	0.9847	5-160	0.43 ± 0.05
Heptanal	0.0179	-0.1162	0.9847	5-80	0.25 ± 0.06
(Z)-4-Heptenal	0.0157	-0.124	0.9821	5-80	1.03 ± 0.23
(E,E)-2,4-Heptadienal	0.0116	-0.1606	0.9838	5-150	1.19 ± 0.09
(E)-2-Octen-1-ol	0.0134	-0.2142	0.9759	5-160	0.68 ± 0.20
(E,E)-2,4-Octadien-1-ol	0.004	-0.0686	0.9761	5-160	0.16 ± 0.03

This linearity was confirmed by using a lack-of fit test. The detection limits (S/N=3) of the method ranged from 0.12 to 1.19 ppb. The limits of quantification (S/N=10) of

the method ranged from 0.40 to 3.97 ppb. Obtained results suggest that the efficiency of the process was scarcely affected by the matrix for the most of compounds evaluated (Table 13). Few compounds, like E-2-hexenal or (E,E)-2,4-heptadienal showed poor recoveries, therefore, standard addition method should be use for their quantification. The method showed also a satisfactory repeatability and inter-day precision since the relative standard deviations of the 26 volatile compounds studied varied from 2.7 to 19.2%, either in samples analyzed in the same day or in different days (Table 14).

Table 14. Identified volatile organic compounds in *Venerupis pullastra* and their repeatability and reproducibility.

Number	**Compound**	**Retention time (min)**	**Identification**	Repeatability n=6	Reproducibility n=6
				% RSD	**% RSD**
1	Pentane	2.26	MS	19.2	11.6
2	2-Thiopropane	3.09	MS	5.2	5.1
3	Butanal	5.64	MS	11.8	13.4
4	2-Butanone	6.05	MS	8.1	6.5
5	3-Methylbutanal	7.84	MS	14.7	15.8
6	2-Ethylfuran	7.99	MS, STD	18.2	7.6
7	Pentanal	9.59	MS	12.1	9.0
8	3-Pentanone	9.68	MS	4.9	6.4
9	1-Penten-3-ol	9.87	MS, STD	5.6	3.4
10	2.3-Pentanodione	9.97	MS, STD	5.9	8.7
11	(E)-2-Pentenal	13.14	MS, STD	9.7	8.1
12	1-Pentanol	13.71	MS	5.8	10.0
13	Hexanal	14.07	MS, STD	18.7	14.1
14	(Z)-2-Penten-1-ol	14.23	MS	4.7	6.5
15	3-Methyl-2-butenal	14.93	MS	16.7	11.0
16	(E)-2-Hexenal	17.55	MS, STD	8.3	9.6
17	(Z)-3-Hexen-1-ol	17.73	MS	2.7	13.2
18	1-Hexanol	18.04	MS	4.3	9.9
19	Heptanal	18.55	MS, STD	16.4	9.6
20	(Z)-4-Heptenal	18.75	MS, STD	17.5	12.9
21	Heptanol	22.20	MS	9.0	8.3
22	(Z)-1,5-Octadien-3-ol	22.27	MS	8.5	5.5
23	(E,E)-2,4-Heptadienal	25.01	MS, STD	8.1	10.0
24	(E)-2-Octen-1-ol	26.37	MS, STD	8.1	8.3
25	2-Nonanone	26.56	MS	5.6	9.1
26	(E,E)-2,4-Octadien-1-ol	26.90	MS, STD	9.8	7.9
IS	3-Methyl-3-buten-1-ol (IS)	12.23	MS, STD		

Application of the developed method to different shellfish species

In order to verify the suitability of the optimized method to analyze the VOCs profile of shellfish, it was applied to characterize the volatile compounds of the 6 shellfish species. Characterizing aromas for freshly harvested shellfish are derived from PUFAs through lipoxygenase mediated reactions, and include both volatile alcohols and carbonyls (Josephson, 1991). Among them, the compounds associated with fresh seafood flavours are mostly 6-, 8-, and 9-carbon aldehydes, ketones and alcohols derived from the unsaturated fatty acid characteristic of seafood by lipoxygenase activities.

Table 15 shows the mean concentrations of the VOCs for each species.

The ANOVA analysis illustrated the significant differences found among the volatile profiles of the different species. A total of 46 different compounds were detected including aldehydes, alcohols, ketones, alkanes, aromatic compounds and ethers. Significant differences were observed in the number, type of volatiles detected and their concentrations in the different species. Only few compounds, such as hexanal, (E)-2-hexenal, (E)-2-pentenal, 1-penten-3-ol, (Z)-2-penten-1-ol, 2,3-pentanedione and 2-ethylfuran were common compounds in all the species. The most abundant compounds in all species were alcohols, which may be formed by action of lipoxygenase on fatty acids or reduction of carbonyl to an alcohol (Pan & Kuo, 1994). Alcohols are generally minor contributors to food flavours because of their high odour threshold, unless they are unsaturated or present at high concentrations (Heat & Reineccius, 1986). The group of aldehydes showed a wide number of different compounds, characterized for low odour thresholds, and which have been described as the main responsible of typical fresh fish flavour (Durnford & Shahidi, 1998). Different ketones were also detected in the six species. Their production has been associated to enzymatic degradation of PUFAs (Cha et al.,1992), amino acids (Kawai, 1996; Spurvey et al.,1998) or microbial oxidation (Pan & Kuo, 1994). The presence of aromatic compounds in molluscs has been also associated to petroleum contamination (Ogata & Miyake, 1980). Compounds such as ethyl furan have been previously described in fish muscle resulting from β-cleavage of the 12-hydroperoxide of linolenate (18:3n-3), the 14-hydroperoxide of eicosapentaenoate (20:5n-3) and the 16-hydroperoxide of docosahexaenoate (22:6n-3) (Medina et al., 1999).

Table 15. Volatile organic compounds profile of 5 species of bivalve molluscs and a crustacean coming from South Galizia coast.

Compounds	*Cerastoderma edule*	*Ensis ensis*	*Mytilus galloprovincialis*	*Ostrea edulis*	*Pollicipes cornucopia*	*Venerupis pullastra*	RSD	P=...
Aldehydes								
Butanal						0.0044	0.0006	
3-Methylbutanal			0.0063			0.0059	0.0007	0.6236
(E)-2-Pentenal	0.1182^{b}	0.0980^{b}	0.1068^{b}	0.1945^{a}	0.0236^{c}	0.0203^{c}	0.0393	0.0019
Hexanal	0.2781^{ab}	0.1683^{b}	0.1678^{b}	0.4074^{a}	0.0479^{c}	0.0612^{c}	0.0789	0.013
(E)-2-Hexenal	0.0720^{b}	0.0538^{bc}	0.0948^{b}	0.2196^{a}	0.0274^{c}	0.0142^{c}	0.0296	<.0001
Heptanal				0.2669^{a}	0.0431^{b}	0.019^{b}	0.0596	0.0041
Benzaldehyde	0.1896^{c}	1.1878^{a}		0.6860^{b}			0.1935	0.0022
(E,E)-2,4-Heptadienal	0.0439^{b}	0.0230^{b}		0.4087^{a}	0.0310^{b}	0.0069^{b}	0.0627	<.0001
2-Octenal	0.0468^{b}			0.3077^{a}			0.0799	0.0162
9,12,15-Octadecatrienal	0.1126				0.0697		0.0255	0.1078
2,6-Nonadienal	0.2551^{b}			0.6033^{a}	0.0235^{c}		0.0953	0.0009
Pentanal	0.0750^{a}		0.0275^{b}	0.0409^{b}		0.0301^{b}	0.0124	0.0092
Propanal			0.0078				0.0001	
Octanal					0.0523		0.0555	
(Z)-4-Heptenal	0.0678^{b}			0.0911^{a}		0.004^{c}	0.0112	0.0002
4-Ethylbenzaldehyde	0.0476	0.0219					0.0090	0.0249
Lylacaldehyde		0.0206^{b}		0.5172^{a}	0.1166^{b}		0.1134	0.0038
3,4,8-Trimethyl-2-nonenal		0.0117			0.0151		0.0095	0.6841
3-Methyl-2-butenal						0.0150	0.0023	
Alcohols								
1-Penten-3-ol	0.1268^{d}	0.2464^{c}	0.2810^{b}	0.0582^{e}	0.0400^{e}	0.3643^{a}	0.0165	<.0001
(Z)-2-Penten-1-ol	0.0338^{c}	0.0645^{b}	0.0949^{a}	0.0796^{ab}	0.0066^{d}	0.0697^{b}	0.0113	<.0001
(Z)-3-Hexen-1-ol						0.0153		
(Z)-1,5-Octadien-3-ol	0.0259^{b}			2.3852^{a}	0.0366^{b}	0.4782^{b}	0.4314	0.0002
3.Cyclohexene-1-ethanol				10.297		0.0541	1.2422	0.0001
1-Hexanol						0.0258	0.0003	
1-Octen-3-ol		0.1265					0.0421	
2-Octen-1-ol				2.3523^{a}		0.0167^{b}	0.2666	0.0004
1-Pentanol			0.0190^{b}			0.0831^{a}	0.0041	0.0004
Heptanol						0.0216	0.0027	
Ketones								
1-Penten-3-one			0.0337^{b}	0.0761^{a}			0.0134	0.0410
2,3-Pentanodione	0.0219^{c}	0.0211^{c}	0.2360^{a}	0.0555^{b}	0.0377^{b}	0.0447^{b}	0.0170	<.0001
3-Octanone				0.0842			0.0273	
2-Butanone			0.0034			0.0058	0.0010	0.0792
(E,E)-3,5-Octadien-2-one		0.0177^{b}			0.0435^{a}		0.0087	0.0221
1-Octen-3-one				0.1103			0.0225	
2-Nonanone		0.0433			0.0377	0.0064	0.0192	0.1459
2-Undecanone		0.0188			0.0151		0.0103	0.6829
3-Pentanone						0.0113	0.0007	
Others								
Pentane			0.0074			0.0166	0.0328	0.541
2-Thiopropane	0.1282^{a}		0.0002^{b}		0.0315^{b}	0.1138^{a}	0.0155	<.0001
Hexane			0.4282	0.4272			0.4593	0.9984
2-Ethylfuran	0.0454^{bc}	0.0338^{bc}	0.0891^{b}	0.3529^{a}	0.0421^{b}	0.0167^{c}	0.0295	<.0001
Carbodisulfide			0.0073				0.0002	
Phenol					0.0533		0.0405	
2-Butylbenzothiazole					0.0124		0.0049	
Indole					0.0197		0.0146	

Lowercase superscript letters indicate statistically significant differences ($p < 0.05$); ns: not significant.

The resulting cleavage of the formed vinyl hydroperoxide produces an alkoxyl radical that by loss of a hydroxyl radical and posterior cyclization results on 2-ethylfuran. Each selected group of volatiles is typically responsible for various flavour notes (Acree & Arn, 2008). Hydrocarbons were also present but these compounds have been reported not to be odour-active components, probably due to their high detection thresholds (Le Guen et al., 2000).

In *O. edulis*, 22 volatile compounds were detected. This species gave the major total concentration of VOCs. The main compounds can be grouped as follows: (i) aldehydes: pentanal, (E)-2-pentenal, hexanal, (E)-2-hexenal, heptanal, (EE)-2,4-heptadienal, (Z)-4-heptenal, 2-octenal, 2,6-nonadienal; (ii) alcohols: 1-penten-3-ol, (Z)-2-penten-1-ol, (Z)-1,5-octadien-3-ol, 2-octen-1-ol, 3-cyclohexene-1-ethanol; (iii) hydrocarbons: hexane; (iv) oxygenated aromatics: benzaldehyde; (v) ketones: 1-penten-3-one, 2,3-pentanedione, 3-octanone, 1-octen-3-one, and (vi) ethers: 2-ethylfuran, lilac aldehyde 2-(5-methyl-5-vinyltetrahydrofuran2- yl)propan*al.* The profile of the major volatiles is different to that shown by Piveteau et al. (2000) and by Pennarun et al. (2002), especially in the volatiles detected in higher concentrations. The main compounds found in this study were alcohols being the major component 3-cyclohexene-1-ethanol, followed by (Z)-1,5-octadien-3-ol and 2-octen-1-ol. Benzaldehyde and lilac aldehyde were also abundant compounds in this species. 3-Cyclohexene-1-ethanol, previously described in leaves of some plants, is associated with odour attributes of fresh and mint (Tanaka et al., 2010). 1,5-Octadien-3-ol is a product of n-3 PUFAs oxidation and was proposed as an intermediate of the EPA degradation via 12-lipoxygenase activity (Durnford & Shahidi, 1998). It shows a low odour threshold (10 ppb) and has been reported to be an active contributor of flavour with a fresh mushroom and moss odour (Kawai, 1996). 2-Octen-1-ol is associated to green flavour notes and was identified as product of the 12-lipoxygenase activity on n-6PUFA such as arachidonic acid in fish tissues (German et al., 1991). Other compounds such as benzaldehyde and lilac aldehyde are linked to pleasant almond odour and floral lilac odour, respectively (Acree & Arn, 2008).

Thirteen compounds [(E)-2-pentanal, (E)-2-hexanal, heptanal, 2,4-heptadienal, 2-octenal, 2,6-nonadienal, (Z)-4-heptenal, lilac aldehyde, 1,5-octadien-3-ol, 2-octen-1-ol, (E,E)-2,4-octadien-1-ol, 1-penten-3-one, and 2-ethylfuran] resulted to be significantly more abundant in oysters than in the other species. In addition to 3-

cyclohexene-1-ethanol, 3-octanone and 1-octen-3-one were only detected in this species.

In *C. edule*, 17 compounds were detected with a strong majority of aldehydes [(E)-2-pentenal, pentanal, hexanal, benzaldehyde, (E,E)-2,4-heptadienal, 2-octenal, 9,12,15-octadecatrienal, 2,6-nonadienal, (Z)-4-heptenal, 4-ethylbenzaldehyde] and alcohols [1-penten-3-ol, (Z)-2-penten-1-ol, (Z)-1,5-octadien-3-ol]. Others such as ketones (2,3-pentanedione), oxygenated aromatic compounds (benzaldehyde), sulfur compounds (2-thiopropane) and furan compounds (2-ethylfuran) were also detected. The more abundant compounds were hexanal and 2,6-nonadienal. The hexanal odour is described as oxidized fatty, green, grassy, powerful and penetrating (Turchini et al., 2010) and its formation from linoleate hydroperoxides is well recognized (Frankel, 1998). 2,6-Nonadienal is characterized by a fatty odour with fresh green cucumber and melon notes. It is described to come from the action of 9-lipoxygenase on eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (German et al., 1991; Hu & Pan, 2000). Additionally, pentanal, originated by the decomposition of linoleate 13-hydroperoxide, resulted to be significantly more abundant in *C. edule* than in the other species.

Analyzing the VOC profile of *M. galloprovincialis*, 17 compounds were detected: 6 were aldehydes [propanal, 3-methyl-2-butanal, pentanal, (E)-2-entenal, hexanal, and (E)-2-hexenal], 3 were alcohols [1-pentanol, 1-penten-3-ol, (Z)-2-penten-1-ol] and 3 were ketones (1-penten-3-one, 2,3-pentanedione, and 2-butanone); 2-thiopropane, pentane, hexane, 2-ethylfuran, and carbodisulfide were also detected. The most abundant compounds were hexane, 1-penten-3-ol, 2,3-pentanedione, hexanal, and (E)-2-pentenal. 1-Penten-3-ol, present in the highest concentrations, has been previously described as products of the action of lipoxygenases on n-3 PUFA (German et al., 1991; Hu & Pan, 2000). E-2-penten-1-ol has been previously identified by Le Guen et al. (2000) as the only alcohol that contributed actively to the aroma of cooked mussels. These authors also described (Z)-4-heptenal detected in trace amounts, and 2,3-butanedione, as impact odourants in flavour of cooked mussel. Fuentes et al. (2009), using simultaneous distillation–extraction techniques for extracting volatiles, have reported the presence of (E)-2-hexenal in *M. galloprovincialis* from Galicia, together with compounds of longer chain and higher molecular weight than those found in this work. These differences could be due to the

different methodologies used for volatiles extraction. Two volatiles, propanal and carbodisulfide, were exclusively detected in Atlantic mussels. The first one is generated by direct autoxidation of methyl linolenate and from15-hydroperoxide formed by photosensitized oxidation of methyl linolenate (Frankel, 1998). Additionally, mussels also showed major proportion of 2,3-pentanedione than the other species studied. Dimethyl disulfide (sulfury odour) has been described as an odorant characteristic of wild mussels (Le Guen et al., 2000).

The VOC profile of *E. ensis* was composed of 16 compounds: 5 aldehydes: (E)-2-pentenal, hexanal, (E)-2-hexenal, (E,E)-2,4-heptadienal, 3,4,8-trimethyl-2-nonenal; 3 alcohols: 1-penten-3-ol, (Z)-2-penten-1-ol, 1-octen-3-ol; 4 ketones: 2,3-pentanedione, (E,E)-3,5-octadien-2-one, 2-nonanone, 2-undecanone; oxygenated aromatics: benzaldehyde, 4-ethylbenzaldehyde; and ethers as lilac aldehyde and 2-ethylfuran. The volatiles benzaldehyde and 1-octen-3-ol were characteristic for this species.

Benzaldehyde was the most abundant compound and its proportion was higher than in the other species, and 1-octen-3-ol was only detected in *E. ensis*. Benzaldehyde could arise from amino acid degradation (Piveteau et al., 2000) and its odour has been described as candy, sweet and almond (Turchini et al., 2010), while 1-octen-3-ol comes from the action of 12-lipoxygenase on arachidonic acid (Hsieh et al., 1988). 1-Octen-3-ol has been cited together with 2,3-pentanedione as marker of lipid oxidation in horse mackerel muscle (Iglesias & Medina, 2008). This species and *P. cornucopia* showed the minor total amount of VOCs.

