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REVIEW ARTICLE

Molluscs and echinoderms aquaculture: biological aspects, current status, technical progress and future perspectives for the most promising species in Italy

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Abstract

Shellfish aquaculture is a widespread activity in the Italian peninsula. However, only two bivalve species are mainly cultured along the coastline of that country: the Mediterranean mussel *Mytilus galloprovincialis* and the Manila clam *Venerupis philippinarum* (*Ruditapes philippinarum*). By contrast, just a few other mollusc species of commercial interest are scarcely reared at a small-scale level.

After analysing the current status of Italian shellfish production, this paper reports and discusses the potential for culturing several different invertebrate species [*i.e.*, the European flat oyster *Ostrea edulis*, the grooved carpet shell *Venerupis decussata* (*Ruditapes decussatus*), the razor clams *Ensis minor* and *Solen marginatus*, the cephalopod *Octopus vulgaris*, and the purple sea urchin *Paracentrotus lividus*] in this country.

In addition, a detailed overview of the progress made in aquacultural techniques for these species in the Mediterranean basin is presented, highlighting the most relevant bottlenecks and the way forward to shift from the experimental to the aquaculture phase. Finally, an outlook of the main economic and environmental benefits arising from these shellfish culture practices is also given.

Introduction

Current status of the Italian shellfish aquaculture

The Italian shellfish production amounted to 181,455 t in 2008 (FAO, 2011), corresponding to 68% of the total Italian aquaculture production and ranking Italy in the 3rd position in Europe, after France and Spain (189,070 and 185,153 t, respectively). Similarly to what happens to finfish production and unlike the two best shellfish producers in Europe, shellfish aquacultural practices are very scarcely diversified in Italy, since the only two species predominantly cultured are the Mediterranean mussel (*Mytilus galloprovincialis*; 123,010 t) and the Manila clam [*Venerupis philippinarum* (*Ruditapes philippinarum*); 58,445 t]. Only sporadic activities of rearing can be listed for other species, currently at a small-scale aquaculture level, as in the case of the oysters *Crassostrea gigas* and *Ostrea edulis*, and the grooved carpet shell *Venerupis decussata* (*Ruditapes decussatus*) or still as an experimental aquaculture example (*Modiolus barbatus*).

About molluscs culture in Italy, old traditions coexists with modern intensive farming techniques (Prioli, 2004). Mussel culture covers a total surface of about 20,000 ha and is realised by 263 companies that give employment to 1,400 people (Prioli, 2008). The cycle is based on the recruitment of wild spat largely available in many areas (namely, Apulia, Veneto and Emilia-Romagna regions) where the grow-out plants are located. The culture of *Mytilus galloprovincialis* is widely diffused along the coasts of the country (included those of Sardinia and Sicily, but not those of Basilicata, Calabria and Tuscany), where different kind of rearing techniques are applied. The traditional ones (fixed systems) are mostly located in sheltered coastal and in lagoon areas (gulf of Trieste, gulf of Taranto, Veneto lagoon, *etc.*), while the new long-lines rearing systems (single long-line *ventia* and Trieste or *multi ventia* long-line in open sea and in partially or fully sheltered areas, respectively) have been more and more diffused in offshore farms. The single *ventia* plants have been spread for 20 years and currently represent around 75% of total linear metres (2,000,000) of long-line quoted in Italy. The culture of the Manila clam started in Italy in the 1980s, when 200,000 juveniles from a North European hatchery were introduced in the Venice lagoon (southern basin) (Cesari and Pellizzato, 1985). Afterwards, this species was introduced in other areas of the Po river delta [Sacca di Goro (Ferrara), Sacca del Canarin-Porto Tolle (Rovigo) and Grado-Marano lagoon (Udine)].

Currently, the Venice lagoon is the most important production site for this bivalve, where 50% of the total Italian production is realised (Zentilin *et al.*, 2008), followed by the area of the Po river delta in Emilia Romagna region (mainly Sacca di Goro) (28% of the total production) and in Veneto region (21%). The Grado-Marano lagoon very scarcely impacts on the total production (1%). Thanks to the optimal conditions found in North Adriatic areas,

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this species spreads spontaneously and now the farmers move from rearing practices to management of production areas in a more or less controlled way. Manila clam culture covers a surface of about 940,000 ha and gives employment to 4000 to 5000 people (Turolla, 2008). Similarly to mussel culture, the spat is mainly collected from the wild (95% of the total utilised), but a hatchery seed production is also realised. Only two hatcheries (*i.e.*, ALMAR and TURBOT, this last producing 10 to 50 million of clam seeds year^{-1}) are currently operating in Italy, while in Europe a total of 34 mollusc hatcheries were recently listed (Robert, 2009).

In agreement with the EU Regulations [CE Reg. 2073/2005 (European Commission, 2005) and CE Reg. 853/2004 (European Commission, 2004)] for the marketing of shellfish, 125 centres for shellfish depuration and 320 centres for shellfish shipping (20 of which are located on boats in the service of equipment) are operational in Italy (Prioli, 2008).

A third cultured species is the grooved carpet shell *Venerupis decussata*, amounting to about 100 t in 2008 (FAO Fisheries and Aquaculture Department, 2011). The interest for the cultivation of that species has significantly increased in the last years due to its higher commercial value in comparison to that of the non-native species Manila clam. Also for oysters, despite their high market request, the amount produced is currently negligible, which puts Italy in the first position among the importer countries (with a stable quantity around 7400 t in the years 2006-2007: http://www.globefish.org/oysters-may-2008.html).

The main problems involving traditional shellfish culture are related to the seasonality of the production (for mussels mainly concentrated between May and September), the lack of traditional processes to obtain a product with a higher added value (currently the amount transferred to industry for processing is lower than 1% of total mussel production) (Prioli, 2004), the extensive seaweed blooms, and the algal blooms responsible for biotoxin risks. The lack of experimentation on the actual possibility to develop effective and economic systems for detoxification is a critical point for the future of this important aquaculture sector, due to serious economic losses during the closure of commercial shellfisheries caused by periodic harmful algal blooms. However, some results recently documented in the literature seem very promising about the possibility to detoxify the molluscs once contaminated (Marcaillou *et al.*, 2010; Medhioub *et al.*, 2010), or to prevent the contamination by innovative rearing systems (Serratore, 2011).

The perspectives of traditional cultures for mussels currently are: i) a positive trend towards the settlement of new production sites along the coastline of those Italian regions offering more favourable conditions for mussel growing; ii) a marked interest for finding new simplified rearing techniques. Also in the case of the clam culture, some priorities can be listed for promoting further development, such as: i) an adequate management of the lagoons where this species is cultured. Indeed, during summer there are often anoxic crises and excessive growth of seaweed due to the peculiar condition of eutrophy; ii) a proper management of the nursery areas, appointed to the production of the seed which is mainly obtained (95%) from the wild (Turolla, 2008).

The high consumption of shellfish associated with the seasonality of the national production (mainly in the case of mussels) and the lack of diversification of production typical of the Italian shellfish aquaculture generate large volumes of imported products, as shown in Table 1. In 2011, the importing of mussels – ranking the first position among the seafood consumed in Italy – dramatically increased (58,300 t, +50% compared to 2010) (http://www.globefish.org/bivalves-june-2012.html), partly due to the current economic crisis that has pushed consumption towards products of lower commercial value.

To give impetus to the sector, some interventions on traditional species and a greater diversification of farmed species are essential. As far as the first aspect is concerned, appropriate business strategies need to be adopted in order to maintain the functional product price and to create new market niches through appropriate techniques of processing and of storage (freezing) of the product.

At a national and regional level, some research has recently been carried out to stimulate the culture of new species of molluscs. Most of the results are documented in grey literature and in final reports written in Italian language, and have had a very scarce impact on the international literature. Althoguh much research has been carried out since the 1990s, no significant progress has been made in the Italian aquaculture landscape. As a matter of fact, for the cultured species it barely changed over the last 20 years. The main issues tackled in the studies of mollusc species which are deemed as promising for Italian aquaculture are summarised in Table 2.

In the following chapters, a detailed analysis of the most promising species for diversification of Italian shellfish culture is reported. Precisely, our attention will focus on some species that are supposed to change the landscape of Italian aquaculture in: i) the short term, as rearing techniques are already established (cf. the oyster *Ostrea edulis* and the

Table 1. Quantity of different species of molluscs imported in Italy in 2008 (FAO, 2011).

Commodity	Quantity, t
Frozen	
Squids (*Ommastrephes sagittatus, Loligo* spp.)	65,518
Octopus	50,945
Squids (*Illex* spp.)	21,399
Cuttlefishes	19,230
Squids nei°	8271
Cuttlefishes (*Sepia officinalis, Rossia macrosoma, Sepiola rondeleti*)	163
Live, fresh or chilled# (prepared or preserved)	
Mussel meat (prepared or preserved, not in airtight containers)	9279
Molluscs nei (prepared or preserved)	7627
Oysters nei	5550
Cuttlefishes and squids nei	4755
Octopus	2764
Squids nei	2760
Squids (*Ommastrephes sagittatus, Loligo* spp.)	1576
Other aquatic invertebrates (prepared or preserved)	1406
Cuttlefishes (other than live, fresh or chilled)	1026
European flat oyster (shucked or not)	740
Squids (other than live, fresh or chilled)	739
Octopus (dried, salted or in brine)	438
Mussel meat (prepared or preserved, in airtight containers)	373
Squids (dried, salted or in brine)	110
Total	204,669

°Not elsewhere identified, according to FAO. #Live, fresh or chilled, unless otherwise specified in brackets.

grooved carpet shell *Venerupis decussata*); or in ii) the medium-long term, as much research has been done or is going to develop the different phases of the breeding cycle (cf. the razor clams *Ensis minor* and *Solen marginatus*, and the common octopus *Octopus vulgaris*).

Lastly, the state of the art of the purple sea urchin *Paracentrotus lividus* is also given. Indeed, because of its significant marketing potential, the aquacultural activities of this species are undergoing a noteworthy improvement and its gonad quality is currently undertaken in many European regions.

Perspectives of Italian shellfish aquaculture

European flat oyster

Distribution, habitat and exploitation

The European flat oyster *Ostrea edulis* (Linnaeus, 1758) belongs to the Ostreidae family (Rafinesque, 1815) and is native of Europe. This species naturally lives in a region going from the Norwegian fjords to Morocco (North eastern Atlantic coasts) and in the Mediterranean sea (Jaziri, 1990) up to the Black sea coasts (Alcaraz and Dominguez, 1985). It is also found in South Africa, North-eastern America (from Maine to Rhode Island), Canada, Nova Scotia, New Brunswick and British Columbia, probably imported from population whose ancestors were from the Netherlands (Vercaemer *et al.*, 2006).

Ostrea edulis is a typical filter feeder, filtering phytoplankton, copepod larvae, protozoans and detritus as food. Being a sessile organism, it lives fixed to a hard substrate and its feeding entirely depends on the resources naturally present in the surrounding environment. As a matter of fact, food is pumped in with the seawater and removed by the gills (Laing *et al.*, 2005), filtering even up to 25 L h^1 depending on animal size and temperature (Korringa, 1952). This species is typical of coastland, estuarine and marine environments and sheltered areas, preferring hard substrates as rocks or artificial structures but also muddy sand, muddy gravel with shells, and hard silt. It lives in brackish and marine seawater, having an optimum of salinity rounding between 17 and 26 PSU (Blanco *et al.*, 1951) up to 40 metres deep.

Ostrea edulis is similar to other species of oysters widely cultivated in many regions of the world, like the Pacific cupped oyster *Crassostrea gigas* (Thunberg, 1793). This latter, however, has a more elongated, distorted and irregular shell and, above all, is characterised by a different sexuality. Oysters are prey of several organisms, including fish, crabs, snails, starfish and flatworms but also of boring sponges, seaworms, molluscs, pea crabs and fouling in general, that can be cause irritation problems or compete for food. With regard to disease, the protist *Bonamia ostrea* is one of the most dangerous pathogens: in 1920 it caused massive mortality events among flat oyster populations (da Silva *et al.*, 2005). These populations were then reintroduced in Europe where the disease was transferred to other established populations.

Reproduction

The European flat oyster is a protandric hermaphrodite (da Silva *et al.*, 2005) and shows an alternation of sexuality within one spawning season: early in the reproductive period it is male, but when it reaches the sexual maturity can alternate between the female and male stages for the rest of its life (Laing *et al.*, 2005). Males are mature after about one year of age when they release sperms into the water depending on temperature values (with a minimum of 14°C to 16°C) (Walne, 1979). Females collect sperms by using their feeding and respiration system (Laing *et al.*, 2005).

The oogenesis can produce up to 1 million eggs per spawning event, releasing them from the gonad and retaining them in the mantle cavity where they can be fertilised by externally released sperms (*i.e.*, larviparous species). After an incubation period of about 8 to 10 days, larvae develop a formed shell, a digestive system and the ciliated swimming and feeding organ (*i.e.*, the *velum*) reaching about 160 μm in size. At this point, they are released into the sea open water where they live at pelagic stage

Table 2. Topics studied about several mollusc species and considered as promising for Italian aquaculture.

Scientific name	Common name	Topics	Country	References
Bolinus brandaris	Purple dye murex	Larval rearing Semi-intensive rearing	Spain Portugal	Ramón and Flos (2001) Vasconcelos *et al.* (2012)
Callista chione	Smooth venus clam	Larval rearing Diet and gonadal development	Spain	Perez-Larruscain *et al.* (2011) Martínez-Pita *et al.* (2011)
Donax trunculus	Wedge shell	Larval rearing under different conditions Diet and lipid composition of broodstock managed in hatchery	Spain	Ruíz-Azcona *et al.* (1996) Martínez-Pita *et al.* (2012)
Haliotis tuberculata	Abalone	Juvenile rearing	Spain	Viera *et al.* (2011)
Hexaplex trunculus	Banded murex	Hatchling and juveniles	Portugal Tunisia	Vasconcelos *et al.* (2004) Lahbib *et al.* (2008)
Modiolus barbatus	Bearded horse mussel	Genetics Artificial reproduction Rearing	Italy	Libertini *et al.* (1996) Turolla *et al.* (2002) Barile *et al.* (2011), Turolla (1998), Rossi *et al.* (1998), Pellizzato *et al.* (1998), Prioli *et al.* (1998), Turolla and Rossi (1999), Rossi (2011)
Pholas dactylus	Common piddock	Artificial reproduction	Italy	Rossi (2011)
Venus verrucosa	Warty venus	Artificial reproduction	Italy	Piccinetti (2011), Rossi (2011)

(8 to 10 days), feeding on phytoplankton for 2 to 3 weeks before settlement (Korringa, 1941, 1952; Laing *et al.*, 2005). The amount of larvae released into the seawater is correlated to the parent size, which ranges between 1.1 and 1.5 millions for oyster from 4 to 7 years old (Walne, 1979).