V. pullastra was the species with a major complex volatile profile. It was composed of 26 compounds; among them 10 were aldehydes: butanal, 3-methylbutanal, pentanal, (E)-2-pentenal, hexanal, 3-methyl-2-butenal, (E)-2-hexenal, heptanal, (E,E)-2,4-heptadienal and (Z)-4-heptenal; 9 were alcohols: 1-penten-3-ol, 1-pentanol, (Z)-2-penten-1-ol, (Z)-3-hexen-1-ol, 1-hexanol, heptanol, (Z)-1,5-octadien-3-ol, (E)-2-octen-1-ol and (E,E)-2,4-octadien-1-ol; 4 were ketones: 2-butanone, 3-pentanone, 2,3-pentanedione and 2-nonanone; ethers as 2-ethylfuran; hydrocarbons as pentane and sulfur compounds as 2-thiopropane. The compounds (Z)-1,5-octadien-3-ol, 1-penten-3-ol and 2-thiopropane were the volatiles found in the highest concentration, and 1-penten-3-ol and 1-pentanol were significantly more abundant in this species compared to the other shellfish species. In *P. cornucopia*, 22 volatile compounds were detected: 9 were aldehydes [(E)-2-pentenal, hexanal, (E)-2-hexenal, heptanal,

(E,E)-2,4-heptadienal, 9,12,15-octadecatrienal, 2,6-nonadienal, octanal and 3,4,8-trimethyl-2-nonenal]; 3 were alcohols [1-penten-3-ol, (Z)-2-penten-1-ol and (Z)-1,5-octadien-3-ol; ethers as lilac aldehyde and 4 were ketones [2,3-pentanedione, (E,E)-3,5-octadien-2-one, 2-nonanone, and 2-undecanone]. The volatiles 9,12,15-octadecatrienal and lilac aldehyde resulted the most abundant compounds in this species. The content of 3,5-octadien-2-one was significantly higher compared to the profiles of the other species. The volatile 3,5-octadien-2-one contributes a fatty fruity odour and has been reported together with 2,4-heptadienal as the main product of EPA degradation via lipid autoxidation (Kawai, 1996). Octanal, indole, phenol and benzothiazole-2-butyl were exclusively detected in *P. cornucopia*. Considering the groups of volatiles, the ANOVA analysis illustrated a clear differentiation of each shellfish species depending on the major groups of compounds present (Table 15). As a general observation, alcohols were predominant in *O. edulis* and *V. pullastra*; aldehydes were the most concentrated compounds in *C. edule*, and aromatic aldehydes in *E. ensis*. *M. galloprovincialis* was predominant in alcohols and ketones and *P. cornucopia* shows another aldehydes as the most abundant compound. In addition, the total amount of volatiles found was higher in *O. edulis* and the minor amount of volatiles was detected for *P. cornucopia*.

Appication of the HSSPME method during *Ostrea edulis* refrigerated storage

The optimized method was also used to monitor *O. edulis* VOCs profile changes during 10 days of 4 °C refrigerated storage as shown in Table 16.

VOCs profile composition of *O. edulis* was already described in the previous paragraph. The general trend shared by all the compounds is the tendency to decrease during the refrigerated storage. Hexane behaviour was an exception from the general trend, keeping stable until the 7th day and then increasing significantly in the 10th day. Another exception was 1-penten-3-ol, that decreased significantly between the 1st and 3rd day of storage and then increased significantly between the 7th and 10th day.

The compounds that significantly decreased during the refrigerated storage were among aldehydes (e)-2-hexenal, heptanal, (z)-4-heptenal, benzaldehyde, (e,e)-2,4-heptadienal, (e)-2-octenal, (e,z)-2,6-nonadienal; among alcohols (e)-2-penten-1-ol, 3-cyclohexene-1-ethanol, 2-octen-1-ol, (z)1,5-octadien-3-ol; among ketones 1-octen-3-one and 3-octanone. The most abundant compound, was 3-cyclohexene-1-ethanol

that has already been found in the leaves of some plants and was described as fresh and minty (Tanaka et al., 2010). (z)1,5-Octadien-3-ol was the second most abundant volatile compound. It has been found to be a product of PUFAn-3 oxidation and was proposed as an intermediate of the EPA degradation via 12-lipoxygenase activity (Durnford & Sahidi, 1998). It has been reported to be an active contributor of flavour with a fresh mushroom and moss odour (Kaway, 1996), characterized by a low odour threshold (10 ppb). (e)-2-Octen-1-ol, together with 3-cyclohexene-1-ethanol and (z)-1,5-Octadien-3-ol characterizes the fresh raw *O. edulis* odour. It has been described as mushroom-like (Cho et al., 2006).

Table 16. Volatile organic compounds variations in oysters (*Ostrea edulis*) stored at 4 °C during 10 days. Amounts are expressed as peak area/internal standard area ratio.

VOCs	Days of storage				RSD
Area ratio	1st	3rd	7th	10th	
Aldehyds					
Pentanal	0.23	0.19	0.16	0.17	0.07
(e)2-Pentenal	1.46	0.96	0.82	0.75	0.28
Hexenal	2.61	2.63	1.64	1.15	0.64
(e)2-Hexenal	1.23^{a}	1.02^{ab}	0.65^{bc}	0.54^{c}	0.27
Heptanal	2.08^{a}	2.19^{a}	1.03^{b}	0.85^{b}	0.48
(z)4-Heptenal	1.05^{a}	0.72^{b}	0.42^{bc}	0.40^{c}	0.16
Benzaldehyde	1.48^{a}	1.82^{a}	0.93^{b}	0.73^{b}	0.40
(e, e)-2,4-Heptadienal	2.98^{a}	1.97^{ab}	1.37^{b}	1.27^{b}	0.65
(e)-2-Octenal	2.18	2.50	1.39	1.29	0.57
(e,z)-2,6-Nonadienal	2.51^{a}	2.31^{ab}	1.40^{bc}	1.12^{c}	0.56
Lylacaldehyde	0.80	1.20	0.58	0.66	0.30
Alcohols					
1-Penten-3-ol	0.54^{a}	0.33^{c}	0.35^{c}	0.42^{b}	0.04
(e)2-Penten-1-ol	0.50^{a}	0.33^{b}	0.29^{b}	0.30^{b}	0.06
3-Cyclohexene-1-ethanol	70.32^{a}	63.15^{a}	41.38^{b}	35.20^{b}	12.05
(e)-2-Octen-1-ol	17.52^{ab}	19.02^{a}	11.21^{bc}	9.45^{c}	3.43
(z)1,5-Octadien-3-ol	23.94^{a}	21.74^{ab}	14.96^{b}	14.88^{b}	3.96
Ketones					
1-Penten-3-one	0.19	0.13	0.16	0.17	0.03
2-3-Pentanedione	0.15	0.15	0.11	0.12	0.03
1-Octen-3-one	0.20^{a}	0.24^{a}	0.14^{b}	0.15^{b}	0.03
3-Octanone	0.33^{a}	0.31^{a}	0.16^{b}	0.15^{b}	0.07
Others					
Hexane	0.10^{b}	0.06^{b}	0.10^{b}	2.03^{a}	0.73
2-ethyl furan	0.88	0.97	0.89	0.71	0.31

Lowercase superscript letters indicate statistically significant differences ($p < 0.05$); ns: not significant.

3.1.4 Conclusions

Oysters coming from Orbetello Lagoon and from the open sea near Manfredonia were compared for fatty acids composition and shelf-life performances during 10 days of 4 °C refrigerated storage. Orbetello oysters presented a higher content of lipids, having a better fatty acids profile, richer in PUFA. In particular the PUFA n-3/PUFA n-6 ratio resulted higher in Orbetello oysters. This characteristic is considered as positive for human consumption, since the recommended PUFA n-3/PUFA n-6 ratio in the human diet is about 1/5 (S.I.N.U., 2011).

From the point of view of microbiological characteristics Manfredonia oysters gave the best results, highly influenced by the quality of the farming water.

The study focused on development of a method for volatile organic compound analysis, adequate for the volatile profile of shellfish allowed to select a very high performant analytical method. The optimized HS-SPME procedure on CAR-PDMS fibre demonstrated to have an extraordinary usefulness to extract the full spectra of aldehydes, alcohols and ketones and other compounds related to flavour. The developed analytical method exhibits a good performance in terms of linearity, repeatability, inter-day precision and recovery, having also the advantage of simplicity, low-cost, organic solvent free and time saving. Type of fiber-coating, sample volume and the time and temperature of incubation influence SPME, so that the optimization of the parameters affecting extraction, fundamental to obtain reliable results, was also realised.

The developed method was then utilised for characterizing the volatile spectra of different shellfish allowing obtaining a good characterization. The six shellfish species studied presented different VOCs profiles that were useful to identificate of the characterizing compounds contributors to flavour. The method was useful for detecting specific volatile compounds of shellfish which have been firstly described in these species. The method also provided the first VOCs profile of Atlantic *E. ensis*, *V. pullastra*, *C. edule* and *P. cornucopia*. The optimized method resulted to be suitable also in monitoring VOCs profile changes of *O. edulis* during refrigerated storage and in evaluating the freshness evolution, because of its sensibility to the changes of concentration of each detected compound.

3.2 Trial 2: growing performances

3.2.1 Effect of the containers

Analyzing the growing performances in open sea, is possible to evaluate the influence of the farming container on the growing performances of the oysters. Tables 17 to 22 show the different growing trends of the oysters along the whole farming experiment in the three different kind of containers: *poches,* baskets and lanterns.

During the 1st sampling session, baskets and lanterns seemed to give the better performances for biometric parameters, but there were no significant differences within the commercial parameters, except for the Walne (1976) condition index (CI(II)) that resulted higher for *poche* oysters.

Table 17. Biometric and commercial characteristics of the oysters coming from Tyrrhenian sea near Porto Ercole grown into three different containers sampled in September 2009.

September 2009	PORTO ERCOLE			RSD
	basket	lantern	*poches*	
Biometric characteristics				
Width (mm)	19.66^{A}	19.49^{A}	17.72^{B}	3.410
Depth (mm)	11.31^{A}	11.09^{A}	10.25^{B}	1.670
Flesh weight (g)	0.28^{A}	0.27^{A}	0.19^{B}	0.116
Shell weight (g)	3.52^{A}	3.45^{A}	2.83^{B}	1.215
Commercial characteristics				
Shell weight (%)	92.70	92.67	93.19	2.247
Shape index	0.35	0.34	0.34	0.0588
ECI	0.43	0.43	0.43	0.07
CI (I)	0.07	0.07	0.07	0.02
CI (II)	0.02^{b}	0.02^{b}	0.02^{a}	0.01

Lowercase superscript letters indicate statistically significant differences ($p< 0.05$).

During the 2nd and 3rd sampling sessions (January and March 2010) the situation changed, and oysters farmed in *poches* and lanterns gave better growing performances. In January biometric characteristics resulted significantly higher for *poches* and lanterns than for baskets, as well as CI(II). During the sampling session of March 2010 oysters farmed in baskets resulted to have the largest and heavier shell, while the oysters farmed in *poches* were significantly meatier than the other two oyster groups: this is in line with CI(I) and CI(II) values.

During the sampling session of May 2010, the situation tended to level out: among the biometric parameters only depth and width resulted significantly higher for basket and lantern oysters and among commercial parameters only the shape index resulted significantly higher for basket oysters.

Table 18. Biometric and commercial characteristics of the oysters coming from Tyrrhenian sea near Porto Ercole grown into three different containers sampled in January 2010.

January 2010	PORTO ERCOLE			RSD
	basket	lantern	*poches*	
Biometric characteristics				
Width (mm)	22.54[b]	26.37[a]	25.68[a]	6.241
Depth (mm)	12.45[b]	13.39[a]	13.40[a]	2.877
Flesh weight (g)	0.54	0.58	0.64	0.446
Shell weight (g)	3.45	3.47	3.43	1.967
Commercial characteristics				
Shell weight (%)	58.28[a]	56.77[a]	53.62[b]	8.973
Shape index	0.34	0.34	0.33	0.0618
ECI	0.42	0.41	0.41	0.07
CI (I)	0.09	0.09	0.09	0.04
CI (II)	0.02[b]	0.02[ab]	0.03[a]	0.01

Lowercase superscript letters indicate statistically significant differences ($p < 0.05$).

From the sampling session of July 2010 until the end of the experiment, the general trend of the growing performances changed again and the *poches* oysters resulted to give the worst results. In July 2010 basket oysters gave the best results for both biometric and commercial characteristics. In particular, from May 2010 to July 2010 the edible part weight increased for basket oysters and decreased for the other two oyster groups Between *poches* and lanterns, the lanterns gave better oysters for shape index.

During the last sampling session (September 2010) oysters farmed in baskets and lanterns presented the best results: biometric characteristics, all resulted significantly higher for basket oysters, while commercial characteristics resulted generally higher for basket and lantern oysters, with a significant higher CI (I) for the former group. The results obtained in this last sampling session, clearly indicate the different life conditions of the oysters farmed in the same water, but into different containers: basket oysters received food and oxygen homogeneously through a correct water flow in the inside of the basket, so that the oysters could accumulate energy reserves.

Table 19. Biometric and commercial characteristics of the oysters coming from Tyrrhenian sea near Porto Ercole grown into three different containers sampled in March 2010.

March 2010	PORTO ERCOLE			RSD
	basket	lantern	*poches*	
Biometric characteristics				
Width (mm)	32.16[a]	29.75[b]	28.39[b]	11.038
Depth (mm)	16.03[a]	14.67[b]	15.93[ab]	3.475
Flesh weight (g)	1.10[b]	1.08[b]	1.40[a]	0.750
Shell weight (g)	6.53	5.84	6.42	3.436
Commercial characteristics				
Shell weight (%)	55.70[a]	56.19[a]	50.59[b]	6.801
Shape index	0.32	0.31	0.30	0.0636
ECI	0.39	0.38	0.39	0.07
CI (I)	0.09[b]	0.11[a]	0.10[a]	0.03
CI (II)	0.03[c]	0.03[b]	0.05[a]	0.01

Lowercase superscript letters indicate statistically significant differences ($p < 0.05$).

Table 20. Biometric and commercial characteristics of the oysters coming from Tyrrhenian sea near Porto Ercole grown into three different containers sampled in May 2010.

May 2010	PORTO ERCOLE			RSD
	basket	lantern	*poches*	
Biometric characteristics				
Width (mm)	33.36[a]	33.73[a]	29.62[b]	8.058
Depth (mm)	17.78[a]	16.434[a]	15.90[b]	4.013
Flesh weight (g)	1.45	1.36	1.36	0.897
Shell weight (g)	8.847	8.46	8.67	4.816
Commercial characteristics				
Shell weight (%)	59.85	57.97	57.18	8.597
Shape index	0.33[a]	0.32[ab]	0.31[b]	0.056
ECI	0.41	0.39	0.39	0.07
CI (I)	0.09	0.09	0.09	0.03
CI (II)	0.03	0.03	0.03	0.01

Lowercase superscript letters indicate statistically significant differences ($p < 0.05$).

Figures 15, 16 and 17 show the trends presented by three different parameters that are considered important to evaluate oysters growing performances, as well as their commercial value: total individual weight, shell length and flesh yield.

The first two parameters presented a similar trend along the 14 months of the growing period, with a similar and gradual increase from September 2009 until May 2010. From May 2010 oysters contained in lanterns and *poches* presented a little decrease in total weight until July 2010 and then an increase until September 2010. From May 2010 to September 2010 oysters contained in baskets presented a strong increase until July 2010 and then a strong decrease for total individual weight and

shell length. This can be explained with an anomalous and not representative sampling during the oysters collection from the baskets in the sampling session of July 2010, when probably only the biggest oysters have been collected. Anyway, these two parameters registered the best results for the oysters contained in baskets and lanterns with significant higher values.

Table 21. Biometric and commercial characteristics of the oysters coming from Tyrrhenian sea near Porto Ercole grown into three different containers sampled in July 2010.

July 2010	PORTO ERCOLE			RSD
	basket	lantern	*poches*	
Biometric characteristics				
Width (mm)	37.30[a]	29.34[b]	25.51[c]	7.751
Depth (mm)	18.99[a]	15.92[b]	13.46[c]	4.215
Flesh weight (g)	2.00[a]	0.99[b]	0.89[b]	0.942
Shell weight (g)	15.80[a]	7.65[b]	6.65[b]	6.544
Commercial characteristics				
Shell weight (%)	61.07[a]	58.74[b]	58.34[b]	7.456
Shape index	0.30[b]	0.32[a]	0.30[b]	0.063
ECI	0.04[b]	0.04[a]	0.04[b]	0.07
CI (I)	0.08	0.08	0.07	0.03
CI (II)	0.02[b]	0.03[a]	0.02[ab]	0.01

Lowercase superscript letters indicate statistically significant differences ($p < 0.05$).

Table 22. Biometric and commercial characteristics of the oysters coming from Tyrrhenian sea near Porto Ercole grown into three different containers sampled in September 2010.

September 2010	PORTO ERCOLE			RSD
	basket	lantern	*poches*	
Biometric characteristics				
Width (mm)	30.97[a]	27.36[b]	26.61[b]	7.093
Depth (mm)	17.94[a]	16.55[a]	14.67[b]	5.168
Flesh weight (g)	1.03[a]	0.79[b]	0.58[c]	0.605
Shell weight (g)	10.34[a]	9.43[a]	6.63[b]	5.654
Commercial characteristics				
Shell weight (%)	58.35[b]	60.65[a]	56.54[c]	6.200
Shape index	0.32	0.32	0.31	0.083
ECI	0.41	0.42	0.40	0.10
CI (I)	0.06[a]	0.05[ab]	0.05[b]	0.03
CI (II)	0.02	0.02	0.02	0.02

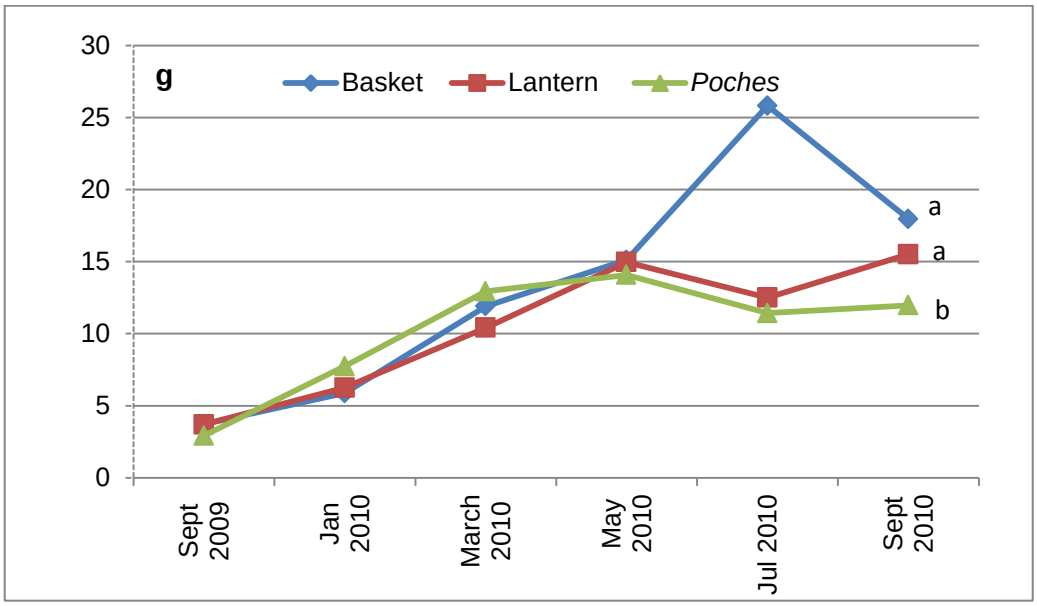
Lowercase superscript letters indicate statistically significant differences ($p < 0.05$).

Flesh weight presented a very different trend as shown by Figure 17: this parameter increased steadily from September 2009 until March 2010 and then

decreased steadily from March 2010 until September 2010. Even for this parameter the best results at the end of the growing period were obtained by the oysters contained in the baskets.

Poches represents one of the most diffused container used for oyster farming on rack structures in tidal and subtidal environments. In open sea water currents are much more strong than in tidal zones and lagoons, so that oysters farmed in the open sea and contained in *poches* without any space division tend to accumulate on one side of the container, with no possibility to exploit the whole available space. In this way the oysters didn't receive the same flow rate, consequently not all the oysters got sufficient food and oxygen and this considerably reduced the final yield.

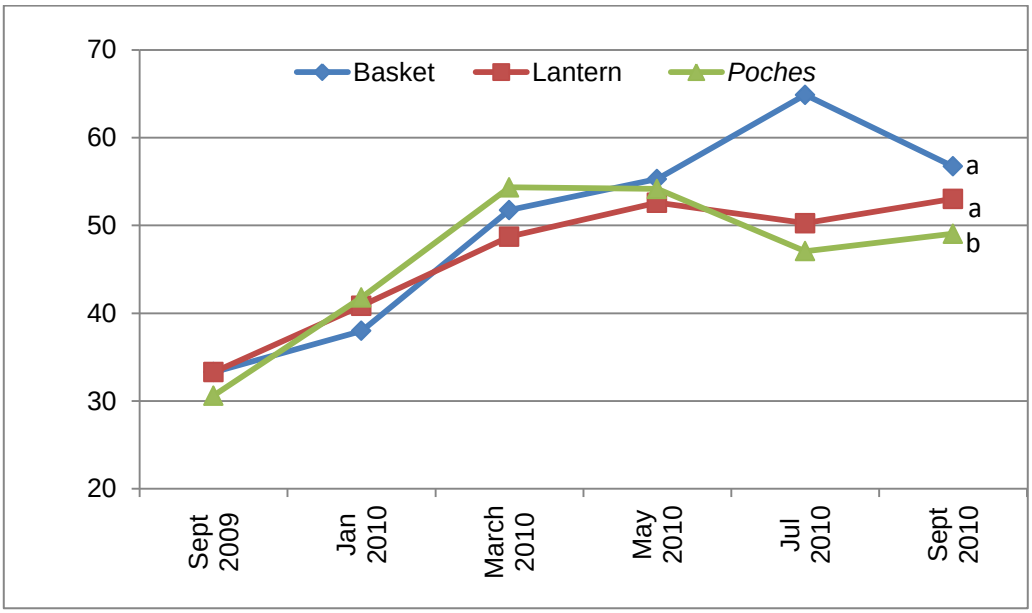
Figure 15. Total individual weight of the oysters farmed in the sea into three different containers during the growing trial.



Only the statistic significance of the last sample session is indicated. Lowercase letters indicate statistically significant differences ($p < 0.05$).

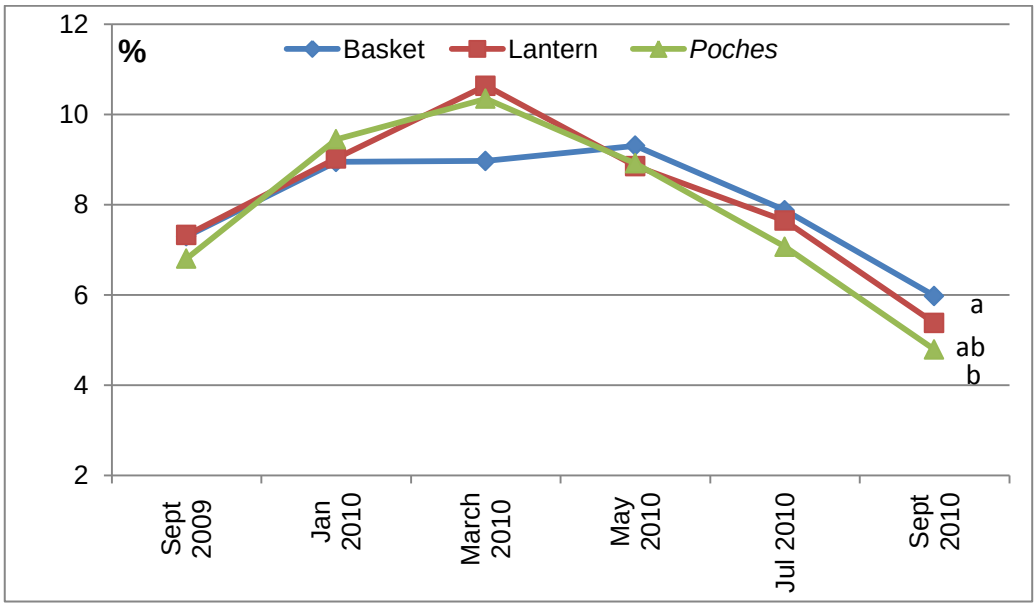
Our results are consistent with those found by Roncarati et al. (2009). Studying the suitability of a new kind of container for oysters farming, very similar to the baskets used in this experiment, the Authors compared the growing performances of the new container with those of *poches* and with those of a tray composed by ten PVC round units placed one upon another. Roncarati et al. (2009) noted that during 11 months of farming, oysters reared in *poches* presented worse biometric characteristics and a lower condition index.

Figure 16. Shell length of the oysters farmed in the sea into three different containers during the growing trial.



Only the statistic significance of the last sample session is indicated. Lowercase letters indicate statistically significant differences ($p < 0.05$).

Figure 17. Flesh yield of the oysters farmed in the sea into three different containers during the growing trial.



Only the statistic significance of the last sample session is indicated. Lowercase letters indicate statistically significant differences ($p < 0.05$).

The importance of the role that the culture container plays in oysters farming has also been demonstrated by Robert et al. (1993) who compared *poches* with Stanway cylinders performances, founding that the cylinders gave a better final product, with better condition index, flesh and shell quality. Other authors (Paynter & Dimichele, 1990; Spencer et al., 1992; Gosling, 2003) investigated the possible damage from handling and sorting in oysters grown in *poches* compared to other kind of container and noted that oysters cultured in *poches* are generally more fragile with friable shells. Consequently the final product appearance and characteristics are in fact critical for successful farming.

3.2.2 Effect of the farming site on growing performances

The biometric and commercial characteristics of the oysters farmed in the Orbetello Lagoon and in the open sea site near Porto Ercole measured during each sampling session are presented in Tables 23 and 24**.**

Lagoon oysters presented an average growth in total individual weight of 0.16 g/day from the seeding to the end of the experiment, while sea oysters grew 0.03 g/day. The big differences between the growth performances of the lagoon and sea oysters are evident if we consider respectively the final length (91.63 vs 53.19 cm) (Figure 18), final total individual weight (73.36 vs 15.28 g) (Figure 19), and total flesh weight (4.50 vs 1.80 g) (Figure 20). Differences between the two groups attenuate if we consider the percentage of the flesh weight on the total weight, that resulted for lagoon and sea oysters 5.87 and 5.48% respectively (Figure 21). The results of the flesh yield of the oysters, that dramatically influence the commercial value of the final product, did not result to differ in a significant way between lagoon and sea oysters during the last two sampling sessions (Figure 21).

Anyway we have to consider the growing performance of the oysters farmed in the Orbetello Lagoon as highly better: this group of oysters reached an average total individual weight and length that are considered suitable for the market (Roncarati et al., 2009; Spencer et al., 1992) in 14 months of farming. During the same period, oysters farmed in the open sea reached an average individual total weight and an

average flesh weight that resulted 4.8 and 2.5 times, respectively, lower than the lagoon oysters.

Figure 18. Shell length of the oysters grown in the Orbetello Lagoon and in open sea near Porto Ercole during the growing trial.

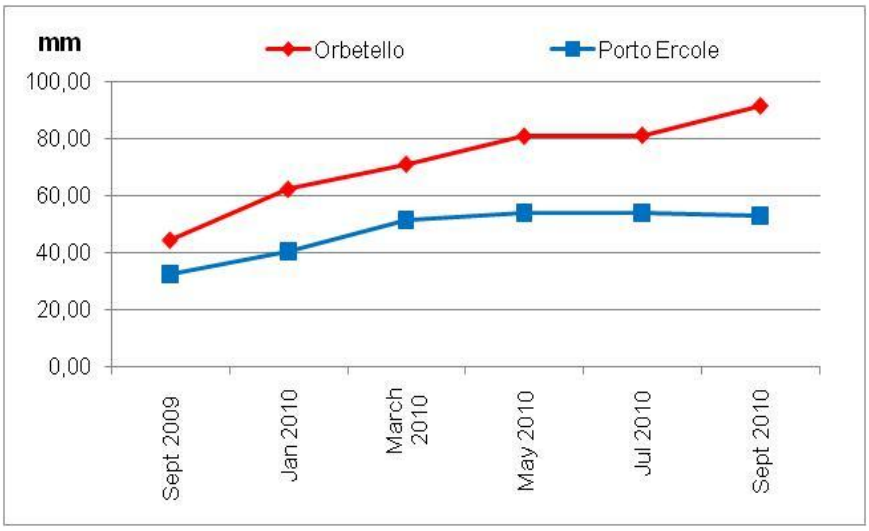


Figure 19. Total individual weight of the oysters grown in the Orbetello Lagoon and in open sea near Porto Ercole during the growing trial.

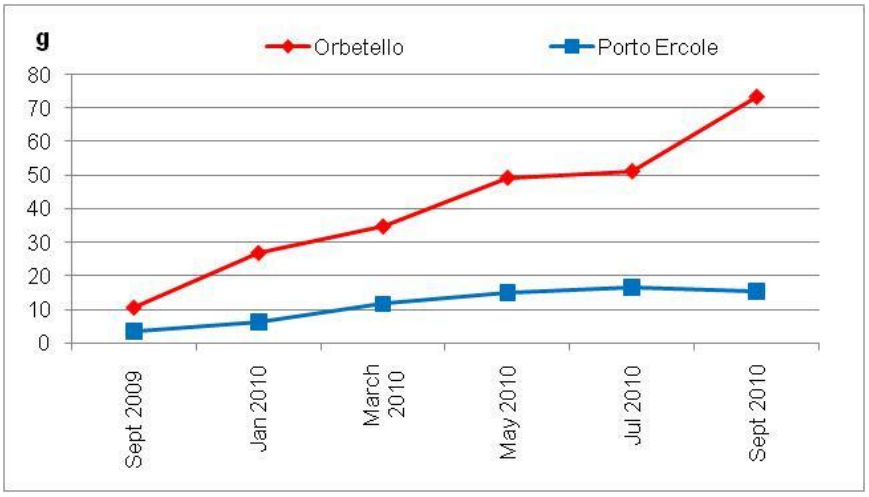


Figure 20. Flesh weight of the oysters grown in the Orbetello Lagoon and in open sea near Porto Ercole during the growing trial.

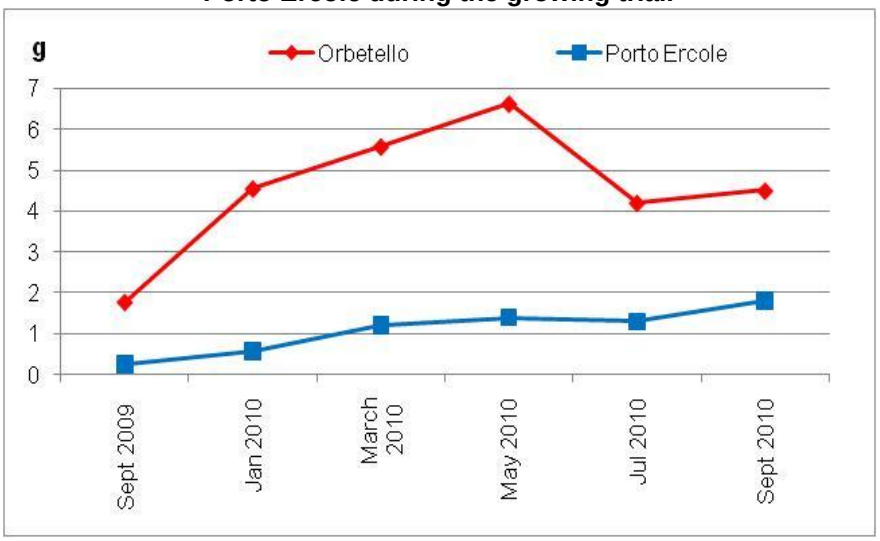
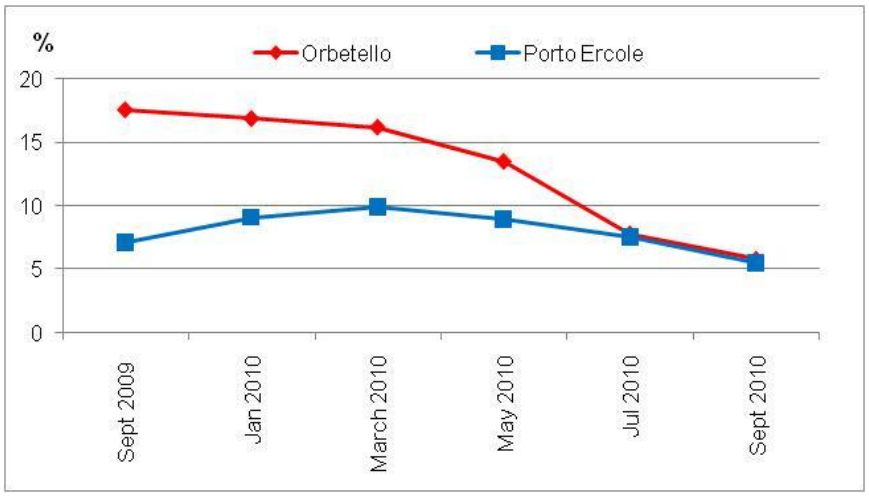


Figure 21. Flesh weight percentage on total individual weight weight of the oysters grown in the Orbetello Lagoon and in open sea near Porto Ercole during the growing trial.



The growing performances of the oysters farmed in the Orbetello Lagoon can be considered good if we consider that in France oyster farming spend 24-36 months to obtain a marketable product (Turolla & Rossi, 2004); in the North Wales, Spencer et al. (1992) from a seed of 3 mm obtained oysters with a total individual weight of 75 g in 4 years farming time, while Askew (1972) from a seed of 3.6 g obtained 60 g oysters in 1 year farming. Valli (1980), conducted a similar experiment to ours in the Grado Lagoon: from a seed weighting 0.03 g farmed for 1 year the author obtained an

average final weighting oysters of 60 g obtaining a growth rate of 0.16 g/day, according to our results. Like in our experiment, Prioli et al. (1995) compared oysters growing performances in two different farming sites and they obtained a commercial-sized product in less than 15 months in the Adriatic Sea near Porto Garibaldi (Fe, Italy).

Comparing the results of the flesh percentage in both the two groups of oysters during the last two sampling sessions, we can notice a significant decrease: this phenomenon can be attributed to spawning that could have caused a mobilization of reserves such as glycogen and protein (Dridi et al., 2007).

Observing the results summarized in Table 23 is evident that the period between the sampling sessions of July and September 2010 was particularly favourable to the growth performances of lagoon oysters; whereas, observing the results reported in Table 24, we can notice that the total individual weight of the sea oysters increased mainly during the period between the sampling sessions of September 2009 and May 2010.

Table 23. Biometric and commercial characteristics of the oysters grown in the Orbetello Lagoon during 14 months.

ORBETELLO

	Jul-2009	Sept-2009	Jan-2010	Mar-2010	May-2010	Jul-2010	Sept-2010	RSD
Biometric characteristics								
Length (mm)	24.62^{f}	44.27^{e}	62.27^{d}	71.03^{c}	80.81^{b}	81.14^{b}	91.42^{a}	13.053
Width (mm)	15.22^{f}	28.46^{e}	40.25^{d}	43.21^{c}	47.86^{b}	47.40^{b}	49.54^{a}	7.638
Depth (mm)	8.36^{f}	18.79^{e}	22.76^{d}	24.23^{c}	25.76^{b}	24.70^{c}	28.94^{a}	4.105
Total weight (g)	1.79^{f}	10.50^{e}	26.78^{d}	34.69^{c}	49.20^{b}	51.16^{b}	73.36^{a}	17.045
Flesh weight (g)		1.77^{d}	4.55^{c}	5.58^{b}	6.63^{a}	4.20^{c}	4.50^{c}	2.424
Shell weight (g)		8.68^{e}	15.76^{d}	19.83^{c}	28.73^{b}	30.79^{b}	43.99^{a}	10.555
Commercial characteristics								
Flesh weight (%)		17.58^{a}	16.90^{ab}	16.22^{b}	13.53^{c}	7.85^{d}	5.87^{e}	3.836
Shell weight (%)		82.50^{a}	59.31^{b}	57.81^{b}	59.10^{b}	60.24^{b}	60.63^{b}	7.734
Shape index	0.35^{b}	0.43^{a}	0.37^{b}	0.37^{b}	0.33^{cb}	0.31^{c}	0.32^{cb}	0.135
ECI		0.52^{a}	0.45^{b}	0.43^{c}	0.41^{d}	0.39^{e}	0.42^{cd}	0.08
CI (I)		0.18^{a}	0.17^{ab}	0.16^{b}	0.14^{c}	0.08^{d}	0.06	0.04
CI (II)		0.05^{b}	0.05^{a}	0.05^{a}	0.05^{b}	0.03^{c}	0.02^{d}	0.02

Lowercase superscript letters indicate statistical significant differences ($p < 0.05$).

Table 24. Biometric and commercial characteristics of the oysters grown in Porto Ercole site during 14 months.

PORTO ERCOLE	Sept-2009	Jan-2010	Mar-2010	May-2010	Jul-2010	Sept-2010	RSD
Biometric characteristics							
Length (mm)	32.37^{d}	40.40^{c}	51.39^{b}	54.04^{a}	54.04^{a}	52.97^{a}	11.984
Width (mm)	18.96^{e}	24.86^{d}	30.10^{b}	32.24^{a}	31.05^{ab}	28.31^{c}	7.638
Depth (mm)	10.88^{d}	13.08^{c}	15.54^{b}	16.71^{a}	16.12^{a}	16.39^{a}	3.525
Total weight (g)	3.47^{e}	6.37^{d}	11.63^{c}	14.99^{b}	16.60^{a}	15.28^{b}	7.489
Flesh weight (g)	0.25^{e}	0.58^{d}	1.19^{c}	1.39^{b}	1.29^{bc}	1.80^{a}	0.65
Shell weight (g)	3.25^{d}	3.45^{d}	6.25^{c}	8.69^{b}	10.03^{a}	8.68^{b}	3.97
Commercial characteristics							
Flesh weight (%)	7.14^{c}	9.15^{b}	9.98^{a}	8.95^{b}	7.59^{c}	5.48^{d}	3.061
Shell weight (%)	92.85^{a}	56.22^{d}	54.16^{e}	58.34^{c}	59.38^{b}	58.51^{b}	7.067
Shape index	0.34^{a}	0.33^{a}	0.31^{b}	0.32^{b}	0.31^{b}	0.32^{b}	0.064
ECI	0.43^{a}	0.41^{b}	0.39^{c}	0.39^{c}	0.39^{c}	0.41^{b}	0.07
CI (I)	0.07^{d}	0.09^{b}	0.10^{a}	0.09^{b}	0.08^{cd}	0.06^{e}	0.03
CI (II)	0.02^{d}	0.02^{c}	0.03^{a}	0.03^{b}	0.02^{c}	0.02^{d}	0.01

Lowercase superscript letters indicate statistical significant differences ($p < 0.05$).

The different trend of the total individual weight between the two groups observed during the period between July and October 2010 is due mainly to the sharp fall of nutrient content in the sea water from June until the end of the summer (Figures 2, 3, 4). Mathiessen (2001) stated that *Crassostrea gigas* presents the higher potential of growing performances during summer time, but growth performance is however highly dependent from environmental conditions, food availability (Ducrotoy et al., 2000) and from filtration rate (Walne, 1979). This explains why the growth of the oysters reared in the sea resulted much slower than the oysters farmed in the lagoon.