By contrast, *Crassostrea gigas* inverts its sex after one spawning season and releases its gametes (eggs or sperms) into the environment at one time or in small amounts over a long period (*i.e.*, oviparous species). Thus, fertilisation occurs externally and the resulting larvae develop in the seawater.

Therefore, during the larval stage life is typically planktonic and, as metamorphosis progresses, oyster moves with an extensible foot in search of a suitable substrate. Once the oyster finds it, it attaches itself by a byssus formation and then by cementation (with a physiological and morphological metamorphosis lasting 3 to 4 days) and starts its sessile life as juvenile, thus becoming spat (Laing *et al.*, 2005). From this event, the growth is quite quick for about 18 months, then it stabilises remaining constant at about 20 g of fresh weight per year and finally slows down after 5 years (Laing *et al.*, 2005). Depending on the environmental conditions, these bivalves can achieve the marketable size of 7 cm in shell length in 4 to 5 years and live in natural beds up to 20 years growing up to 20 cm of size.

Aquacultural activities

Oyster spat can be obtained by both wild stocks and hatchery production. Like in other bivalves, sexual maturation and subsequent reproduction is obtained by modifying the temperature of the water (*i.e.*, increasing it) and by administering the phytoplankton *ad libitum*, thus imitating the natural reproductive cycle. Compared to the conditioning of other species, the fertilisation of *O. edulis* specimens is more difficult due to a lower larval survival rate, so that a period of incubation is necessary. In general, spat is cultured using traditional techniques for bivalves at nursery stages and, when it reaches the size of 5-6 mm, it can be moved to open water to grow up.

On the contrary, natural spat harvesting is based on the employment of collectors. Some examples are mussel shells sown in density of about 30 to 60 m^3 ha^{-1} (the Netherlands), or tubular nets containing mussel shells (about 600) suspended under steel frames in shallow waters (France). Recently, PVC dishes are used in intertidal areas. Therefore, seed can be transferred to the growing or fattening area. Yet, this is not always necessary for the seeding area that can also become the growing and fattening area in some facilities. Breeding methods are generally categorised into *on-bottom* and *off-bottom*, each of them having its advantages and disadvantages. Thus, it should be better to choose the most suitable method for the selected site and for the specific financial possibility. On-bottom techniques require that oysters are seeded directly in subtidal or intertidal grounds with a stable, non-shifting bottom (Quayle, 1980), at a density of about 50-100 kg ha^{-1}. Seeding is generally carried out in the period between May and June, when molluscs are about 1 cm long (1 year old), and here they reach the marketable size. Traditionally, cotton nets or steel frames are used to preserve the culture from predators. The on-bottom method certainly is the simplest and cheapest one, but mortality, stock loss caused by predation and siltation events are highest, and even harvesting is difficult.

On the other hand, off-bottom techniques allow oysters to be cultured in suspension. This method is certainly more expensive than the first one and requires more maintenance, but it is compensated by the rapid growth and high quality of the cultured oysters. The technique consists of using floating structures, rafts, long-line systems, suspended ropes, lanterns or plastic baskets pending from a raft/rope, where oysters are located in. Product is thinned out as it grows. Harvesting should be programmed when oysters are in their best conditions, with full and creamy meat. From on-bottom cultures, molluscs can be dredged or handily collected, whereas in the off-bottom ones they can be handily-picked. Finally, before marketed, they are temporarily stored in clean water and subjected to depuration procedures as all other bivalve molluscs.

Rearing in Europe

In Europe, several rearing experiments with this species have been carried out in the last decades. In particular, much attention has been paid to both survival and growth of experimental batches of hatchery-reared *O. edulis* larvae and spat (Davis and Calabrese, 1969; Laing and Millican, 1986; Spencer and Gough, 1978; Utting, 1988; Beiras and Pérez Camacho, 1994; Beiras *et al.*, 1994; Berntsson *et al.*, 1997; Rodstrom and Jonsson, 2000), and to the biochemical composition of larvae fed on different food regimes (Ferreiro *et al.*, 1990; Millican and Helm, 1994).

On the other hand, a few studies have been conducted on its reproduction (Mann, 1979; Wilson and Simons, 1985; Ieropoli *et al.*, 2005), while many growth trials were tested in different European countries like Ireland (Wilson, 1987), Germany (Pogoda *et al.*, 2011), Croatia (Zrnćić *et al.*, 2007), Malta (Agius *et al.*, 1978), Turkey (Yildiz *et al.*, 2011), Spain (González and González, 1985; Pérez Camacho and Roman, 1985; Cano and Rocamora, 1996), and most of all Italy (Pellizzato and Da Ros, 1985; Lubrano Lavadera *et al.*, 1999; Pellizzato *et al.*, 2005; Pais *et al.*, 2007, 2012a; Carlucci *et al.*, 2010). Nevertheless, commercial farming activities of this species are still scarce in Italy. The Pacific cupped oyster *Crassostrea gigas* is preferred for aquacultural purposes although in recent years this industry has experienced high mortality rates of up to 80% due to Type 1 Ostreid Herpes Virus (OsHV-1) (EFSA, 2010).

Grooved carpet shell

Distribution, habitat and exploitation

The grooved carpet shell *Venerupis decussata* (Linnaeus, 1758) is a bivalve belonging to the Veneridae family (Rafinesque, 1815). This species is found all through the Mediterranean sea, but it is widely distributed along the western Atlantic coasts from Norway to Congo and also in the northern part of the Red sea where it migrated from the Suez Canal.

This species typically lives buried in sandy and silt-muddy bottom, inhabiting the areas near and below the mean sea level (intertidal zone and subtidal zone, respectively), and buried 15 to 20 cm into the sediment. Moreover, it continuously filters surrounding water through its two siphons protruded from the substrate, picking up organic particles and phytoplanktonic cells as nourishment and to allow gas exchange between oxygen and carbon dioxide that occur with breathing. Overall, clams bear quite well the variations of chemical and physical variables of water, such as temperature, salinity, dissolved oxygen, turbidity, typical of lagoon environments or estuarine areas where they live. As a consequence, their favourite sites are generally located away from areas with high hydrodynamic, and from windy areas where the substrate where they are buried can be destabilised. Nevertheless, it is important the presence of a slight and a constant current that allows good water exchange and the constant flow of food. For this reason, clams can live on a variety of substrates although a mixture of sand, silt and granules is the most suitable composition which allows a good oxygenation and a comfortable softness of the bottom. It is important to emphasize that other filter feeders species (*e.g.* Bivalves, Hydroids, Bryozoans, Serpulids, *etc.*), can compete with a clam population for food availability. At the same time, another form of competition can take place during the recruitment, depending on the availability of suitable substrates (Paesanti and Pellizzato, 2000; FAO, 2004).

In Europe, the harvesting of *Venerupis decussata* mainly occurs in countries like Spain and France, but also in Italy, especially in Sardinia, where the semi-extensive culture of the allochthonous species *Venerupis philippinarum* has been banned by the Regional Government in order to protect the native Mediterranean carpet clam (Chessa *et al.*, 2005; Pais *et al.*, 2006b).

Reproduction

Even though occasional cases of hermaphroditism can be observed (Delgado and Pérez Camacho, 2002) especially in juvenile forms (Lucas, 1975), this clam is strictly gonochoristic and the reproduction takes place externally in the aqueous medium, mainly in summer when temperature is higher and food is abundant. Resulting larvae are freely floating for 10 to 15 days until they settle as spat (about 0.5 mm in length) and continue their growth to adult form, once they found a suitable substrate.

Like most of other marine bivalves, *Venerupis decussata* is characterised by a cyclical pattern of reproduction, which can be divided into different phases: gametogenesis and vitellogenesis, spawning and fertilisation, larval development and growth. Each bivalve species evolved a number of adaptive strategies (genetic or not) to coordinate these events with the environment in order to maximise the reproductive process (Newell *et al.*, 1982). In this regard, numerous studies show that gametogenic cycle in marine invertebrates is strictly conditioned by the interaction between exogenous factors (*i.e.*, temperature, salinity, light, availability of food, parasitic infestations) as well as by internal factors (Rodríguez-Moscoso and Arnaiz, 1998). Temperature is certainly one of the most important factors influencing the reproductive cycle (Sastry, 1975), defining both the starting point and the rate of gonadal development, whereas food availability can determine the extension of the reproductive process (Lubet, 1959). These two factors are subject to natural seasonal fluctuations and their variability is closely related to the energy available for growth and reproduction. In particular, clam reproduction requires abundant energy for providing a suitable gonadic development so that the success directly depends on ingested food or on previously stored reserves (Delgado and Pérez Camacho, 2005). In general, when food is abundant, reserves accumulated before and after gametogenesis (*i.e.*, glycogen, lipid and protein) are utilised to produce gametes when metabolic demand is high (Bayne, 1976). As a consequence, gametogenesis can differ from location to location depending on the geographic area considered: for example, in adult clams from southern Europe the cycle generally starts in March, gonads become ripe in May-June and spawning occurs in summer, following a phase of inactivity in winter (Shafee and Daoudi, 1991).

Aquacultural activities

Until a few decades ago, the management of this species was exclusively related to the availability of natural seed. However, nowadays the manipulation of its gonadal cycle is a quite common practice. In fact, artificial spawning techniques and larval rearing programs have been recently developed. These methods are applied in highly specialised systems – the hatcheries – where breeders (previously selected from natural beds on the basis of their appearance, size and shape) are stocked into tanks for 30 to 40 days at 20°C of temperature, and richly fed with phytoplanktonic algae. In order to guarantee the continuous availability of this nourishment for breeders and future larvae, hatcheries have to possess algal culture systems.

The specimens selected are abundantly fed to maximise their gonadic maturation until they are ready to reproduction. At this phase, the release of gametes is induced by a thermal shock of the water of about 10°C (from 18 to 28°C), repeated for one or more cycles of about 30 min each. Generally, males emit before females and fertilisation occurs in small containers. The eggs obtained are counted, filtered and placed into little aquariums (about 10 L in volume), where *veliger* larvae appear after 8 days. Subsequently, they are filtered through a 100 μm mesh, daily fed with phytoplankton for the first week and then every 2 days. At the *pediveliger* stage, clams have a diameter of about 180 to 220 μm, they already have the foot but the *velum* is still present. Indeed, they spend most of their time swimming and sometimes they are fixed on the container surfaces. After about 3 weeks, the metamorphosis process is completed and the spat stage is reached (about 250 μm in size). The little molluscs can be now reared in greenhouses, fed with phytoplankton or with pumping environmental water into inland tanks, where they are placed inside small containers having a rigid mesh as bottom (*i.e.*, nursery).

From this stage onwards, methods of farming may be different depending on the features of the hatchery (*e.g.* standing water, constant water flow, downwelling and upwelling forced water flow). As said above, spat can be obtained both from natural populations in the vernal period (digging them with sand by a small rank and riddling it to retain the seed) and from hatcheries. When a size of about 1 mm is reached, a new phase of rearing can start using a controlled system: the so-called pre-fattening. Clams grow up to about 10 to 15 mm in 2 to 4 months and it would be convenient to complete their weaning period outside, pumping natural seawater or brackish water since their maintenance into the hatchery is quite difficult for both management and for economic reasons. Once they have reached this size, depending on the preferences and the possibilities of the farmer, molluscs can be transferred to the ground (with a density of about 5000 individuals m^{-2}) or in special facilities that allow their growth in suspension, such as net bags (*pôches*) or stacked baskets (at lower density). Moreover, if they are sown directly on the substrate, it is advisable to protect the seed from predation by plastic nets. In this way, clams are able to attain a size of 20 to 25 mm in about 2 months. At this phase, management regards only the preparation and maintenance of breeding substrate (*i.e.*, cleaning and removal of algae or predators) or the control of the suspension systems (*i.e.,* attachment and clearing of encrusting organisms or fouling).

The last procedure of the production cycle is fattening, *i.e.*, when carpet shells grow in the bottom within the sediment. In this way, molluscs live following their natural pattern, filtering water and then feeding until they achieve the commercial size of at least 25 mm in length. According to the environmental conditions and breeding, the fattening stage can be completed in a period of 12 to 28 months. After reaching the commercial size, clams can be gathered in different ways depending on the type of farming. When and where it is possible, fishermen manually collect the bivalves by walking using a rake equipped with an appropriate net, whose mesh is sized to hold the molluscs and allow the escape of the sediment. Alternatively, the harvest can be made from boats (with oars or engines) furnished with an extended rake.

Rearing in Europe

During the last decades, clam aquaculture has conspicuously developed in Europe and particularly in Italy where, after its introduction into the northern Adriatic lagoons, the Pacific carpet clam *Venerupis philippinarum* (Adams and Reeve, 1850) has been intensively exploited due to its rapid growth and propagation (Paesanti and Pellizzato, 2000). On the contrary, farming activities of the grooved carpet shell *Venerupis decussata* are still limited at present, although intensive research on the rearing of this species has been carried out throughout the continent. In particular, several studies have been conducted both on gametogenesis (Xie and Burnell, 1994; Rodríguez-Moscoso and Arnaiz, 1998; Ojea *et al.*, 2004; Serdar and Lök, 2009) and reproductive cycle (Breber, 1980; Beninger and Lucas, 1984; Laruelle *et al.*, 1994; Urrutia *et al.*, 1999; Delgado and Pérez Camacho, 2003, 2007) of this species.