3.2.3 Conclusions

Comparing the three different kinds of container tested in the open sea farming site near Porto Ercole, baskets resulted to give the better performances. Baskets have the advantage to allow the good circulation of the water in the inside and the optimal distribution of the oysters, so that the animals always receive food and oxygen

uniformly, reducing the reciprocal competition, as already observed by Roncarati et al. (2009) and by Turolla & Rossi (2004).

The results obtained from the growing trial, underline the trophic differences existing between the Orbetello Lagoon and the open sea farming site near Porto Ercole. The better trophic characteristics of the water of the Orbetello Lagoon deeply influenced the growing performances of the oysters during the whole trial, confirming the findings of other Authors dealing with the effect of the trophic characteristics of the farming environment on growing performances of the farmed oysters. In particular, the scarcity of nutrients that characterized the open sea farming site during the spring-summer period, played a fundamental role in differentiating the growing performances in the two farming sites. The difference between the two growing performances was wide, if we consider that lagoon oysters reached 11 months earlier the same total weight reached by the sea oysters at the end of the trial.

3.3 Trial 3: finishing

3.3.1 Growing performances during sea finishing

Orbetello Lagoon is an environment rich of nutrient, where oysters can grow healthy and fast. Despite this, it is also a critical environment, where algal blooms often occur, especially during the spring and the summer. Algal blooms can result dangerous for human health, since the oysters, as all bivalve molluscs, are filter-feeders and can accumulate toxins and pathogens (Rippey, 1994) that are harmful for human health, but not for the animals. Oysters farmed in marine areas classified by Italian D. lgs. 530/92 as B or C must be depurated or stabulated, respectively, before commercialization. Depuration procedure represents a cost in addition to the costs of production. The possibility to grow oysters in the Orbetello Lagoon (classified as B) and then move the animals in an open sea site for finishing and depuration permit to take advantage of both the high nutrient availability of the lagoon and of the high water quality of the open sea.

In order to verify the feasibility of the oysters transfer from lagoon to the open sea for finishing, biometric and commercial characteristics as well as quality characteristics of oysters from both the finishing sites (lagoon and sea) were

evaluated during each sampling session (September, corresponding to the starting point, and in October and December 2010).

The biometric and commercial characteristics changes of the oysters during the finishing period in lagoon and in the open sea are summarized in Table 25.

Table 25. Biometric and commercial characteristics of oysters farmed in Orbetello Lagoon and Tyrrhenian sea near Porto Ercole analyzed during the finishing trial.

	September 2010	**October 2010**		**December 2010**		**RSD**
		Orbetello	**Porto Ercole**	**Orbetello**	**Porto Ercole**	
Biometric characteristics						
Total weight (g)	*61.56*	96.10[a]	67.91[b]	71.79	74.25	19.24
Soft part weight (g)	*4.51*	8.78[a]	4.56[b]	7.18[a]	4.72[b]	2.35
Shell weight (g)	*39.78*	60.23[a]	42.74[b]	46.81	45.94	12.75
Commercial characteristics						
Flesh weight %)	*7.26*	9.04[a]	6.55[b]	9.98[a]	6.15[b]	1.96
Shell weight (%)	*67.39*	62.76	62.69	65.21[a]	62.05[b]	7.47
Shape index	*0.320*	0.324	0.320	0.379[a]	0.302[b]	0.14
ECI		0.42	0.41	0.45[a]	0.40[b]	0.07
CI (I)		0.09[a]	0.07[b]	0.10[a]	0.06[b]	0.02
CI (II)		0.03[a]	0.02[b]	0.03[a]	0.02[b]	0.01

Lower case superscript letters indicate statistically significant differences ($p < 0.05$).

Only one month after the transfer, the oysters remained in the lagoon presented a significantly heavier edible part, significantly higher edible part yield, CI (I) and CI (II) indicating a better general health conditions of the animals. At the end of the finishing trial the total individual weight resulted higher for the oysters finished in the sea (74.25 vs 71.79 g), even if not in a significant way, but all the other parameters gave significant better results for the oysters finished in the lagoon. These differences are due to the already mentioned differences between the farming environments, but the transfer of the oysters from the lagoon to the sea site may also have influenced the final result. The handling procedures performed to select oysters' size together with the sudden change of environmental and farming conditions (temperature, salinity, water current flow, container), represented a stressful situation for the oysters moved to the sea, so that the animals had to use energy to face stressors and to adjust to the new different environment.

The general oysters' marketable quality can be also expressed in relation to the shape of shell, that can dramatically influence the product value when animals are commercialised live or half shell (Batista et al., 2008). The lagoon oysters were significantly meatier, having higher CI values, and showed a better shape (due to the higher depth to length ratio) compared to the oysters from the sea site. This ratio was found to be a parameter more effective than others in categorizing good and bad shell shapes (Brake et al., 2003; Xiong et al., 2010). In oysters this is an extremely plastic trait, affected by the type of substrate on which they grow, the degree of crowding and husbandry procedures, and also by the different physico-chemical parameters of water (Galtsoff, 1964; Quayle, 1988). However the shell shape of both sample groups can be considered as good, since the ratio resulted higher than the threshold of 0.316 proposed by Brake et al. (2003) to discriminate the oysters good-shaped, whereas a mean value significantly lower than 0.219 identifies bad-shaped oysters.

The higher quality level of samples from lagoon is clearly expressed by the values of the parameters characterizing the marketable quality, such as the weight of the soft part (15.40% of the total weight in the lagoon samples while in the sea samples was around the half, i.e. 7.68%), and the values of all the three Condition Indexes.

Environmental farming conditions strongly affect bivalve characteristics and this was evident in our results as well as in the results of other authors. Fuentes et al. (2009) found significant differences in the size, meat yield and shell percentage of blue mussels farmed in three different Spanish sites. Mussels with higher length, width, and height presented lower meat yield and shell percentage, and showed different energy storage. Chávez-Villalba et al. (2010), comparing the growth performances and the condition index between oysters cultivated in a lagoon site and in a near gulfside site throughout a year, found a higher average condition index in the gulfside group of oysters, and a significantly better growth performance in the lagoon group. The authors concluded that the gulfside environment allowed oysters to accumulate energy reserves in the edible part during the farming period.

Table 26 shows the biometric and commercial characteristics of oysters finished in the lagoon and in the sea site and collected in February 2011, after 5 months of finishing in Orbetello Lagoon and in the open sea near Porto Ercole and used to perform the 4°C refrigerated storage trial analysis. The sea group of oysters had

significantly higher values for biometric characteristics, except for soft part weight and all the commercial characteristics resulted significantly better for the oysters finished in lagoon.

Table 26. Biometric and market characteristics of lagoon- and sea-finished oysters.

	Lagoon	Sea	RSD
Morphological characteristics			
Total weight (g)	69.36^{b}	88.82^{a}	24.22
Soft part weight (g)	9.92^{a}	6.59^{b}	2.61
Shell weight (g)	50.10^{b}	57.02^{a}	14.25
Length (mm)	86.92^{b}	102.57^{a}	11.68
Width (mm)	52.55^{b}	62.38^{a}	9.32
Depth (mm)	31.50	32.55	4.99
Commercial characteristics			
Soft part weight (%)	15.40^{a}	7.68^{b}	5.15
Shell weight (%)	77.28^{a}	67.90^{b}	23.94
Depth / Length	0.37^{a}	0.32^{b}	0.07
Economic Condition Index	0.430^{a}	0.397^{b}	0.046
Condition Index I	0.135^{a}	0.072^{b}	0.022
Condition Index II	0.058^{a}	0.028^{b}	0.011

Lowercase superscript letters indicate statistically significant differences ($p< 0.05$).

In this study, the analyzed biometric and commercial characteristics clearly show that the lagoon oysters used the higher feed availability from the eutrophic water of lagoon to accumulate energy reserves in the edible part. The sea oysters used energy to build up shell tissues, and maybe to face stressors like the high flow speed of the water characterizing the open sea environment. In a previous research carried out in the same sites, Parisi et al. (2006) found relevant differences in the trophic condition of the water of the two environments, both for the phytoplankton quality (35 *taxa* in the lagoon and 105 *taxa* in the sea) and quantity (on average $2x10^{6}$ cell L^{-1} in lagoon and $2x10^{5}$ cell L^{-1} in sea).

As during the growing trial *poches* gave the worst results, we decided to exclude this kind of tray from the finishing in the sea, where only lanterns and baskets were used to perform the trial. In the lagoon we continued to use *poches* because it was the only tray suitable for an environment where the mean water depth has been of 1 m.

Table 27 shows the effect of the two different containers used in the open sea on biometric and commercial characteristics of oysters.

Table 27. Effect of two different kind of containers on oysters' biometric and commercial characteristics during the finishing trial performed in the Tyrrhenian sea near Porto Ercole.

	September 2010	**October 2010**		**December 2010**		**RSD**
		basket	**lantern**	**basket**	**lantern**	
Biometric characteristics						
Total weight (g)	*61.56*	70.96[a]	64.82[b]	75.80	73.02	16.25
Soft part weight (g)	*4.51*	4.92[a]	4.19[b]	4.42	4.77	1.79
Shell weight (g)	*39.78*	44.25	41.16	46.77	45.29	11.07
Commercial characteristics						
Soft part weight (%)	*7.26*	6.90	6.18	5.74	6.48	1.85
Shell weight (%)	*67.39*	62.78	62.61	61.80	62.25	8.65
Shape index	*0.320*	0.326	0.313	0.281[b]	0.319[a]	0.06
ECI		0.42	0.41	0.37[b]	0.42[a]	0.01
CI (I)		0.07	0.06	0.06	0.07	0.02
CI (II)		0.02	0.01	0.02	0.02	0.01

Lower case superscript letters indicate statistically significant differences ($p < 0.05$).

One month after the transfer, oysters farmed in baskets presented significantly higher total individual weight and edible part weight. At the end of the finishing period, significant differences were registered for the shape index and the ECI, both resulting higher for the oysters farmed in the lanterns. The differences in flesh weight and percentage were not significant but, at the end of the finishing trial, the values registered resulted to be higher for the oysters grown in lanterns (4.77 vs 4.42 g and 6.68 vs 5.74%, respectively). This is probably due to the fact that the net of the lanterns allows the oysters not to slide when affected by the currents, unlike the oysters contained into the baskets. This represents a stress reduction for the oysters, resulting in a better general health condition. Turolla & Rossi (2004), comparing the effects of the use of lanterns and rectangular trays during oysters fattening, also observed better growing performances of adult oysters in lanterns. During oysters finishing in lanterns, did not occur the net trapping by the shells, that affected in a negative way the shells of the oysters analyzed during the growing trial. The reason is probably that oysters used for the finishing trial were adult oysters, grown in a high trophic environment (the lagoon) and with a strong shell.

3.3.2 Chemical characteristics

The gross chemical composition, and the levels of total, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFAs) and n-3 fatty acids, n-6 fatty acids, eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6n-3) in oysters cultivated in lagoon and in the sea are shown in Table 28. Gross chemical composition presented significant differences between the lagoon and sea finishing oysters: a higher moisture content was recorded in the sea finished oysters (79.57 vs 78.97%), as well as protein and ash content (55.11 vs 49.11% and 16.96 vs 10.72%, respectively).

Table 28. Chemical characteristics of the oysters coming from Orbetello Lagoon and Tyrrhenian sea near Porto Ercole analyzed during the finishing trial.

	Orbetello	Porto Ercole	RSD
Moisture(%)	78.97^{b}	79.57^{a}	0.45
Raw protein(% d. m.)	49.11^{b}	55.11^{a}	3.71
Total lipids (% d. m.)	11.82^{a}	9.34^{b}	1.54
Ashes (% d. m.)	10.72^{b}	16.96^{a}	3.29
Fatty acids (% on total F. A.)			
C14:0	2.28^{b}	6.63^{a}	0.36
C16:0	16.55^{a}	11.95^{b}	2.11
C18:0	3.82	3.84	0.44
C18:1 n-9	5.40^{a}	3.93^{b}	0.58
C18:1 n-7	3.39^{b}	3.96^{a}	0.31
C20:1 n-7	2.29^{b}	2.63^{a}	0.29
C20:4 n-6	1.55^{b}	2.31^{a}	0.29
C20:5 n-3	7.53^{b}	9.57^{a}	1.31
Unknown 2	1.90^{b}	2.71^{a}	0.39
C22:5 n-3	1.61	1.74	0.18
C22:6 n-3	13.41	14.95	2.47
SFA	32.99	29.86	3.78
MUFA	20.66	20.22	1.78
PUFA	41.04	43.82	5.26
PUFAn-3	31.21	31.74	5.11
PUFAn-6	6.82^{b}	7.52^{a}	0.58
PUFAn-3/PUFAn-6	4.59	4.24	0.73
PUFAn-6/PUFAn-3	0.24	0.24	0.05
Healthiness indexes			
h/H ratio	2.02^{b}	2.57^{a}	0.41
IA	0.47	0.40	0.09
IT	0.07^{a}	0.06^{b}	0.02

The following fatty acids C13:0,C13:0-trimetil, C14:0 metil-9, C15:0 metil-14, C16:0 metil-14, C18:2n-4, C18:3n-4, C18:4n-1, C20:2n-xx, C20:3n-6, C20:3n-3, C20:4n-3, C22:0, C22:1n-9, and C22:5n-6 are not shown in the table but considered in the groups.
Lowercase superscript letters indicate statistically significant differences ($p< 0.05$).

Our findings accord to the range of results reported by Cruz-Romero et al. (2008b) on the control group of oysters, while Orban et al. (2004) found higher moisture and ash content (89.7 and 22.3% respectively) and a lower content of total lipids (6.79%) in adult Pacific oysters, confirming that environmental conditions deeply influence the biochemical composition of the oysters.

Total lipids resulted to be higher in the lagoon oysters (11.82 vs 9.34%), resulting in agreement with other authors findings (Jeong et al., 1990; Piveteau et al., 1999; Saito & Marty, 2010).

The level of SFA was higher in the lagoon oysters; this trend was influenced by the amount of C16:0 which was the most abundant and also the only SFA to be significantly higher in lagoon oysters (16.55 vs 11.95%). C14:0 showed an inverted trend, being significantly more abundant in sea oysters (6.63 vs 2.28%). C16:0 was found to be the most abundant fatty acids among SFA and also among the all fatty acids of *C. gigas* by Orban et al. (2004) and Piveteau et al. (2000). Pazos et al. (1996) found C16:0 as the most abundant SFA, but among the total fatty acids was the second being the C20:5n-3 the most abundant.

MUFA and PUFA were higher in the oysters finished in the sea, but as for SFA, none of them resulted significantly different. MUFAs did not show a coherent behavior: significant differences were recorded for C18:1n-9, higher in lagoon oysters (5.40 vs 3.93%), and for C18:1n-7 and C20:1n-7 which recorded a higher level in sea oysters (3.96 vs 3.39% and 2.63 vs 2.29%). Other MUFA (C18:1n-5 or 6, C20:1n-11 and C20:1n-9) were more abundant in lagoon oysters, but not in a significant way.

PUFA were the most abundant group of fatty acids both in lagoon and sea oysters, according to the results obtained by many authors (Abad et al., 1995; Pazos et al., 1996; Piveteau et al., 2000; Orban et al., 2004; Saito & Marthy, 2010). Even in this case, every single PUFA presented a different behaviour from the others. C18:3n-6, C20:4n-6 and C20:5n-3 recorded significantly higher levels in the sea finished oysters. C18:2n-6, C18:3n-3 and C18:4n-3 amounts resulted significantly higher in lagoon oysters. C22:6n-3 resulted more abundant in the sea samples, but not in a significant way. PUFA n-6 group resulted significantly more abundant in sea samples (7.52 vs 6.82%) as well as PUFAn-3 group resulted, but not in a significant way.

Saito & Marthy (2010) found that diatoms are the highest EPA source for *C. gigas* and that dinoflagellates are responsible for DHA amount in *C. gigas*. We can explain

the significantly higher amount of EPA and the higher amount of DHA with a possible higher concentration of diatoms and dinoflagellates in the sea than in the lagoon water.

The levels of PUFAn-3 and PUFAn-6 and their ratios (n-3/n-6), are considered important to human health (Piggott & Tucker, 1987; Torstensen et al., 2004; Uauy & Valenzuela, 2000).

To assess the nutritional properties of the fatty acid profile of oysters AI, TI, h/H ratio, PUFAn-3/PUFAn-6 and PUFAn6/PUFAn-3 were determined.

AI and TI, which relate atherogenic to antiatherogenic and ithrombogenic to antithrombogenic fatty acids, respectively, resulted both higher (and then worst) in lagoon samples (0.47 vs 0.40 and 0.07 vs 0.06, respectively) but only the values of TI were significantly different. AI values agree with a previous work on rainbow trout by Turchini et al. (2010) that found a range values between 0.37 and 0.45. The values of these indices, in both the lagoon and sea farmed oysters, are lower than those found in beef or chicken (Ulbricht & Southgate, 1991) and also lower than those found in wild and farmed sharpsnout sea bream by Rueda et al. (2001), thus indicating that oysters can be considered healthy food in terms of the fatty acid composition and the risk of cardiovascular diseases.

PUFAn-3/PUFAn-6 resulted 4.59 vs 4.24 respectively for lagoon and sea farmed oysters and PUFAn6/PUFAn-3 resulted 0.24 for both groups, not presenting any significant differences between the two sample groups. Anyway our results of PUFAn-3/PUFAn-6 ratio values agree with those reported by Orban et al. (2004), who found values ranging from 4.21 and 8.69 during different periods of oyster farming during the year. PUFAn-6/PUFAn-3 ratio presented very low values in both sample groups. This characteristics are indeed vey desirable in food, especially in industrialized countries, where the high PUFAn-6/PUFAn-3 and the low PUFAn-3/PUFAn-6 ratios of the human diet promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of PUFAn-3 (a lower PUFAn6/PUFAn-3 ratio), exert suppressive effects (Simopoulos, 1991).

The hypocholesterolemic and hypercholesterolemic fatty acid ratio (hH) considers the specific effects of fatty acids on cholesterol metabolism. Higher values of this ratio, which are desirable, were found in the sea oysters (2.57 vs 2.02). These values

are higher than those found by Mendes et al. (2010) on seven freshwater fish species, where the maximum value was 1.81.

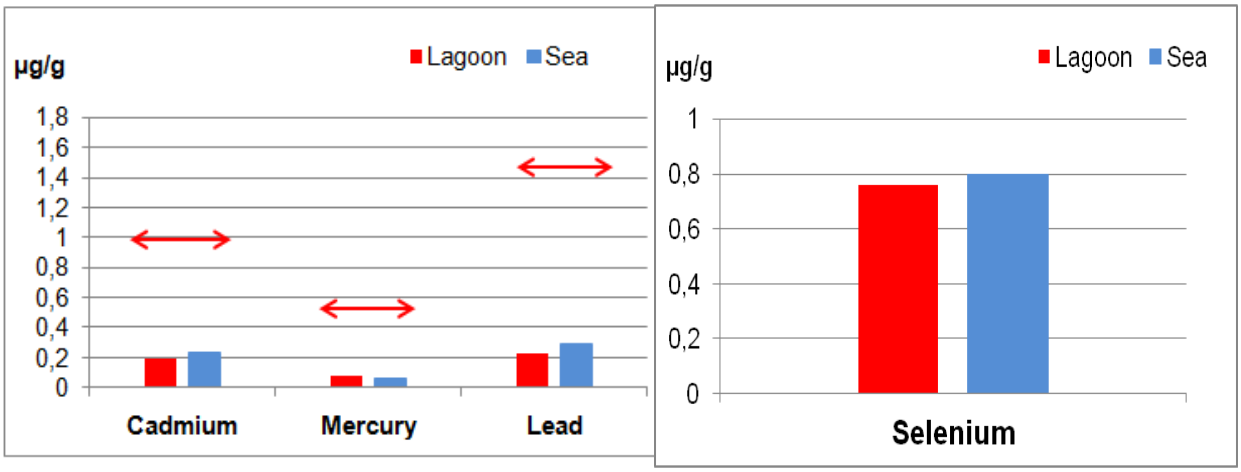
Heavy metals (cadmium, lead and mercury) and selenium content in the edible part of the oysters were also determined. It is commonly recognized that oysters have a great capacity to accumulate metals in seawater from three sources (Galstoff, 1951; Boyden, 1975; Choi et al., 1993; Ettanjani et al., 1993): inorganic metal ions, organometallic ion complexes and metal ion accumulated by phytoplankton. Since oysters are filter-feeders, high levels of metals in the polluted environments are accumulated in their soft tissues and a long time of depuration in clean water is needed in order to obtain a satisfactory reduction of the heavy metals concentration (Chan et al., 1999). If heavy metals cannot be eliminated trough the commonly used depuration systems in a better quality water (Boisson et al., 2003; Ng et al., 2008), is important to measure how much metals can be accumulated in oysters soft parts in a specific farming site in order to evaluate if the final product is suitable or not for human consumption.

In both Orbetello Lagoon and Porto Ercole sites the heavy metals content resulted to be far below the maximum contents allowed by the Commission Regulation (EC) No. 629/2008, that is 1.0, 0.5 and 1.5 mg/kg wet weight, respectively for cadmium (Cd), mercury (Hg) and lead (Pb), as shown in Figure 22.

Unlike Cd, Hg and Pb, selenium (Se) is a desirable mineral in oyster tissues. From the analysis of our samples resulted a Se content of 0.76 e 0.80 μg g^{-1} of fresh edible part weight, respectively for lagoon and sea oysters. At the end of the finishing period oysters presented a mean edible part weight of 7.18 in the lagoon and 4.72 g in the sea, so that the mean content of Se for each oyster was respectively 5.43 and 3.76 μg.

Guérin et al. (2011), considering 20 trace elements in French market seafood including oysters, found a Se content of 0.44 μg/g of fresh edible part weight. This value is much lower than ours (0.76 and 0.80 μg/g), which resulted also higher than some commonly consumed fish species, like sea bass (0.25 μg/g), sea bream (0.21 μg/g) and salmon (0.12 μg/g). Also Orban et al. (2004) found a selenium content lower than ours in oysters farmed in the Lagoon of Venice, which amounted to 0.46 μg/g.

Figure 22. Content of three main metals and selenium in the soft portion of the oysters from Orbetello Lagoon and Porto Ercole sites of the finishing trial. Red arrows stand for the law threshold for each metal. Amounts are expressed as µg/g of the fresh soft portion weight.



3.3.3 Sensory analysis

Finishing the oysters in an environment different from the farming one, has not only the aim to resolve the problems of the hazards and to save money; indeed it is also performed to give to the final product some particular and specific characteristics, such as particular taste and colour, that can be appreciated by the consumers.

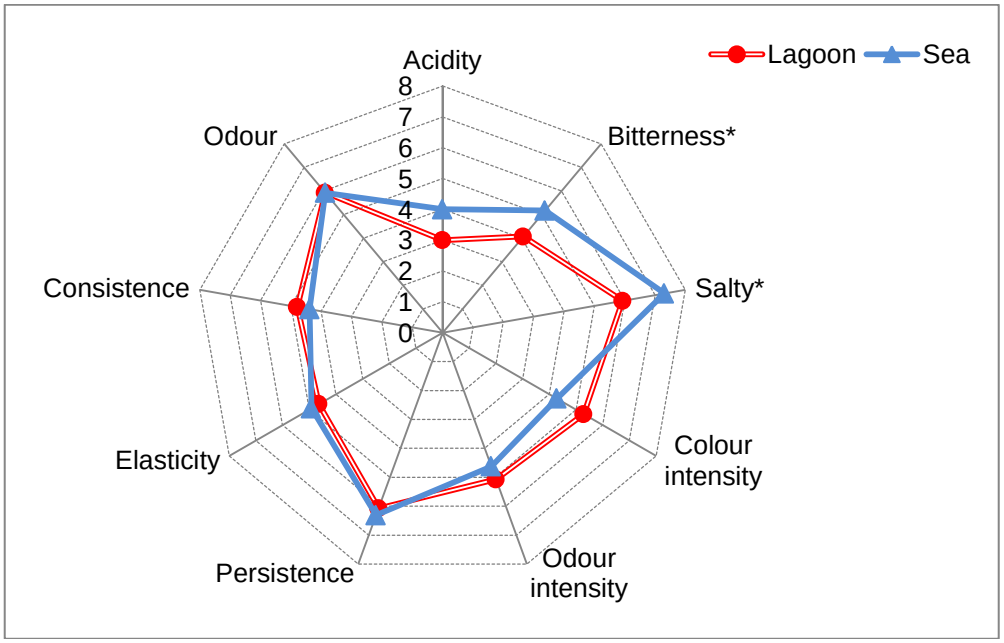
Figure 23 presents the results of the panel test performed on fresh oysters.

Oysters finished in the sea presented a significant higher intensity of two descriptors, salty and bitter. Other descriptors did not show any significant differences between the two groups. Anyway the two groups of oysters presented some important differences in colour intensity, sour and odour intensity, even though not significant from a statistical point of view. The colour of the oysters coming from the lagoon were described as white-creamy, while the sea samples were darker and described as light grey. The global panel opinion resulted significantly better for lagoon oysters with a final score of 6.4 vs 5.0 (Figure 24).

Odour intensity was a little bit stronger in lagoon oysters, and was described as fresh seaweed while sour taste was stronger in the sea samples. Existing literature dealing with fish and shellfish farming has always emphasized how different farming conditions (Brown & Hartwick, 1988; Pennarun et al., 2002; Orban et al., 2004; Dridi

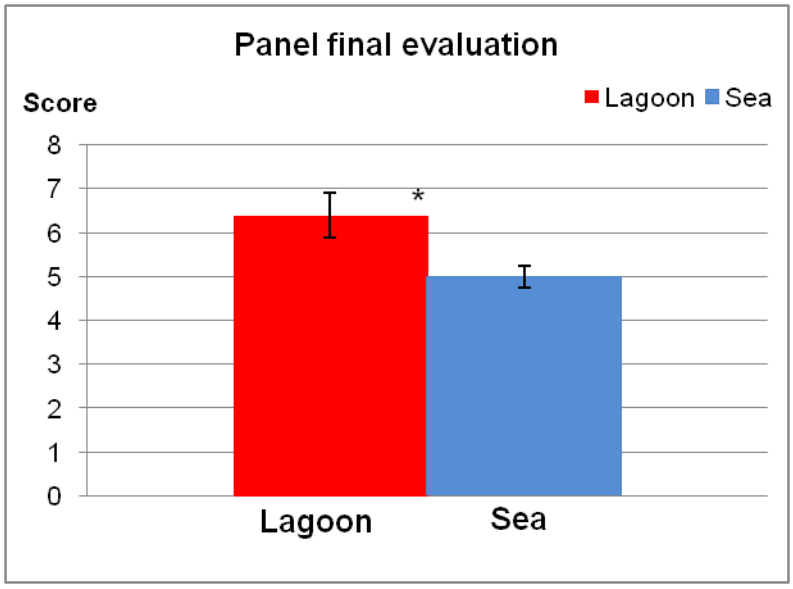
et al., 2007), as well as different storage conditions (Aaraas et al., 2004) and post harvest processing (Cruz-Romero et al., 2004; Cao et al., 2009a), strongly affect biochemical, microbiological and sensory characteristics of the final product. This fact is much more evident in bivalve molluscs because of their filter feeding habits and their highly perishable flesh.

Figure 23. Results of sensory analysis test performed by the panel on the edible part of the oysters finished in the Orbetello Lagoon and in Porto Ercole sites.



Asterisks indicate statistically significant differences (p< 0.05).

Figure 24. Total final evaluation of the panel on the oysters finished in the Orbetello Lagoon and in Porto Ercole sites.



Asterisk indicates statistically significant differences (p< 0.05).

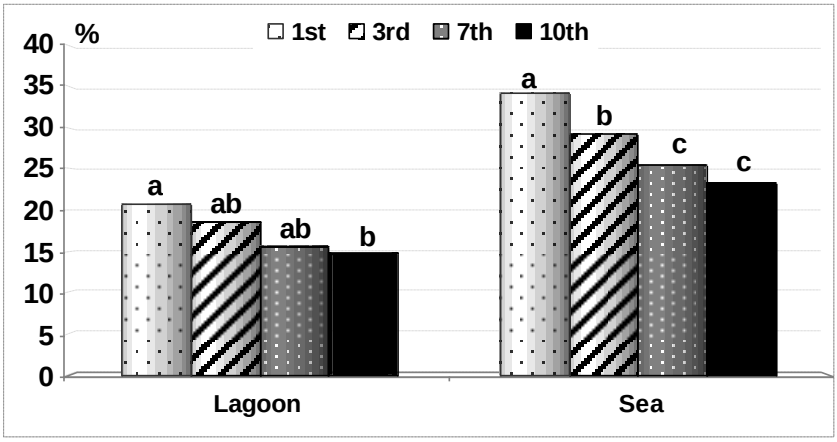
3.3.4 Indicators of changes during the shelf life

Intervalvar liquid content

Oysters lost intervalvar liquor during the storage time. This is a common phenomenon that occurs when oysters have some health problem (Poulet et al., 2003) that, in this case, could be caused by the prolonged air exposure during the refrigerated storage, so that the adductor muscle relaxes allowing the liquor coming out from the valves. Figure 25 shows the loss of intervalvar liquor from the oysters during the refrigerated storage period.

Lagoon oysters had less intervalvar liquor than sea oysters because the volume of the shell was filled with a higher percentage of soft tissues. Both lagoon and sea oysters presented a progressive loss of intervalvar liquor; observing the significant differences between the different days of storage in the two sample groups we can notice that lagoon oysters presented a higher ability to retain intervalvar liquor.

Figure 25. Intervalvar liquid of the oysters finished in the Orbetello Lagoon and Porto Ercole sites, analyzed during the 10 days of refrigerated storage at 4 °C.



Within each site lowercase letters indicate statistically significant differences ($p < 0.05$).

Mantle and gills colour

Colour is an important characteristic from a marketable point of view, because it plays a fundamental role in product perception by the consumer and it is much more true for fish and shellfish products (Harada, 1991). Colour measurement is an important tool, useful to characterize quality, but also to evaluate fish and shellfish freshness (Skrede & Storebakken, 1986).

In Table 29 the results obtained from the instrumental analyses of the colour of the oyster mantle and gills are presented. Analyzing the colour differences between lagoon and sea oysters, regardless the effect of refrigerated storage, the mantle presented significant differences for all the colour parameters, with *L**, *a**, *b** and chroma values higher in the lagoon group and hue values higher in the sea group. This means that the mantle of the oysters finished in the lagoon appeared more bright and that its colour was warmer compared to the mantle of the oysters finished in the sea. Gills were less influenced by the farming site effect, since only *L** values resulted significantly higher for the lagoon group, in agreement with the colour characteristics of the mantle. The results obtained by the instrumental measurement of the colour are quite in agreement with the difference perceived by the panellist in the sensory analysis, previously discussed.

Table 29. Effects of finishing site and day of storage and the relative interaction (site x day) on colour characteristics of the mantle and gills and on pH value of the oysters.

	Site (S)		Days of storage (D)				S x D	RSD
	Orbetello	Porto Ercole	1st	3rd	7th	10th	P=…	
Colour								
Mantle								
L*	52.61^{a}	40.25^{b}	50.04^{a}	39.79^{b}	46.12^{a}	49.76^{a}	ns	9.00
a*	-0.13^{a}	-1.28^{b}	-0.77	-0.65	-0.81	-0.58	ns	1.54
b*	18.89^{a}	15.15^{b}	16.50^{b}	19.33^{a}	18.16^{a}	14.07^{c}	<.0001	3.41
Chroma	18.94^{a}	15.30^{b}	16.63^{b}	19.41^{a}	18.29^{a}	14.17^{c}	<.0001	3.37
Hue	90.49^{b}	95.47^{a}	92.60	92.45	92.64	94.23	ns	6.04
Gills								
L*	45.64^{a}	38.70^{b}	38.37^{c}	47.75^{a}	39.43^{c}	43.13^{b}	<.0001	5.69
a*	-0.42	-0.35	-0.46	-0.76	-0.18	-0.15	ns	1.41
b*	17.92	17.56	17.98	17.97	18.68	16.34	<.0001	4.17
Chroma	18.01	17.65	18.09	18.03	18.77	16.42	<.0001	4.10
Hue	93.04	91.97	93.85	92.73	92.15	91.30	ns	6.49
pH	6.42^{b}	6.56^{a}	6.36^{b}	6.50^{a}	6.54^{a}	6.58^{a}	0.0080	0.22

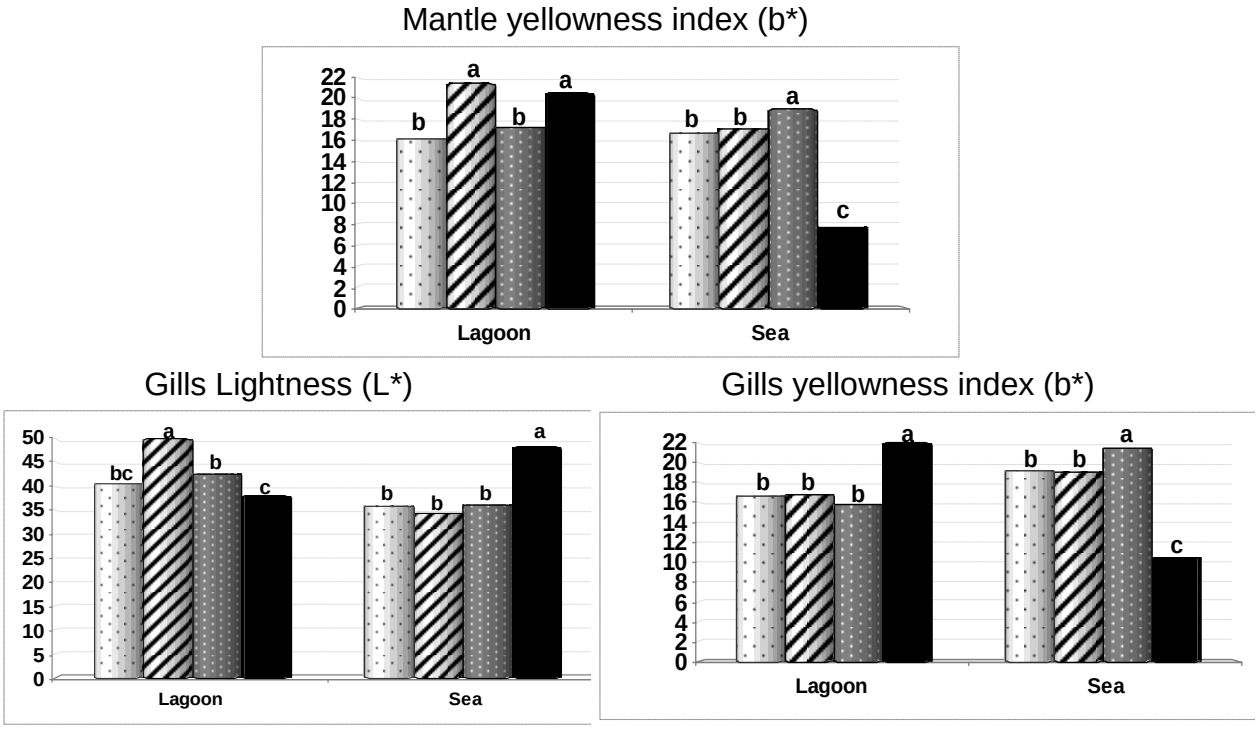
Lowercase letters indicate statistical significant differences (p< 0.05).

Considering the effect of the storage significant differences were found for mantle *L*, b** and chroma values: while *b** and chroma values presented a similar trend during the storage, with higher values during the 3rd and 7th days, *L** presented the higher values during the 1st and 10th days. This means that the mantle tended to

appear more yellow, with a higher colour saturation and with a darker appearance during the 3rd and 7th days of storage.

The gills appearance was less influenced by the storage time; only *L** values presented significant changes, with the higher value and consequently the most bright appearance occurring during the 3rd day of storage.

Figure 26. Changes in colour characteristics of mantle and gills in oysters finished in the Orbetello Lagoon and in Porto Ercole sites during the storage at 4 °C.



Within each site, different letters indicate statistically significant differences ($p < 0.05$).

The interaction site x day resulted significant for yellowness index (b*) and for chroma of both mantle and gills, and for lightness (L*) of the gills only. As shown in Figure 26, where the behaviour of the interaction are displayed, yellowness index presented a coherent trend between mantle and gills during the 10 days of storage in the same sample group and opposite between the groups. In lagoon farmed oysters yellowness index was significantly higher at the end of the storage period, while in the sea samples at the 10th day of storage corresponds to the lower value. Even gill lightness presented an opposite trend between the two sample groups, during the

refrigerated storage, showing the lower value for the lagoon oysters and the higher value for the oysters finished in the sea at the 10th day.

Colour measurements are widely used in literature to evaluate quality changes during processing. It is also used to the same purpose during storage but in a limited way. Average values of *L**, *a** and *b** measured on the mantle of the oysters analyzed in the present research are consistent with the values measured on the control group by Cruz-Romero et al. (2004), investigating on HHP treatment of oysters in order to extend oysters' shelf life.

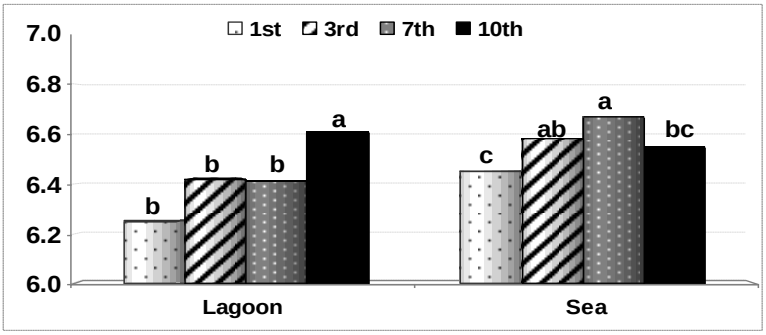
pH

During the refrigerated storage pH levels (Table 29) were influenced in a significant way by the farming site as well as the days of storage. The oysters farmed in the sea site presented a higher mean pH value (6.56 vs 6.42), and during the refrigerated storage at 4°C it tended to slightly increase from an initial value of 6.36 to a value of 6.58, recorded during the 10th storage day.