Much research has been carried out also on broodstock conditioning (Ojea *et al.*, 2008; Matias *et al.*, 2009) and on diets for *Venerupis decussata* larvae (Pérez Camacho *et al.*, 1994; Matias *et al.*, 2011) and seed (Albentosa *et al.*, 1996a, 1996b, 1996c, 1997, 1999; Lamela *et al.*, 1996; Jara-Jara *et al.*, 1997; Fernández-Reiriz *et al.*, 1998, 1999; Pérez Camacho *et al.*, 1998, 2007; Enes and Borges, 2003) in controlled conditions. Furthermore, a number of successful trials have demonstrated the feasibility of rearing this bivalve in different European countries (Walne, 1976; Breber, 1985; Pastore *et al.*, 1996) using different culture techniques in the sea as well as in coastal lagoons (Chessa *et al.*, 1998, 2005; Pais *et al.*, 2006b; Serdar *et al.*, 2007).

Razor clams

Only two genera of razor clams are commercially exploited in Europe: genus *Ensis* (*E. arcuatus*, *E. minor*, and *E. siliqua*), belonging to the Pharidae family; and genus *Solen* (*S. marginatus*), belonging to the Solenidae family. The razor clams have a high and increasing commercial value due to the high prices reached in European and international markets (Barón *et al.*, 2004). Spain, Italy, France, Portugal and the Netherlands are considered as the most important countries involved in this market. In 2004, the import value of the razor clam market within the EU25 was 550 million € (BIM, 2005). The world landings of these species are low compared with other traditional shellfish species (*i.e.*, oysters, scallops, or clams), and the fishing pressure on wild populations is increasing. Signs of severe exploitation of natural stocks are documented (Gaspar *et al.*, 1998; Tuck *et al.*, 2000; Fahy and Gaffney, 2001) also in Italy, where razor clams (*E. minor* and *S. marginatus*, a less valuable species) are widely distributed, and the quantity harvested from the wild is decreasing. This species is of interest for aquaculture both for improving natural stocks and for producing food. The aquaculture potential of *E. arcuatus* and *S. marginatus* has been recently assessed by a number of specific trials carried out mainly in Spain, a country importing large quantity of this seafood (47% of the European importation value, according to data obtained from Eurostat information database). The experiments carried out on the production of razor clam species diffused in Spain, using hatchery and semi-intensive aquaculture techniques, are deeply summarised in a recently published report (Guerra Diaz *et al.*, 2011).

Despite interest in razor clam aquaculture, little is known about its growth and reproduction, even though the studies of the cultivation of 3 razor clams (*S. marginatus*, *E. siliqua* and *E. arcuatus*) date back to 1990. Available literature is scarce and mainly represented by documents for internal use (reports, MSc, or PhD thesis), or by posters and short presentations documented as short abstracts in international or, more often, national meetings. The availability of these last documents is reduced also for the use of native languages other than English. Some documents are in form of grey literature, thus reducing the possibility for exchanging results among researchers. Currently, no information is available about the aquaculture potential of *E. minor*, which is located exclusively in the Mediterranean sea basin.

Reproduction, larval and *post*-larval rearing

In *Solen marginatus* the spawning takes place in a few weeks during spring, in *Ensis minor* in March-April in the southern Adriatic sea, while in *E. siliqua* there is only one spawning period (May-June) (Guerra Diaz *et al.*, 2011). The increase of seawater temperature during broodstock conditioning helps the maturation process in most of the species, except for *E. arcuatus* that is conditioned to ripeness at low temperature. In some species (*S. marginatus*, *E. siliqua*), the ripe adult can be successfully induced to spawn using thermal shock (Loosanoff and Davies, 1963; Martinez-Patiño *et al.*, 2007; da Costa and Martinez-Patiño, 2009), while in *E. arcuatus* the change of water levels by simulating tides is the only effective method (da Costa *et al.*, 2008).

The management of eggs for fecundation and of larvae during rearing can be carried out according to the same protocol utilised for other bivalve species. Larval culture duration is very short in *S. marginatus* (8 days), due to the high levels of stored reserves in eggs (da Costa *et al.*, 2011b), and a larval survival ranging from 28 to 81% (53% on average) was recently achieved by da Costa and Martinez-Patiño (2009) in specimens obtained from adults spawned in hatchery by induction. As a consequence of the large size of eggs and the short length of the larval stage, a different pattern in the use of gross biochemical and fatty acid reserves during larval development compared to other razor clams and bivalve species was recently found (da Costa *et al.*, 2011b). A dramatic reduction in survival was obtained by the same authors in 1-month-old spats (8.6%), the bottleneck phase resulting at the *post*-larvae age corresponding to 1 mm in length (15 to 22 day from settlement). Using seed 1.3 mm in length, da Costa and Martinez-Patiño (2009) achieved better survival when the rearing was done without substrate than when 2 types of sand were utilised. In *E. arcuatus*, recently da Costa *et al.* (2011a) achieved the settlement of larvae on day 20, a survival from egg to newly settled postlarvae ranging between 4.8 and 24.8% (14.35% on average), and a very low (4.8%) survival from settlement (day 20) to 3-month-old spat (15.5 mm in length).

Grow-out

The effect of substrate (fine-grain sand or coarse sand, 150 to 600 or 300 to 1200 μm grain diameter, respectively) in comparison to the absence of substrate was tested by da Costa *et al.* (2011a) in seed culture of *E. arcuatus* (3.76 mm in length) in nursery during a experiment lasting 30 days. No differences among treatments resulted for length, while the fine sand induced a lower weight and the absence of substrate a lower survival. Substrate and stocking density influenced the *E. arcuatus* juveniles performance (length and survival), that resulted the worst at high density (36 g per a 5 L bottle) and with fine sand as substratum (*vs* coarse sand). The on-growing in cage buried in sand, in natural environment, from the initial size of 60 to 80 mm highlighted high mortality and high sensitivity to changes in salinity. For those reasons, the choice of the site is highly affecting juveniles grow-out and performance.

Da Costa and Martinez-Patiño (2009) in *S. marginatus* broodstock from the wild managed spawning induction and fecundation, larval and spat rearing in hatchery, and assessed growth performance of juveniles produced in hatchery when transferred to natural beds. Two-year-old razor clams showed a survival ranging from 50 to 83% and after 2 to 3 years from seeding they reached the commercial size (80 mm). Since some of those specimens were utilised as broodstock, the culture cycle of *S. marginatus* was closed. In this species, a greater tolerance for salinity variations was found in comparison to *E. arcuatus*.

In experimental condition, *E. arcuatus* reached a length ranging from 90 to 100 mm (da Costa *et al.*, 2011a) after two years of cultivation, while *S. marginatus* reached the commercial size only after 3 years (da Costa and Martinez-Patiño, 2009). In *E. minor*, Froglia (1975) found that the size of 100 mm was reached in 2-year-old individuals. After 2 years, the growth is reduced as a consequence of the gametogenic activity both in *E. arcuatus* and in *S. marginatus*.