As the interaction between farming sites and storage days resulted significant, in Figure 27 the different trend registered for pH values in the two oyster groups is shown. In the sea group pH values grew steadily until the 7th day and then decreased significantly by the 10th day. In the lagoon group, the pH level was significantly lower (6.26) at the beginning of the storage period, compared to the sea group (6.45), then grew until values that remained quite constant until the 7th day, and restarted growing until the 10th day (6.61).

The differences between pH values in the two groups were relevant until the 7th day, while the values registered for both at the 10th day were more similar. The pH value trends of this trial are inconsistent with the results of Cao et al. (2009b), that found a slight decrease during 18 days of refrigerated storage at 5 °C. On the contrary, they are consistent with those obtained by Cruz-Romero et al. (2008a), that stored their oyster samples at 2 °C observing a little increase of pH value during the first 15 days of storage, and then a decrease. That inhomogeneous behaviour could be attributed to the different storage conditions (like temperature) applied in the different trials and probably also to the handling techniques practiced, which deeply affect the shelf life of a perishable product like raw oysters.

Figure 27. pH values registered on the mantle of oysters finished in the Orbetello Lagoon and in the Porto Ercole sites analyzed during the 10 days of refrigerated storage at 4 °C.



Within each site lowercase letters indicate statistically significant differences ($p < 0.05$).

pH, a parameter easy to measure, is considered very informative about the health condition in molluscs since it can discriminate the living specimens from the dead ones (Aaraas et al., 2004). The different trends found in this trial for pH of the two oyster groups showed that specimens from lagoon reacted in a better way to air exposure during the chilled storage, consistently with the findings obtained for other parameters previously analyzed (intervalvar liquor percentage, colour). The stability of the pH values during the storage indicates the maintenance of extracellular pH (Aaraas et al., 2004); this is typically performed by many intertidal bivalve molluscs (Newell, 1970) with the aim to maintain the respiratory gas exchange and thus tolerate emersion during low tide (Delaporte et al., 2005). Oysters coming from the sea, by the 7th day of storage probably did not further tolerate the air exposure, while oysters coming from the lagoon showed a good stability of pH during 10 days of air exposure. However, in both groups, pH was kept widely above 6, which is the limit of acceptability for oysters suggested by Banks et al. (1977) throughout the whole refrigerated storage period. The values of pH, during the 10 days of storage, were found to be always indicators of live oysters (Aaraas et al., 2004), both for oysters from the lagoon and from the sea.

Volatile organic compounds profile

The results of VOC profile analysis of the oysters is shown in Table 30. Five groups of chemical compounds were detected, i.e aldehydes, alcohols, ketones, alkanes and furans, with a different number of chemical molecules within each group (n. 12, 6, 6, 2, and 2, respectively).

The profile of volatiles was the same in both groups of oysters, but the amounts of each VOC tended to be significantly more abundant in samples from lagoon, accordingly to the higher odour intensity detected by the panel. The group of alcohols was the most abundant, followed by the aldehydes. 1,5-Octadien-3-ol and 2,6-nonadienal were the most abundant compounds among the alcohols and the aldehydes, respectively. 1,5-Octadien-3-ol together with 3-cyclohexene-1-ethanol were the VOCs showing the highest differences in quantity between the samples from lagoon and from sea. The first volatile is a product of PUFAn-3 oxidation and was proposed as an intermediate of the EPA degradation via 12-lipoxygenase activity (Durnford & Sahidi, 1998). It has been reported to be an active contributor of flavour with a fresh mushroom and moss odour (Kaway, 1996), characterized by a low odour threshold (10 ppb).

In the two groups of oysters, the volatile amount increased during the refrigerated storage, according to a similar trend in both groups for the most of volatiles, that showed similar values during the first three days and then, achieving a maximum value by the seventh day. The most abundant volatiles was 1,5-octadien-3-ol, that tended to increase significantly (+70%) during the storage period.

Significant interactions site x day of storage resulted only for (e,z)-2,6-nonadienal, 1-penten-3-ol, (e)-2-penten-1-ol and 2-thiopropane, whose concentration trends are showed in Figure 28. (e,z)-2,6-Nonadienal, an aldehyde characterized by a fatty odour, with fresh green cucumber and melon notes, duplicated its initial value during the storage. It is described to come from the action of 9-lipoxygenase on eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids (German et al., 1991; Hu & Pan, 2000).

1-Penten-3-ol, previously described as product of the action of lipoxygenases on PUFAn-3 (German et al., 1991; Hu & Pan, 2000), did not present any significant trend along the storage period in lagoon oysters, while it tended to increase in sea oysters, similarly to (e)-2-penten-1-ol. An opposite behaviour was registered for 2-thiopropane; more abundant in lagoon oysters, it increased in a significant way only in lagoon samples. 3-Cyclohexene-1-ethanol was previously described in leaves of some plants and associated with odour attributes of fresh and mint (Tanaka et al., 2010). This alcohol characterizes the aromatic profile of *Ostrea edulis* (Table 15), but also in *Crassostrea gigas* it was found to be the second most abundant compound of the

VOCs profile. During the ten days of refrigerated storage, the 3-cyclohexene-1-ethanol experimented a high and significant increase (62%).

Comparing the VOCs profile changes during refrigerated storage in *O. edulis* farmed in the Galician coast (Table 16) and in *Crassostrea gigas* farmed in the Tyrrhenian coast (Table 30) is interesting to notice that the general trend had opposite behaviour: while the VOCs of the *Crassostrea gigas* tended to increase their concentration during storage, concentration of VOCs of the *O. edulis* tended to decrease.

No information has been found about VOCs behaviour of fresh oysters during refrigerated storage, but is possible to hypothesize that different origin, postharvest handling methods and the difference of species determined the different behaviour of VOCs. Different farming environments and postharvest handling surely determined different microbial populations producing flavour and off-flavour compounds in different ways from oyster proteins and lipids in the two species. Moreover, the different composition of available food and the genetic differences surely affected the chemical composition of the two species, having different enzymatic supply, so that producing VOCs by different enzymatic pathways during storage.

Wierda et al. (2006) studied the VOCs profile of stored salmon, finding that volatile alcohols and aldehydes decreased during storage, while Prost et al. (2004) studying the effect of storage on sardines aroma quality, found that the odour active compounds responsible for oxidized flavours of sardines during storage increased their concentration.

According to the differences in the quantities of single VOCs, the volatile profile was able to discriminate the origin of oysters, confirming the findings of literature (Cardinal et al., 2000; Ratel et al., 2008). It was also useful to follow the freshness evolution during the storage (Doré, 1991; Zhang et al., 2009).

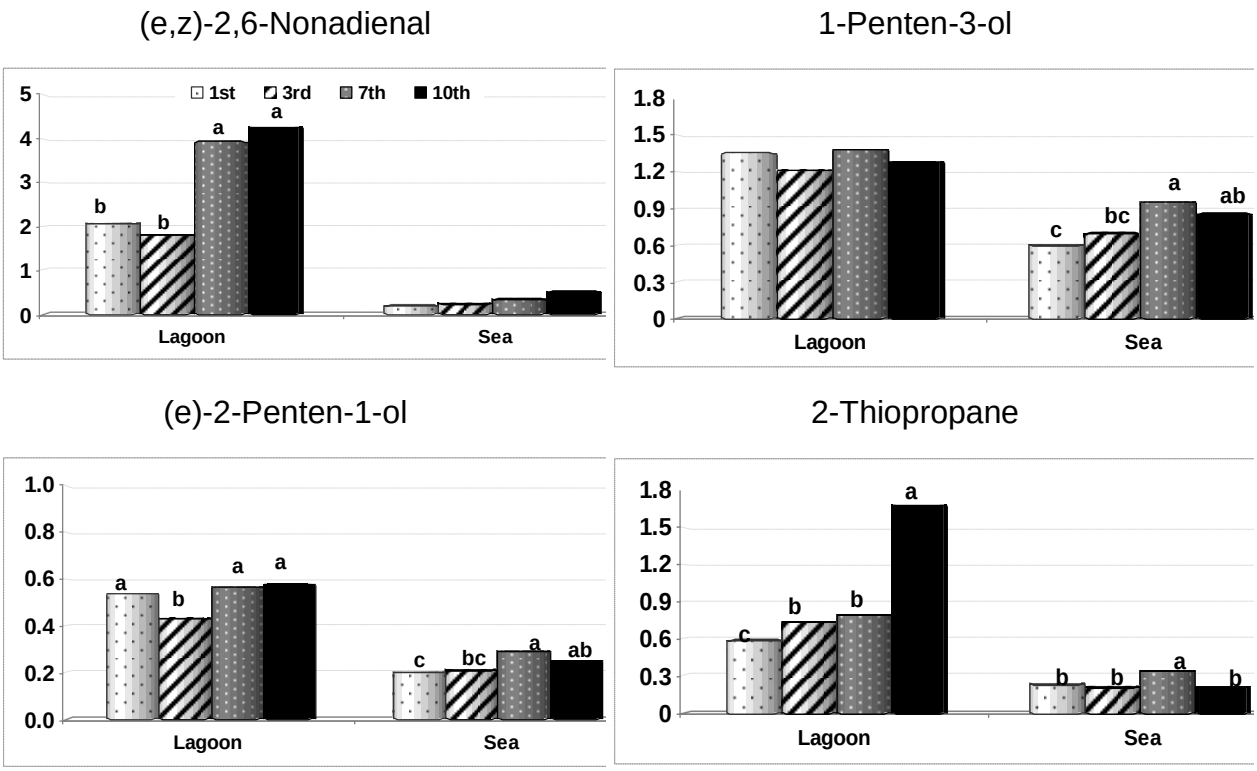
It is noteworthy that the sensorial analysis performed in this trial was able to significantly distinguish the two oyster groups on the basis of descriptors related to taste, that resulted more effective in comparison to the descriptors related to the other senses. The panellists assigned higher scores to the lagoon oysters for odour intensity, in agreement with their volatile profile analysis, where the lagoon oysters have a significant higher concentration of volatile compounds even though the volatile profile of the two groups of oysters was very similar.

Table 30. Effects of finishing site and day of storage and the relative interaction (site x day) on volatile organic compounds (VOCs) profile of the edible part of the oysters. Values are expressed as peak area/I.S. area.

VOCs	Site (S)		Days of storage (D)				S *x* D	RSD
	Orbetello	Porto Ercole	1st	3rd	7th	10th	P=…	
Aldehydes	*15.98^{a}*	*4.63^{b}*	*7.91^{b}*	*7.09^{b}*	*13.22^{a}*	*13.00^{ab}*	*ns*	*4.27*
Pentanal	0.63^{a}	0.31^{b}	0.45^{ab}	0.35^{b}	0.60^{a}	0.49^{ab}	ns	0.13
(e)-2-Pentenal	1.84^{a}	0.81^{b}	1.08^{b}	0.97^{b}	1.67^{a}	1.56^{a}	ns	0.34
Hexanal	1.54^{a}	0.47^{b}	0.87	0.81	1.16	1.18	ns	0.41
(e)-2-Hexenal	1.37^{a}	0.23^{b}	0.60^{b}	0.61^{b}	0.99^{a}	1.00^{a}	ns	0.25
Heptanal	1.36^{a}	0.42^{b}	0.71	0.65	1.10	1.10	ns	0.42
(z)-4-Heptenal	0.78^{a}	0.22^{b}	0.35^{b}	0.28^{b}	0.66^{a}	0.70^{a}	ns	0.20
(e,e)-2,4-Heptadienal	0.40^{a}	0.07^{b}	0.19	0.15	0.34	0.26	ns	0.18
(e)-2-Octenal	0.39^{a}	0.13^{b}	0.18	0.17	0.34	0.35	ns	0.14
(e,z)-2,6-Nonadienal	3.02^{a}	0.31^{b}	1.13^{b}	1.02^{b}	2.13^{a}	2.39^{a}	0.015	0.64
Benzaldehyde	1.32^{a}	0.59^{b}	0.71	0.63	1.23	1.24	ns	0.47
Lylacaldehyde	1.05^{a}	0.78^{b}	0.63^{b}	0.66^{b}	1.09^{a}	1.29^{a}	ns	0.21
Alcohols	*24.67^{a}*	*9.79^{b}*	*13.51^{b}*	*13.20^{b}*	*20.38^{a}*	*21.84^{a}*	*ns*	*3.11*
1-Penten-3-ol	1.31^{a}	0.78^{b}	0.98^{b}	0.96^{b}	1.17^{a}	1.08^{ab}	0.039	0.10
1-Pentanol	0.32^{b}	0.42^{a}	0.42	0.32	0.44^{a}	0.32^{a}	ns	0.08
(e)-2-Penten-1-ol	0.53^{a}	0.24^{b}	0.37^{b}	0.32^{c}	0.43^{a}	0.41^{a}	0.010	0.03
1,5-Octadien-3-ol	17.42^{a}	7.33^{b}	9.34^{b}	9.30^{b}	14.79^{a}	16.08^{a}	ns	2.27
Z-2-Octen-1-ol	0.26^{a}	0.11^{b}	0.13^{b}	0.13^{b}	0.22^{a}	0.26^{a}	ns	0.06
3-Cyclohexene-1-ethanol	4.82^{a}	0.91^{b}	2.27^{b}	2.17^{b}	3.33^{a}	3.68^{a}	ns	0.72
Ketones	*2.79^{a}*	*1.37^{b}*	*1.70^{c}*	*1.82^{bc}*	*2.38^{ab}*	*2.44^{a}*	*ns*	*0.50*
1-Penten-3-one	0.47^{a}	0.36^{b}	0.37	0.37	0.48	0.43	ns	0.08
2,3-Pentanedione	0.56^{a}	0.28^{b}	0.36	0.40	0.44	0.46	ns	0.06
1-Octen-3-one	0.10^{a}	0.08^{b}	0.06^{b}	0.06^{b}	0.11^{a}	0.12^{a}	ns	0.02
2,3-Octanedione	0.79^{a}	0.44^{b}	0.50	0.55	0.69	0.73	ns	0.18
2-Nonanone	0.48^{a}	0.13^{b}	0.22	0.23	0.37	0.39	ns	0.12
E,E-3,5-Octadien-2-one	0.40^{a}	0.10^{b}	0.19	0.21	0.29	0.31	ns	0.08
Alkanes	*1.43*	*1.34*	*1.31^{b}*	*1.36^{b}*	*1.09^{b}*	*1.79^{a}*	*<.0001*	*0.24*
2-Thiopropane	0.95^{a}	0.25^{b}	0.41^{d}	0.47^{c}	0.56^{b}	0.94^{a}	<.0001	0.05
Hexane	0.49^{b}	1.09^{a}	0.90	0.89	0.52	0.85	ns	0.26
Furans	1.17^{a}	0.47^{b}	0.69	0.74	0.92	0.93	ns	0.26
2-Ethylfuran	1.07^{a}	0.42^{b}	0.63	0.68	0.83	0.84	ns	0.25
2-Methylfuran	0.11^{a}	0.05^{b}	0.07	0.06	0.09	0.09	ns	0.02

Within each criterion, lowercase superscript letters indicate statistically significant differences ($p < 0.05$); ns: not significant.

Figure 28. Concentration of the volatile compounds in edible part of oysters finished in the Orbetello Lagoon and in the Porto Ercole sites analyzed during the 10 days of refrigerated storage at 4 °C. The values are expressed as Peak Area/IS Area.



Within each site, different letters indicate statistically significant differences ($p < 0.05$).

Microbiological analyses

The results of microbiological analyses performed on the oysters during the 10 days of 4 °C refrigerated storage are shown in Table 31. All the considered bacteria groups (total aerobic bacteria load, *Pseudomonas* spp. and Micrococcaceae) resulted to be influenced in a significant way by the effect of both the farming site and the storage time. The interaction between these factors also resulted significant. Total aerobic bacteria load and *Pseudomonas* spp. resulted more abundant in the lagoon oysters (5.85 vs 5.68 Log CFU g^{-1} and 3.97 vs 3.37 Log CFU g^{-1}, respectively), while Micrococcaceae load were more abundant in sea oysters (5.03 vs 4.70 Log CFU g^{-1}). All the three bacteria groups tended to increase in a significant way their population during the refrigerated storage.

Figure 29 shows the population trend of each group during 10 days of 4 °C refrigerated storage. Total aerobic bacteria load trend presented significant

differences between the two sampling groups during the whole storage time. The initial total aerobic bacteria load of oysters was found to be 5.20 and 4.16 log CFU g^{-1}, respectively in lagoon and sea groups. These values are consistent with the values reported in a previous work by Cao et al. (2009b), that considers an initial microbial count ranging between 2 and 5 log CFU g^{-1} as normal.

Table 31. Effects of finishing site and day of storage and the relative interaction (site x day) on bacterial load of the edible part of the oysters. Values are expressed as Log CFU g^{-1}.

Bacteria load	Farming site		Days of storage			SxD	RSD
	Orbetello	Porto Ercole	1st	7th	10th	P=...	
Total aerobic bacteria load	5.85^{a}	5.68^{b}	4.68^{c}	5.94^{b}	6.68^{a}	<.0001	0.18
***Pseudomonas* spp.**	3.97^{a}	3.37^{b}	2.35^{c}	4.12^{b}	4.54^{a}	<.0001	0.27
Micrococcaceae	4.70^{b}	5.03^{a}	3.56^{c}	5.06^{b}	5.98^{a}	<.0001	0.27

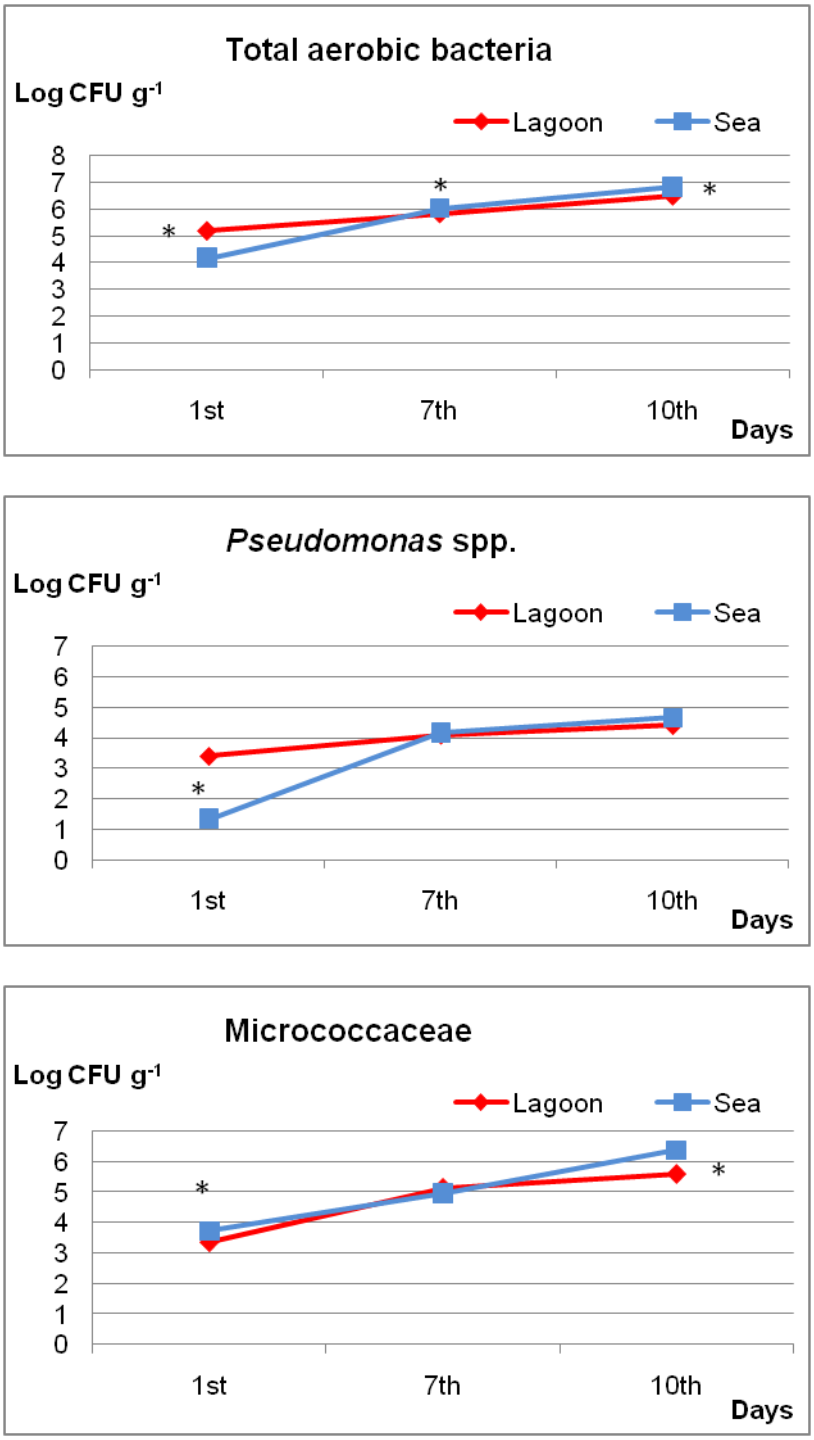
Lowercase superscript letters indicate statistically significant differences ($p< 0.05$).