Critical points

Among the limiting factors for aquaculture of razor clams, the followings can be listed: short reproduction period, requirement of substrate and, for the on-growing phase, appropriate seed holding systems, low survival times in absence of soft substrate, difficulties for checking growth due to burying behaviour, need for appropriate substrate. At the moment, postlarval and seed survival obtained is very low for *S. marginatus*, and mainly for *E. arcuatus*. For *S. marginatus*, low survival during the on-growing of juveniles has been also achieved. The information related to larval and postlarval nutrition and their influence on growth and survival presently is very scarce. In addition to these aspects which constitute a major bottleneck for the reproduction management, the requirement of an appropriate substrate for the culture process can also be envisaged as an obstacle for promoting the development of culture in hatchery. Therefore, further investigations on the specific requirements at different stages of the development for the diverse species could improve the results and the potential for culture of razor clams.

Octopus

Statistics

The benthic octopuses of the family Octopodidae (Order: Octopodida, Leach, 1818) are one of the most familiar groups amongst the cephalopods. In this family, there are over 200 species, which range in size from pygmy *taxa* mature at <1 g (*e.g.*, *Octopus wolfi*) to giant forms exceeding 100 kg (*e.g.*, *Enteroctopus dofleini*). These species inhabit all marine habitats from tropical intertidal reefs to polar latitudes and in the deep sea to nearly 4000 m (Villanueva and Norman, 2008). Only a few cephalopods are commercially fished on a large scale (Kreuzer, 1984): squid (genus *Loligo*) is by far the main species, representing 73% of cephalopod world catches; cuttlefish (genus *Sepia*) is the second with 15%; and octopus the third with 8.8%. *Octopus vulgaris* Cuvier, 1797 (order Octopoda, suborder Incirrata) is one of the most important species in terms of commercial value and landings. Thirty-one per cent of the total production is put out by Mexico (11,855 t), which is the world leader, followed by Portugal (9,965 t; 27%), Spain (5,792 t; 16%) and Italy (3,018 t; 8%) (FAO, 2010).

Potential for aquaculture

Octopus vulgaris presents many characteristics: easy adaptation to captivity due to their benthic mode of life, reclusive behaviour, and low swimming activity; high growth rates (Aguado-Giménez and García García, 2002; Iglesias *et al.*, 2006, 2007) (between 3 and 15% body weight day^{-1}); high food conversion rates (incorporating 40 to 60% of ingested food into tissue) (Mangold and Boletzky, 1973); high fecundity (producing from 100 to 500 thousand eggs per female) with well developed hatchlings compared to other molluscs; high market size and price (García García *et al.*, 2004) in areas where cephalopod consumption is high; and a non-geographically limited request by the markets (Berger, 2010).

The life cycle of this octopus species was carried out for the first time in captivity by Iglesias *et al.* (2002) using *Artemia* and zoeae of spider crab (*Maja squinado*) as live feed. The authors obtained a survival of 31.5% day^{-1} *post*-hatching, a weight of 0.5 to 0.6 kg in 6-month-old animals and of 1.6 kg in 8-month-old animals (Iglesias *et al.*, 2002).

Reproduction

Octopuses are dioecious. *Octopus vulgaris*, as other benthic octopuses (*O. mimus*), produces numerous small eggs (2.7 mm in length) that hatch into planktonic, free-swimming hatchlings (1.2 mg) very different in their morphology, physiology, ecology and behaviour compared to the adult stage. Other octopuses (*i.e.*, *O. maya*, *O. bimaculoides*), by contrast, produce relatively few, large eggs resulting in better developed hatchlings with a benthic habit that resembles the adult (Villanueva and Norman, 2008). They readily mate in captivity and easily spawn, and females reared to the sexual maturity can produce viable spawns (Iglesias *et al.*, 2000, 2004). No further information is available about reproduction in captivity. However, reproduction is not considered as a limiting factor for this species and Iglesias *et al.* (2000) recommend a correct feeding of broodstock before mating and a correct management of females after eggs deposition. The optimal water conditions for broodstock (maintained at a 1:1 ratio of males and females or 1:3 ratio, according to Iglesias *et al.*, 2007) are temperature ranging from 13°C to 20°C and salinity around 32-35 PSU. Normally rectangular tanks (5 to 10 m^3), maintained with low light to obtain spawning as swiftly as possible, have been utilised (Iglesias *et al.*, 2000; De Wolf *et al.*, 2011) and a diet based on frozen crustaceans (80%), fish (15%) and bivalve molluscs (5%) seemed to be favourable to obtain highly viable spawns (Iglesias *et al.*, 2002). Males show a copulatory activity and egg deposition occurs on the walls and on the roof of den where the broodstock move. The eggs, cared for by the females, hatch after 34 days at 20±1°C, in the Mediterranean area (Villanueva, 1995), while more time is taken for hatching in captivity as the results obtained by Iglesias *et al.* (2000) showed.

Currently, the rearing of paralarvae is not possible, and the impossibility of obtaining benthic juveniles on a commercial scale in captivity (Navarro and Villanueva, 2000, 2003; Iglesias *et al.*, 2004, 2007) forces farmers to catch from the wild the juveniles that will be utilised in industrial on-growing. This behaviour is highly objectionable from an ethical point of view because it potentially increases fishing pressure on octopus stocks.

Larval rearing and feeding

Many rearing systems have been experimented, different for tank colour, size and shape, larval and prey densities and environmental factors (light, water flow and temperature), resulting in different survival of paralarvae. However, the phase of paralarvae rearing is considered inadequately explored and survival of paralarvae older than 50 days *post*-hatch in captivity conditions is very rare, and referred only by Iglesias *et al.* (2002, 2004) that reared octopus until 8 months by *Artemia* and zoeae. In Italy, De Wolf *et al.* (2011) carried out many trials fom 2003 to 2007. They produced eggs and paralarvae in captivity and reared juveniles that reached the age of 160 days, by using standard aquaculture procedures for feeding (based on *Artemia* and rotifers as live prey) and rearing paralarvae. Currently, the most recognised opinion is that nutritional aspects are the most important factor influencing the performance and mortality of paralarvae (Iglesias *et al.*, 2007). For feeding paralarvae different prey were experimented: *Palaemon serrifer* zoeae (Itami *et al.*, 1963), *Artemia* (Imamura, 1990), *Artemia* enriched with *Nannochloropsis* sp. (Hamazaki *et al.*, 1991) or *Tetraselmis suecica* or *Chlorella* sp., *Liocarcinus depurator* and *Pagurus prideaux* (Villanueva, 1994, 1995; Villanueva *et al.*, 1995, 1996), *Artemia* with spider crab *Maja brachydactyla* zoeae (Moxica *et al.*, 2002; Iglesias *et al.*, 2004), *Artemia* supplemented with copepods or juvenile mysids. Rotifer *Brachionus plicatilis* too was recently tested (De Wolf *et al.*, 2011). The survival rate largely varied with prey (species, strain, size, culture technique) and with trial, usually resulting very low after 1 or 2 months from hatching: 8.3% (Moxica *et al.*, 2002) and 0.8 to 9% (Villanueva, 1994, 1995; Villanueva *et al.*, 1995, 1996), respectively. As regards to live prey, spider crab was preferred and induced better performance, probably for its high content in the essential fatty acids (EFA), particularly in arachidonic acid (ARA) (Iglesias *et al.*, 2007). A mix of *Artemia* and inert diet (Navarro and Villanueva, 2000), and millicapsules (Villanueva *et al.*, 2002; Navarro and Villanueva, 2003) was also tested, thus obtaining reduced growth performance and a survival limited to first month.