Even if during the first day the lagoon oysters had a higher total aerobic bacteria load, we observed that during the storage period a faster increase of the bacteria load in the sea group occurred, so that at the 7th and 10th day of storage we observed a significant higher bacteria load in the sea samples. At the end of the storage period oysters presented total aerobic bacteria count values of 6.51 and 6.84 log CFU g^{-1}, respectively for the lagoon and the sea finished oysters. This different behaviour of total bacteria population in the two sample groups can be explained with the different farming environments: oysters cultivated in the lagoon had a higher food supply, physical stressors, like water currents, were absent and change of salinity is generally well tolerated by *Crassostrea gigas* (Brown & Hartwick, 1988). This hypothesis is consistent with the values of condition indexes confirming the healthier condition of the oysters farmed in the lagoon.

Then, even though the initial total aerobic bacteria load of sea samples were significantly lower, the final total aerobic bacteria load resulted significantly higher compared to the lagoon samples, because of their lower ability to face the air exposure during storage and to restrain bacteria activity. Both Orbetello Lagoon and Porto Ercole oysters reached an acceptable quality level, according to the indication of Kim et al. (2002), that suggested a total aerobic bacteria load of 7 log CFU g^{-1} as

an acceptable quality limit for oysters.

Figure 29. Bacterial load changes during 10 days of chilled storage at 4 °C on oysters finished in the Orbetello Lagoon and in Porto Ercole site.



Within each site, asterisks indicate statistically significant differences ($p < 0.05$).

Pseudomonas spp. load showed a coherent trend to total aerobic bacteria and this is consistent with the results obtained by various authors that found *Pseudomonas* spp. to be the main responsible, together with *Shewanella*, of the spoilage of shellfish (Cao et al., 2009a) and fish products (Gram & Huss, 1996; Gennari et al., 1999). The differences between the two sampling groups were significant only during the first day of storage, with a significant higher concentration of *Pseudomonas* spp. in Orbetello Lagoon oysters. In Porto Ercole samples *Pseudomonas* spp. concentration grew over three times from the 1st to the 7th day of storage, from 1.31 to 4.15 log CFU g^{-1}, while in lagoon sample it grew in a milder way (from 3.40 to 4.42 log CFU g^{-1}).

Micrococcaceae load estimated in the two sampling groups showed significant differences during the 1st and 10th day of storage, and the higher concentration was recorded in both cases in the oysters coming from the sea.

3.3.5 Conclusions

A farming system based on the synergistic integration of two different environments, with a finishing period in the open sea just before commercialization, could allow the exploitation of the high growing performances occurring in the lagoon, and could also be a valid solution to the problems that are common to occur in lagoon environment. The most common problems for a fish or shellfish farm in a lagoon environment are the high temperature of the water, occurring mostly during summer, that can lead to animal death (Royer et al., 2007; Chávez-Villalba et al., 2010; Soletchnik et al., 2005; Samain et al., 2005) as well as algal blooms at times responsible for biotoxin production, and that can create hypoxic conditions and harmful compounds originating from the organic matter accumulated on the bottom.

In this study the effect of three months finishing period in a sea site on oysters previously grown in lagoon was analyzed by the growing performances, whereas quality characteristics and shelf-life were considered in oysters finished for a longer period (150 days). The finishing period in sea site produced oysters with worse commercial characteristics, as well as edible part yield and shape index, so that the final product would have a lower value on the market. The comparison between the two kinds of containers (lanterns and baskets) experimented for the finishing in the sea site resulted to be more positive for the lanterns, that gave higher shape index and condition index.

Considering the indexes related to fatty acid profile assessing the nutritional properties of the final product, sea oysters presented TI and h/H values more positive for the human health. Also EPA and DHA percentage on total fatty acids were higher in sea oysters. This means that the lagoon environment, compared to the sea, was more suitable for oysters growth and offered a more abundant food supply, but induced a slight worsening of the nutritional quality of the edible part.

During 10 days lagoon oysters reacted in a better way to the refrigerated storage out of water, presenting more stable pH value trend, a higher ability to retain intervalvar liquor, and a higher ability to restrain bacteria proliferation. This is probably due to the better nutritional state of the lagoon oysters, thanks to the more favourable trophic conditions of the environment where their finishing was realised. In addition, a better ability of the lagoon oysters to face on hypoxia during the out of water refrigerated storage, due to the higher adaptation of the molluscs to a wide range of fluctuations of environmental parameters, could be involved in the better preservation observed.

The scores attributed by the panellists to sensory descriptors, even if in many cases not significantly different, are generally more positive for lagoon oysters.

VOCs profile analysis displayed different quantities of volatiles but a similar qualities of volatiles in the oysters finished in the two sites. Surely further studies are needed to define the relationship with sensory descriptors and microorganisms responsible for spoilage.

The finishing period in the sea produced a decline of all the market and sensory characteristics and also of the shelf life performances during the storage period. Only the nutritional value of the fatty acid profile improved. This results show that in order to perform an oysters' finishing period further research should be conducted to try other sea farming sites, more suitable for oysters finishing, in order to improve the quality characteristics of the oysters farmed in the lagoon and obtain a high quality product.

4. REFERENCES

AOAC (2000). Official methods of analysis (17th ed.) Gaithersburg, MD: Association of Official Analytical Chemists.

Aaraas, R., Hernar, J.I., Vorre, A., Lunestad, B.T., Skeie, S., Slinde, E., & Mortensen, S. (2004). Sensory histological and bacteriological changes in flat oysters, *Ostrea edulis*, L ., during different storage conditions. *Journal of Food Science* 69(6), 205-210.

Abad, M., Ruiz, C., Martinez, D., Mosquera, G., & Sanchez, L. (1995). Seasonal variations of lipid classes and fatty acids in flat oyster, *Ostrea edulis* , from San Cibran (Galicia, Spain). *Comparative Biochemistry and Physiology* 110C(2), 109-118.

Acree, H., & Arn, T. (2008). Cornell University, USA, Flavornet and human odor space, http://www.flavornet.org, accessed October 24, 2008.

Arthur, L.C., & Pawlszyn, J. (1990). Solid phase microextraction with thermal desorption using fused silica optical fibers. *Analytical Chemistry* 62(19), 2145-2148.

Ashie, I.N.A., Smith, J.P., & Simpson, B.K. (1996). Spoilage and shelf- life extension of fresh fish and shellfish. *Critical Reviews in Food Science and Nutrition* 36, 87–121.

Askew, C.G. (1972). The growth of oysters *Ostrea edulis* and *Crassostrea gigas* in Emsworth Harbour*. Aquaculture* 1, 237-259.

Banks, A., Dawson, J.A., Feiger, E.A., Novak, A.F., Poters, J.A., Stavin, J.W., & Waterman, J.J. (1977). Freezing of shellfish. In: Fundamentals of Food Freezing (N.N. Desrosier, D.K. Tressler, eds). Avi, Westpon, Connecticut. 318–356

Batista, F.M., Ben-Hamadou, R., Fonseca, V.G., Taris, N., Ruano, F., Reis-Henriques, M. A., & Boudry, P. (2008). Comparative study of shell shape and muscle scar pigmentation in the closely related cupped oysters *Crassostrea angulata*, *C. gigas* and their reciprocal hybrids. *Aquatic Living Resources* 21, 31-38.

Boisson, F., Goudard, F., Durand, J.P., Barbot, C., Pieri, J., Amiard, J.C., Fowler, S.W. (2003). Comparative radiotracer study of cadmium uptake, storage, detoxification and depuration in the oyster *Crassostrea gigas*: potential adaptive mechanisms. *Marine Ecology Progress Series* 254, 177–186.

Booth, J.D. (1983). Studies on twelve common bivalve larvae and bivalve spawning seasons. *New Zealand Journal of Marine Fresh-Water Research* 17, 231-265.

Boyden, C.R. (1975). Distribution of some trace metals in Poole Harbour, Dorset. *Marine Pollution Bulletin* 6(12), 180-187.

Brake, J., Evans, F., & Langdon, C. (2003). Is beauty in the eye of the beholder? Development of a simple method to describe desirable shell shape for the Pacific oyster industry. *Journal of Shellfish Research* 22(3), 767–771.

Briones-Labarca, V., Perez-Won, M., Zamarca, M., Aguilera-Radic, J.M., & Tabilo-Munizaga, G. (2012). Effects of high hydrostatic pressure on microstructure, texture, colour and biochemical changes of red abalone (*Haliotis rufecens*) during cold storage time. *Innovative Food Science and Emerging Technologies* 13, 42-50.

Brown, J.R., & Hartwick, E.B. (1988). Influences of temperature, salinity and available food upon suspended culture of the Pacific oyster, *Crassostrea gigas*. 2. Condition index and survival. *Aquaculture* 70, 253–267.

Buzin, F., Baudon, V., Cardinal, M., & Barille, L. (2011). Cold storage of Pacific oysters out of water : biometry, intervalval water and sensory assessment. *International Journal of Food Science and Technology* 46, 1775-1782.

Cao, R., Xue, C.-Hu, Liu, Q., & Xue, Y. (2009a). Microbiological, chemical, and sensory assessment of Pacific oysters (*Crassostrea gigas*) stored at different temperatures. *Czech Journal of Food Science* 27(2), 102-108.

Cao, R., Xue, C.-Hu, & Liu, Q. (2009b). Changes in microbial flora of Pacific oysters (*Crassostrea gigas*) during refrigerated storage and its shelf-life extension by chitosan. *International Journal of Food Microbiology* 131(2-3), 272-276.

Cardinal, M., Viallon, C., Thonat, C., & Berdagué, J.-L. (2000). Pyrolysis-mass spectrometry for rapid classification of oysters according to rearing area. *Analusis* 28, 825-829.

Cha, Y.-J., Baek, H.H., & Hsieh, T.C.-Y. (1992). Volatile components in flavour concentrates from crayfish processing waste. *Journal of the Science of Food and Agriculture* 58(2), 239-248.

Chan, K.W., Cheung, R.Y. H., Leung, S.F., & Wong, M.H. (1999). Depuration of metals from soft tissues of oysters (*Crassostrea gigas*) transplanted from a contaminated site to clean sites. *Environmental Pollution* 105, 299-310.

Chávez-Villalba, J., Arreola-Lizárraga, A., Burrola-Sánchez, S., & Hoyos-Chairez, F. (2010). Growth, condition, and survival of the Pacific oyster *Crassostrea gigas* cultivated within and outside a subtropical lagoon. *Aquaculture* 300(1-4), 128-136.

Cho, I.H., Kim, S.Y., Choi, H.-K., & Kim, Y.-S. (2006). Characterization of aroma-active compounds in raw and cooked pine-mushrooms (*Tricholoma matsutake* Sing.). *Journal of Agriculture and Food Chemistry* 54, 6332-6335.

Choi, H.G., Park, J.S., & Lee, P.Y. (1993). Study on the heavy metal concentration in mussels and oysters from the Korean coastal waters. *Bulletin of the Korean Fisheries Society* 25(6), 485-494.

Chong, C.Y., Abu Bakar, F., Russly, A.R., Jamilah, B., & Mahyudin, N.A. (2011). The effects of food processing on biogenic amines formation. *International Food Research Journal* 18(3), 867-876.

CIE (Commission Internationale de L'Eclairage) (1976). Colorimetry. Pub. N° 15, Bureau Central de la CIE, Vienna, Austria.

Colby, J.-W., Enriquez-Ibara, L.G., & Flick, G.J. (1995). Shelf life of fish and shellfish. In: Shelf life studies of foods and beverages, chemical, biological, physical and nutritional aspects (G. Charalambous, Ed.). New York, NY: Elsevier. Pp. 85–143.

Colwell, R.R., & Liston, J. (1960). Microbiology of shellfish. Bacteriological study of the natural flora of Pacific oysters (*Crassostrea gigas*). *Applied and Environmental Microbiology* 8(2), 104-109.

Cook, D.W. (1991). Microbiology of bivalve molluscan shellfish. In Microbiology of marine food products (D.R. Ward, C. Hackney, Ed.). New York: Van Norstrand Rheinhold. 301–330.

Cruz-Romero, M., Smiddy, M., Hill, C., Kerry, J.P., & Kelly, A.L. (2004). Effects of high pressure treatment on physicochemical characteristics of fresh oysters (*Crassostrea gigas*). *Innovative Food Science and Emerging Technologies* 5, 161−169.

Cruz-Romero, M.C, Kerry, J.P., & Kelly, A.L. (2008a). Changes in the microbiological and physicochemical quality of high-pressure-treated oysters (*Crassostrea gigas*) during chilled storage. *Food Control* 19, 1139-1147.

Cruz-Romero, M.C., Kerry, J.P., & Kelly, A.L. (2008b). Fatty acids , volatile compounds and colour changes in high-pressure-treated oysters (*Crassostrea gigas*). *Innovative Food Science and Emerging Technologies* 9, 54-61.

Cruz-Romero, M., Kelly, A.L., & Kerry, J.P. (2007). Effects of high-pressure and heat treatments on physical and biochemical characteristics of oysters *(Crassostrea gigas*). *Innovative Food Science and Emerging Technologies* 8, 30-38.

Dalgaard, P., Gram, L., & Huss, H.H. (1993). Spoilage and shelf-life of cod fillets packed in vacuum or modified atmospheres. *International Journal of Food Microbiology* 19, 283–294.

De Moreno, J.E.A., Moreno, V.J., & Brenner, R.R. (1976). Lipid metabolism of the yellow clam, *Mesodesma mactroides*: 2. Polyunsaturated fatty acid metabolism. *Lipids* 11, 561–566.

Delaporte, M., Soudant, P., Moal, J., Kraffe, E., Marty, Y., & Samain, J.-F. (2005). Incorporation and modification of dietary fatty acids in gill polar lipids by two bivalve species *Crassostrea gigas* and *Ruditapes philippinarum*. *Comparative Biochemistry and Physiology Part A* 140, 460-470.

Doré, I. (1991). A guide to oysters, mussels, scallops, clams and similar products for the commercial user. New York: Van Nostrand Reinhold. Pp. 69–109.

Dridi, S., Salah, M., & Elcafsi, M. (2007). Seasonal variation in weight and biochemical composition of the Pacific oyster , *Crassostrea gigas* in relation to the gametogenic cycle and environmental conditions of the Bizert Lagoon, Tunisia. *Aquaculture* 263, 238-248.

Ducrotoy, J.P., Elliott, M., & De Jonge, V.N. (2000). The North Sea. *Marine Pollution Bullettin* 41(1–6), 5–23.

Duflos, G., Moine, F., Coin, V.M., & Malle, P. (2005). Determination of volatile compounds in whiting (*Merlangius merlangus*) using head-space solid-phase microextraction-gas chromatography-mass spectrometry. *Journal of Chromatographic Science* 43, 304-312.

Dunford, E., & Shahidi, F. (1998). Flavours of fish meat. In: Flavor of meat, meat products, and seafoods (F. Shahidi, Editor). New York: Blackie Academic & Professional. Pp.131–158.

Ettanjani, H., Amiard, T.C., & Amiard, J.C. (1993). Experimental study of the transfer of two trace elements (silver, copper) in a marine trophic chain: water to particles (natural sediment, microalgae) to filter-feeding molluscs (*Crassostrea gigas* Thunberg). *Water Air and Soil Pollution* 65(3-4), 215-236.

Felhusen, F. (2000). Review: The role of seafood in bacterial food borne diseases. *Microbes and Infection* 2, 1651–1660.

Folch, J., Lees, M., & Sloane Stanley, H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *The Journal of Biological Chemistry* 226, 497-509.

Frankel, E.N. (1998). Lipid oxidation. Dundee, UK: The Oily Press Ltd.

Fuentes, A., Fernandez-Segovia, I., Escriche, I., & Serra, A.J. (2009). Comparison of physico-chemical parameters and composition of mussels (*Mytilus galloprovincialis* Lmk.) from different Spanish origins. *Food Chemistry* 112, 295-302.

Galtsoff, P.S. (1964). The American oyster *Crassostrea virginica* Gmelin. *Fisheries Bulletin (United States)* 64, 1-480.

Galstoff, P.S. (1951). The oyster industry of the world. In: Marine Products of Commerce (D.K. Tressler, J.W. McLemon, Eds.). Reinhold, New York, pp. 550-575.

Gennari, M., Tomaselli, S., & Cotrona, V. (1999). The microflora of fresh and spoiled sardines (*Sardina pilchardus*) caught in Adriatic (Mediterranean) Sea and stored in ice. *Journal of Food Microbiology* 16, 15–28

German, J.B., Zhang, H., & Berger R. (1991). Role of lipoxygenases in lipid oxidation in foods. In Lipid oxidation in food (A.J. St.Angelo, J. Vercellotti, T. Jacks & M. Legendre. Ed.). ACS Symposium Series, Vol. 500, Chapter 5. Pp. 74–92.

Giese, A.C., Hart, M.A., Smith, A.M., & Cheung, M. A. (1967). Seasonal changes in body component indices and chemical composition in the Pismo clam *Tivela stultorum*. *Comparative Biochemistry and Physiology* 22, 549-561.

Giese, A.C. (1966). Lipids in the economy of marine invertebrates. *Physiological Review* 46, 244-298.