Even though much research was carried out on this topic, high mortality and scarce growth performance have been obtained. At the moment, the high diversity of the experimental protocol of the trials and the lack of standardised methodologies do not let find the most effective diet for adequately feeding paralarvae. The low performance of this stage of culture can be also due to inappropriate techniques utilised during rearing. An inadequate size of tanks and the hydrodynamic behaviour of water can be responsible for mortality or damages in paralarvae arms and mantle (Rasmussen and McLean, 2004). In large volume tanks, higher survival and longevity was obtained by De Wolf *et al.* (2011) and better growth by Sanchez *et al.* (2011), the larger volumes mitigating the fluctuations in water temperature and in other water parameters. A drastic reduction of survival (4 to 0.6%) was noticed when density increased from 3 to 15 ind. L^{-1} (De Wolf *et al.*, 2011). The negative effect of high density could be explained by the releasing of paralysing substances or by the competition for space during prey location and capture.

Useful information was obtained about the nutritional requirements of paralarvae by the comparison of the chemical profiles of mature ovaries, eggs at different developmental stages, fresh hatchlings and wild juveniles with the chemical profiles of paralarvae cultured for one month with different live *natural* prey and with those of the prey administered (Navarro and Villanueva, 2000, 2003; Villanueva *et al.*, 2002, 2004, 2009; Villanueva and Bustamante, 2006). Special attention was paid to polyunsaturated fatty acids (PUFA), especially docosahexaenoic (DHA), DHA/eicosapentaenoic (EPA) ratio, phospholipids, cholesterol, to some essential amino acids (lysine, leucine and arginine representing about 50% of the total essential amino acids of paralarvae), mineral (copper) and vitamins (Vitamin A, but especially Vitamin E; Villanueva *et al.*, 2009). The requirement of Vitamin E, which is higher than for other marine molluscs and fish larvae, is probably due to the high percentage of PUFA in paralarval and juvenile cephalopods, that consequently need of a strong antioxidant system.

Grow-out

The on-growing of octopus started in Galicia in the mid-1990s with wild sub-adults 750 g in weight. The first findings on on-growing experimental trials date at the end of the last century and have been obtained in Spain (Iglesias *et al.*, 1997, 2000), Portugal, and Italy (Cagnetta and Sublimi, 2000).

Rearing parameters

Much information on the behaviour of octopus, which can be usefully used for an appropriate design of tanks and equipment for rearing, is obtained by observing octopus in laboratory or directly in nature.

Even though octopus shows a rapid and easy adaptation to life in captivity (Iglesias *et al.*, 2000) in different kinds of containers [aquaria, cylindrical-conical containers, raceways, floating cages and also benthic cages, as recently showed by Estefanell *et al.* (2012)], the habit to attach to any surface can be a problem for handling the specimen maintained in captivity, also as a consequence of their tendency to escape from containers. To counteract the aggressive behaviour and to respect the natural habits of octopuses that prefer dark sites, inside tanks or floating cages, shelters should be put in adequate number. The feeding habits and the utilisation in captivity of live food or dead whole marine organisms need frequently cleaned tanks or the utilisation of self-cleaning tanks (Vaz-Pires *et al.*, 2004).

About water quality parameters, octopus shows a very low tolerance to low concentrations of salts, normally living in a range fluctuating from 35 to around 27 g L^{-1} (Boletzky and Hanlon, 1983). For on-growing, the temperature should be kept between 10°C and 20°C, and better performances are achieved at the higher temperatures in this range. Temperature above 23°C can be responsible for high mortality, so that rearing in the Mediterranean area can be critical in summer months. This parameter affects the most important zootechnical parameters (growth, food conversion and ingestion).

The results obtained by Cerezo Valverde *et al.* (2003) highlighted that ammonia excretion is very important in this species compared to finfish, such as sea bass or sea bream. Thus, checking this parameter during rearing is fundamental. The same can be said for oxygen (Cerezo Valverde and García García, 2004), mainly in *post*-prandial period (from 6 to 16 hours after the meal ingestion), due to high consumption found in octopus. At a temperature of 17°C to 20°C, the optimum oxygen saturation resulted ranging from 100 to 65%, suboptimal saturation from 65 to 35%, dangerous below 35%, and lethal below 11% of saturation (Cerezo Valverde and García García, 2005). *Octopus vulgaris* shows a great resistance to hypoxia, similarly to other cephalopod species. The resistance to low oxygen levels was higher in small sized octopuses and at low temperature, this parameter playing an important role by increasing the lethal oxygen value. Given the relevant changes produced in the most suitable oxygen levels, temperature variations are highly critical for octopus. For this reason the utilisation of benthic cages could reduce the abrupt changes of environmental parameters associated with rearing in floating cages (Estefanell *et al.*, 2012). Males and females showed a different behaviour, the latter having greater oxygen consumption, conversely to what the same authors previously found on immature males and females (Cerezo Valverde and García García, 2004).

The separation according to sex improved culture yield, as the non-fertilised females continued to grow to commercial size without interferences due to the stopping of feeding during eggs care. The rearing performance of female can be further improved by using high light intensity in order to delay the sexual maturation and to achieve a greater somatic growth (Iglesias *et al.*, 2002), because egg formation generates higher metabolic requirements during sexual maturation of females (O'Dor and Wells, 1978). In other experiments, differences in growth and food intake between males and female were not found (Aguado-Giménez and García Garcia, 2002).

In relation to the hierarchical behaviour of octopus, the influence of rearing density has been studied. Domingues *et al.* (2010) tested growth and survival during on-growing of octopus at 3 densities (4 *vs* 8 *vs* 15 kg m^{-3}) in an experiment lasting 70 days. No differences were found for growth, but a lower survival characterised the highest density group. Even though the groups showed similar feeding rates, the food conversion rates were lower in medium and high density octopuses, probably for their more stressing and uncomfortable condition. Previously, Otero *et al.* (1999) tested 10 and 20 kg m^{-3} as stocking density, suggesting a density no higher than 10 kg m^{-3} to obtain the best growth performance. A modulation of density in relation to water temperature (higher density at lower temperature) and to culture system (higher in cage than in tank) should be considered.