Girova, T.D., & Gochev, V.K. (2009). Detection and enumeration of spoilage and pathogenic microorganisms in refrigerator stored meat products. *Biotechnol. & Biotechnol. Eq.* 23, 896-899.

Gonzalez-Rodriguez, M.N., Sanz, J.J., Santos, J.A., Otero, A., & Garcia-Lopez, M.L. (2002). Numbers and types of microorganisms in vacuum-packed cold-smoked freshwater fish at the retail level. *International Journal of Food Microbiology* 77, 161-168.

Gosling, E. (2003). Bivalve molluscs. Biology, ecology and culture. Fishing News Books.

Gram, L., & Huss, H.H. (1996). Microbiological spoilage of fish and fish products. *International Journal of Food Microbiology* 33, 121–137.

Gram, L., & Huss, H.H. (2000). Fresh and processed fish and shellfish. In: The microbiological safety and quality of food (B.M. Lund, T.C. Baird-Parker, & G.W. Gould, Eds.).1, 472–506.

Guérin, T., Chekri, R., Vastel, C., Sirot, V., Volatier, J.-L., Leblanc, J.-C., & Noël, L. (2011). Determination of 20 trace elements in fish and other seafood from the French market. *Food Chemistry* 127, 934–942.

Guillen, M.D., Errecalde, M.C., Salmeron, J., & Casas, C. (2006). Headspace volatile components of smoked swordfish (*Xiphias gladius*) and cod (*Gadus morhua*) detected by means of solid phase microextraction and gas chromatography–mass spectrometry. *Food Chemistry* 94, 151-156.

Harada, K. (1991). Fish colour. *Australian Fisheries* 50,18−19.

Heat, H.B., & Reineccius, G. (1986). Flavor Chemistry and Technology, Basingstoke: Macmillan (Chapter 4).

Horseley, R.W. (1973). The bacterial flora of the Atlantic salmon (*Salmo salar,* L.) in relation to its environment. *Journal of Applied Bacteriology* 36, 377-386.

Hsieh, R.J., German, J.B., & Kinsella, J.E. (1988). Lipoxygenase in fish tissue:some properties of 12-lipoxygenase from trout gill. *Journal of Agricultural Food Chemistry* 36, 680-685.

Hoz, L., Lopez-Galvez, D.E., Fernandez, M., Hierro, E., & Ordonez, J.A. (2000) Use of carbon dioxide enriched atmospheres in the refrigerated storage (2°C) of salmon (*Salmo salar*) steaks. *European Food Research and Technology* 210, 179–188.

Hu, S.P., & Pan, B.S. (2000). Modification of fish oil aroma using a macroalgal lipoxygenase. *Journal of the American Oil Chemists' Society* 77, 343-348.

Hu, Y., Xia, W., & Liu, X. (2007). Food chemistry changes in biogenic amines in fermented silver carp sausages inoculated with mixed starter cultures. *Food Chemistry* 104, 188-195.

Iglesias, J., & Medina, I. (2008). Solid-phase microextraction method for the determination of volatile compounds associated to oxidation of fish muscle. *Journal of Chromatography A* 1192, 9-16.

Imai, T., & Sakai, S. (1961). Study of Japanese oyster *Crassostrea gigas*. *Tohoku Journal of Agricultural Research* 12, 107-172.

ICMSF (International Commission on Microbiological Specifications for Foods) (1980). Fish and shellfish and their products. In: Microbial ecology of foods. London Academic Press. Pp. 567–605.

Jay, J.M. (2000). Modern food microbiology (6th ed.). Gaithersburg, Maryland: Aspen Publishers Inc.

Jeong, B., Ohshima, T., Koizumi, C., & Kanou, Y. (1990). Lipid deterioration and its inhibition of Japanese oysters *Crassostrea gigas* during frozen storage. *Nippon Siusan Gakkaishi* 56(12), 2083-2091.

Josephson, D.B. (1991). In: Seafood. Volatile compounds in foods and beverages (1st ed.) (H. Maarse, Ed.). Marcel Dekker Inc., New York. Pp.179-202.

Kataoka, H., Lord, H.L., & Pawliszyn, J. (2000). Applications of solid-phase microextraction in food analysis. *Journal of Chromatography A* 880(1-2), 35-62.

Kawai, T. (1996). Fish flavor. *Critical Reviews in Food Science and Nutrition* 36, 257-298.

Kim, Y.-M., Paik, H.-D.,& Lee, D.-S. (2002). Shelf-life characteristics of fresh oysters and ground beef as affected by bacteriocin-coated plastic packaging film. *Journal of the Science of Food and Agriculture* 82(9), 998–1002.

Kyrana, V.R., & Lougovois, V.P. (2002). Sensory, chemical and microbiological assessment of farm-raised European sea bass (*Dicentrarchus labrax*) stored in melting ice. *International Journal of Food Science and Technology* 37, 319–328.

Le Guen, S., Prost, C., & Demaimay, M. (2000). Characterization of odorant compounds of mussels (*Mytilus edulis*) according to their origin using gas chromatography–olfactometry and gas chromatography–mass spectrometry. *Journal of Chromatography A* 896, 361-371.

Lenzi, M., Palmieri, R., & Porrello, S. (2003). Restoration of the eutrophic Orbetello lagoon (Tyrrhenian Sea, Italy): water quality management. *Marine Pollution Bulletin* 46, 1540-1548.

López-Caballero, M.E., Pérez-Mateos, M., Montero, P., & Bonderías, A.J. (2000). Oyster preservation by high-pressure treatment. *Journal of Food Production* 63, 196-201.

Matthiessen, G.C. (2001). Oyster culture. Fishing News Books.

Medina, I., Satué-Gracia, M., & Frankel, E. (1999). Static headspace gas chromatographic analyses to determine oxidation of fish muscle lipids during thermal processing. *Journal of the American Oil Chemists' Society* 76(2), 231-236.

Mendes, M., Filho, R., & Tala, E.M. (2010). Nutritional value of seven freshwater fish species from the Brazilian Pantanal. *Lipids* 87(12), 1461-1467.

Mendes, R., Huidobro, A., & Lopez-Caballero, M.E. (2002). Indole levels in deepwater pink shrimp (*Parapenaeus longirostris*) from the Portuguese coast. Effects of temperature abuse. *European Food Research and Technology* 214, 125–130.

Morrison, R., & Smith, M.L. (1964). Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. *Journal of Lipid Research* 5, 600-608.

Newell, R.C. (1979). Biology of intertidal animals. 3rd ed. Faversham, Kent, U.K.: Marine Ecological Surveys Ltd. 782 p.

Newell, R.C. (1973). Factors affecting the respiration of intertidal invertebrates. *American Zoologist* 13, 513–28.

Newell, R.C. (1970). Biology of intertidal animals. London: Logos Press, 555 p.

Ng, T.Y.T., Chuang, C.Y., & Wang, W. (2008). Biokinetics of cadmium in different populations of the Pacific oyster *Crassostrea gigas* Trace. Hong Kong University of Science and Technology (HKUST) and Integral Consulting, Appendix A. In: Final Project Report-Characterization of the Cadmium Health Risk, Concentrations and Ways to Minimize Cadmium Residues in Shellfish: Sampling and Analysis of Cadmium in U.S.West Coast Bivalve Shellfish, USDA Cooperative State Research Education and Extension Service.

Nollet, L.M.L., & Toldrá, F. (Ed.) (2009). Handbook of Seafood and Seafood Products Analysis. CRC Press. Pp. 928.

Ogata, M., & Miyake, Y. (1980). Gas chromatography combined with mass spectrometry for the identification of organic sulphur compounds in shellfish and fish. *Journal of Chromatographic Science* 18, 594–605.

Orban, E., Di Lena, G., Masci, M., Nevigato, T., Casini, I., Caproni, R., Gambelli, L., & Pellizzato, M. (2004). Growth, nutritional quality and safety of oysters(*Crassostrea gigas*) cultured in the lagoon of Venice (Italy). *Journal of the Science of Food and Agriculture* 84(14), 1929-1938.

Pan, B.S., & Kuo, J.M. (1994). Flavour of shellfish and kamaboko flavorants. In: Seafoods: chemistry, processing technology and quality (F. Shahidi & J.R. Botta, Eds.). Blackie Academic & Professional: London, UK. Pp. 85–114.

Parisi, G., Giorgi, G., & Nuccio, C. (2006). Environmental parameters and growth performance in Mediterranean mussels (*Mytilus galloprovincialis,* Lamarck) farmed in two different sites in Tuscany (Italy). In: Aqua 2006, World Aquaculture Society Congress, Firenze, pp. 702.

Paynter, K.T., & Dimichele, L. (1990). Growth of tray-cultured oysters (*Crassostrea virginica* Gmelin) in Chesapeake Bay. *Aquaculture* 87, 289–297.

Pazos, A.J., Ruiz, C., Garcia-Martin, O., Abad, M., & Sanchez, J.L. (1996). Seasonal variations of the lipid content and fatty acid composition of *Crassostrea gigas* cultured in El Grove, Galicia, N.W. Spain. *Comparative Biochemistry and Physiology* 114B(2), 171-179.

Pennarun, A.L., Prost, C., & Demaimay, M. (2002). Aroma extracts from oyster *Crassostrea gigas*: comparison of two extraction methods. *Journal of Agricultural and Food Chemistry* 50, 299-304.

Piggot, G., & Tucker, B. (1987). Science opens new horizons for marine lipids in human nutrition. *Food Reviews International* 3, 105−138.

Piveteau, F., Gandemer, G., Baud, J.-P, & Demaimay, M. (1999). Changes in lipid and fatty acid compositions of European oysters fattened with *Skeletonema costatum* diatom for six weeks in ponds. *Aquaculture International* 7, 341-355.

Piveteau, F., Le Guen, S., Gandemer, G., Baud, J.-P., Prost, C., & Demaimay, M. (2000). Aroma of fresh oysters *Crassostrea gigas*: Composition and aroma notes. *Journal of Agricultural and Food Chemistry* 48(1964), 4851-4857.

Pogoda, B., Buck, B.H., & Hagen, W. (2011). Growth performance and condition of oysters (*Crassostrea gigas* and *Ostrea edulis*) farmed in an offshore environment (North Sea, Germany). *Aquaculture* 319(3-4), 484-492.

Poulet, S.A., Lennon, J., Plouvenez, F., Jalabert, F., Correc, G., Cueff, A., & Lacoste, A. (2003). A nondestructive tool for the measurement of muscle strength in juvenile oysters *Crassostrea gigas. Aquaculture* 217, 49-60.

Prioli, G., Piva, P., & Maffei, M. (1995). Sperimentazione di un sistema ottimale per l'allevamento di ostriche in mare aperto. Relazione Scientifica Finale, pp. 64.

Prost, C., Hallier, A., Cardinal, M., Serot, T., & Courcoux, P. (2004). Effect of storage time on raw sardine (*Sardina pilchardus*) flavor and aroma quality. *Journal of Food Science* 69(5), 198-204.

Quayle, D.B. (1988). Pacific oyster culture in British Columbia. *Canadian Bullettin of Fisheries and Aquatic Sciences* 218, 241 pp.

Ratel, J., Berge, P., Berdague, J.-L., Cardinal, M., & Engel, E. (2008). Mass spectrometry based sensor strategies for the authentication of oysters according to geographical origin. *Journal of Agricultural and Food Chemistry* 56, 321–327.

Rice, T., Buck, D.H., Gorden, R.N., & Tazik, P.P. (1984). Microbial pathogens and human parasites in an animal waste polyculture system. *Prog. Fish-Cult.* 46, 230-238.

Rippey, S.R. (1994). Infectious diseases associated with molluscan shellfish consumption. *Clinical Microbiological Reviews* 7, 419–425.

Risticevic, S., Lord, H., Górecki, T., Catherine, L., Arthur, C.L., & Pawliszyn, J. (2010). Protocol for solid-phase microextraction method development. *Nature Protocols* 5, 122–139.

Robert, R., Trut, G., Borel, M., & Maurer, D. (1993). Growth, fatness and gross biochemical composition of the Japanese oyster, *Crassostrea gigas*, in Stanway cylinders in the Bay of Arcachon, France. *Aquaculture* 110, 249–261.

Roch, P. (1999). Defence mechanisms and disease prevention in farmed marine invertebrates. *Aquaculture* 172, 125–145.

Roncarati, A., Felici, A., Dees, A., Forlini, L., & Melotti, P. (2009). Trials on Pacific oyster (*Crassostrea gigas* Thunberg) rearing in the middle Adriatic Sea by means of different trays. *Aquaculture International* 18(1), 35-43.

Royer, J., Ropert, M., & Costil, K. (2007). Spatio-temporal changes in mortality, growth and condition of the Pacific oyster, *Crassostrea gigas*, in Normandy (France). *Journal of Shellfish Research* 26, 973–984.

Rueda, F.M., Hernandez, M.D., Egea, M.A., Aguado, F., Garcia, B., & Martinez, F.J. (2001). Differences in tissue fatty acid composition between reared and wild sharpsnout sea bream, *Diplodus puntazzo* (Cetti, 1777). *British Journal of Nutrition* 86, 617-622.

Saito, H., & Marty, Y. (2010). High levels of icosapentaenoic acid in the lipids of oyster *Crassostrea gigas* ranging over Both Japan and France. *Journal of Oleo Science* 59(6), 281-292.

Samain, J.F., Boudry, P., Dégremont, L., Soletchnick, P., Ropert, M., Bédier, E., Martin, J.L., Moal, J., Mathieu, M., Pouvreau, S., Lambert, C., Escoubas, J.M., Nicolas, J.L., Le Roux, F., Renault, T., Burgeot, T., & Bacher, C. (2005). Information on the distribution, causes and significance of the summer mortality syndrome in the Pacific oyster (*Crassostrea gigas*) and other bivalve species. Report on the Working Group on Pathology and Diseases of Marine Organisms, ICES CM 2005/F:02, 8–12 March, La Tremblade, France. 1206 pp.

Santos-Silva, J., Bessa, R.J.B., & Santos-Silva, F. (2002). Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. *Livestock Production Science* 77, 187-194.

SAS® (2007). SAS/STAT software, release 9.1. Cary, NC: SAS institute, Inc.

Shalaby, A.R. (1996). Significance of biogenic amines to food safety and human health. *Food Research International* 29(7), 675–690.

Sharpe, A.N., & Jackson, A.K. (1972). Stomaching: a new concept in bacteriological sample preparation. *Applied Microbiology* 24(2), 175-178.

Simopoulos, A.P. (1991). Omega-3 fatty acids in health and disease and in growth and development. *The American Journal of Clinical Nutrition* 54, 438–463.

S.I.N.U. (2011). L.A.R.N. www.sinu.it.

Skrede, G., & Storebakken, T. (1986). Instrumental colour analysis of farmed and wild Atlantic salmon when raw, baked and smoked. *Aquaculture* 53, 279–286.

Soletchnik, P., Lambert, C., & Costil, K. (2005). Summer mortality of *Crassostrea gigas* (Thunberg) in relation to environmental rearing conditions. *Journal of Shellfish Research* 24, 197-207.

Soudant, P., Ryckeghem, K.V., Marty, Y., & Moal, J. (1999). Comparison of the lipid class and fatty acid composition between a reproductive cycle in nature and a

standard hatchery conditioning of the Pacific Oyster *Crassostrea gigas*. *Comparative Biochemistry and Physiology Part B* 123, 209-222.

Spencer, B.E., Edwards, D.B., & Millican, P.F. (1992). Growing hatchery-reared Pacific oysters (*Crassostrea gigas* Thunberg) to marketable size in trays–observation on coasted small-scale culture methods and rough-handling trials. *Aquaculture* 106, 261-274.

Spencer, B.E. (1990). Cultivation of Pacific oysters. Laboratory Leaflet, MAFF Directorate of Fisheries Research, Lowestoft n. 63., 47 pp.

Spurvey, S., Pan, B.S., & Shahidi, F. (1998). Flavour of shellfish. In: Flavour of meat, meat products and seafoods (2nd ed.). (F. Sahidi, Ed.). Blackie Academic & Professional: New York. 159-196.

Tanaka, F., Miyazawa, T., & Ujiie, Y. (2010). Effect of cultivation conditions on odor character and chemical profile of shiso (*Perilla frutescens*) flavor. 19th World Congress of Soil Science, Soil Solutions for a Changing World. 1–6 August 2010, Brisbane, Australia.

Torstensen, B.E., Frøyland, L., Ørsnrud, R., & Lie, Ø. (2004). Tailoring of a cardioprotective muscle fatty acid composition of Atlantic salmon (*Salmo salar*) fed vegetable oils. *Food Chemistry* 87, 567−580.

Travaglia, C., & Lorenzini, M. (1985). Monitoring algae growth by digital analysis of LANDSAT data: the Orbetello Lagoon case study. In: RSC Series. FAO, Rome. 19 pp.

Turchini, G.M., Giani, I., Caprino, F., Moretti, V.M., & Valfré, F. (2010). Discrimination of origin of farmed trout by means of biometrical parameters, fillet composition and flavour volatile compounds, *Italian Journal of Animal Science* 3, 123-140.

Turolla, E., & Rossi, R. (2004). Esperienze di allevamento dell'ostrica concava (*Crassostrea gigas*) in Alto Adriatico. Ed. C.Ri.M., Centro Ricerche sui Molluschi, Goro (Fe). 31 pp.

Uauy, R., & Valenzuela, A. (2000). Marine oils: the health benefits of n-3 fatty acids. *Nutrition* 16, 680−684.

Ulbricht, T.L.V., & Southgate, D.A.T. (1991). Coronary heart disease: seven dietary factors. *The Lancet* 338, 985-992.

Valli, G. (1980). Riproduzione ed accrescimento di alcune specie di molluschi eduli nelle lagune di Grado e di Marano. *Nova Thalassia* 4, 49-65.

Waldock, M.J., & Holland, D.L.(1984). Fatty acid metabolism in young oysters, *Crassostrea gigas*: polyunsaturated fatty acids. *Lipids* 19, 332–336.

Walne, P.R. (1976). Experiments on the culture in the sea of the Butterfish *Venerupis decussata* L. *Aquaculture* 8, 371-381.

Walne, P.R. (1979). Culture of bivalve molluscs: 50 years experience at Conwy. Whitefriars Press, London and Tonbridge.

Wierda, R.L., Fletcher, G., Xu, L., & Dufour, J. (2006). Analysis of volatile compounds as spoilage indicators in fresh King salmon (*Oncorhynchus tshawytscha*) during storage using SPME−GC−MS. *Journal of Agricultural and Food Chemistry* 54, 8480-8490.

Xiong, G., Lee, D.J., Moon, K.R., & Lane, R.M. (2010). Shape similarity measure using turn angle cross-correlation for oyster quality evaluation. *Journal of Food Engineering* 100, 178–186.

Zhang, Z., & Pawlszyn, J. (1993). Analysis of organic compounds in environmental samples by Headspace Solid Phase Microextraction. *Journal of High Resolution Chromatography* 16, 689-692.

Zhang, Z., Li, T., Wang, D., Zhang, L., & Chen, G. (2009). Study on the volatile profile characteristics of oyster *Crassostrea gigas* during storage by a combination sampling method coupled with GC/MS. *Food Chemistry* 115, 1150-1157.

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