Feeding

Lee (1994) studied the feeding behaviour of cephalopods and highlighted the importance of visual stimuli, chemical and textural properties of food, and nutritional quality of the diet, this last affecting the ingestion. Octopuses prefer live food, giving better performance with mixed diet than with monodiet. Cagnetta and Sublimi (2000) obtained a better growth when crabs were used in comparison to a diet based on squid or fish, even though squid simplify the management of tank for cleaning, due to reduced waste. In a recent paper, Estefanell *et al.* (2012) showed how feeding octopused on monodiet based on bogue (*Boops boops*) discarded from fish farms produced high growth (1.8 to 1.9% day^{-1}) and high survival (91 to 97%), and the best biomass increment (178 to 212%) and food conversion rates (2.3 to 2.6) ever recorded for *O. vulgaris* under industrial rearing conditions. The species can be easily adapted to dead food (Boucaud-Camou and Boucher-Rodoni, 1983) and to commercial dehydrated foods, as pelleted diets (Lee, 1994), that can be appreciated and ingested in relation to the specific texture characteristics. Different digestibility for lipids (46%) and proteins (96%) justifies the nutritional superiority of food poorer in lipids and richer in protein that is the main source of energy in octopus (Lee, 1994; O'Dor *et al.*, 1983). The knowledge of aminoacid composition of food could be essential for evaluating the adequacy of protein to satisfy energetic requirements. Crabs are also preferred to fish for specific pre-ingestional (size, shape, texture, flavour) and/or *post*-ingestional stimuli (digestibility, assimilation or energetic benefit) that this food can generate (Lee, 1994). The high variability of natural food tested in trials referred in different papers is responsible for the large differences found in documented growth responses and for non-consistent results (Aguado-Giménez and García García, 2002).

Even if research on artificial diets for cephalopods started 2 decades ago (in the early 1990s), the lack of an appropriate artificial diet for nutritional requirements of octopus still represents a limiting factor for its fattening phase.

Quality

The shelf life of octopus is very short, even at low positive temperatures, due to high protein deterioration which is responsible for an increase in nitrogen released during storage. Hurtado *et al.* (1999) reported a shelf life lasting 6 to 7 days after catch at 2.5°C and Barbosa and Vaz-Pires (2003) found a shelf life of 10 days at 0°C. Different methods and different parameters have been tested for monitoring quality and quality evolution during the shelf life, based on physical, chemical, and microbiological analysis. A sensorial scheme based on the Quality Index Method was developed by Barbosa and Vaz-Pirez (2003). In their trail, the authors found that the rejection of octopus kept in crushed ice occurs at day 8. Several attempts were carried out to increase the shelf life by an appropriate treatment (high pressure, heat combined to high pressure, gamma radiation) and to improve the textural properties inducing the softening of meat (Hurtado *et al.*, 2001a, 2001b, 2001c; Sinanoglou *et al.*, 2007). Recently, Mendes *et al.* (2011) tested the active packaging based on soluble CO_2 stabilisation (SGS) methodology to obtain ready-to-eat products. Even though no extension of shelf life was obtained, the bacteriostatic effect allowed an effective extension of the period of use by date. Besides the short shelf life, octopus shows other peculiarity as regards its quality, for example the high solubility of fibrillar protein, responsible for a considerable leaching when processing in water is performed, reducing nutritive value and affecting sensorial characteristics.

The edible portion of octopus is very high, compared to fish or crustaceans (80 to 85 *vs* 40 to 75 *vs* 40% to 45%) (Kreuzer, 1984). Also, its composition presents very low content in fat (0.54 to 0.94%, according to the season), where the total polyunsaturated fatty acids of the n-3 species (PUFAn-3) range between 42 and 47% (Ozogul *et al.*, 2008), and EPA and DHA represent a high percentage of total fatty acids (Zlatanos *et al.*, 2006; Ozogul *et al.*, 2008).

Critical points

The high market request in the Mediterranean, Latin America and Asian countries, the declining landings by fishery, the features of subadults and adults constitute excellent starting points for proposing octopus as a new candidate for aquaculture. Currently, the production from aquaculture is constantly increasing, even though it is still scarce (30 t).

Some reviews summarising useful information for the different steps of the octopus cycle in aquaculture conditions are available. Unfortunately, the results achieved until now are not conclusive and some bottlenecks reduce the feasibility of farming this species in a manner completely independent from the wild.

The high mortality in the paralarvae stage (Iglesias *et al.*, 2006) and the inexistence of adequate artificial feeds for paralarvae and subadults (Domingues *et al.*, 2005, 2006, 2007; Rosas *et al.*, 2007; García *et al.*, 2011) currently are the two major bottlenecks for the commercial aquaculture of this species (García García and García García, 2011). Evaluating the economic viability of *O. vulgaris* on-growing, García García *et al.* (2004) found that juveniles represented around 41% of total costs. Even though that incidence is highly variable depending on catches, the on-growing activity is now retained a high risk business with low profits. The success of paralarval rearing in order to achieve economic and environmental sustainability for octopus aquaculture is essential.

The management of this species in captivity (*i.e.*, during the different steps of the culture until slaughtering) should take into account that, like other cephalopods, *O. vulgaris* has some behavioural characteristics (Mather, 2008) which highlight the needs for the animal welfare consideration. In UK, Canada and Australia an appropriate legislation is already at stake (Berger, 2010).

Purple sea urchin

Distribution, habitat and exploitation

The purple sea urchin *Paracentrotus lividus* (Echinodermata: Echinoidea) is widely distributed in the Mediterranean sea and along the North-eastern Atlantic coast, from Scotland and Ireland to southern Morocco (Boudouresque and Verlaque, 2001). This sea urchin lives on rocky substrates and in seagrass meadows, from shallow waters down to about 20 m depth. It is a species of commercial importance, with a high market demand for its roe, particularly in the Mediterranean basin (Régis *et al.*, 1986) and more recently in other European non-Mediterranean areas (Byrne, 1990; Barnes and Crook, 2001). In the last decades, its populations have shown a wide scale decline in many European countries due to overfishing (Boudouresque and Verlaque, 2001). In Italy, the harvesting of *P. lividus* is a widespread activity mainly exerted in southern regions (Tortonese, 1965; Guidetti *et al.*, 2004; Gianguzza *et al.*, 2006; Pais *et al.*, 2007) and, despite the fishery of this species being regulated by a number of decrees (*i.e.*, fishing periods, minimum size of harvestable individuals and quantity per day per fisherman), the harvesting of *P. lividus* is intensively practised, particularly due to high tourism demand. For this reason, shallow rocky reef populations of *P. lividus* are mainly exploited by both authorised fishermen and poachers equipped with SCUBA, but also by occasional collectors throughout the year (Pais *et al.*, 2012b).

Reproduction

In general, *Paracentrotus lividus* biology has been well studied, and much research has been carried out to determine all the phases of the reproductive cycle from many European countries (Byrne, 1990; Lozano *et al.*, 1995; Spirlet *et al.*, 1998b; Sanchez-Espana *et al.*, 2004; Pais *et al.*, 2006a). This Echinoid has external fertilisation and gametes are released in the water. The development of the embryo is quite fast and is a multi-phase process.

In controlled conditions, adult sea urchins are taken out from the rearing seawater (18°C to 20°C) in order to obtain gametes. To do so, 0.5 to 1 mL (according to the specimen size) of a 5×10^{-1} M KCl solution are injected inside the mouth using a syringe. Afterwards, sea urchins are gently rotated putting them upside down on glass beakers containing sterilised and ultra-filtered (Millipore filters 0.22 μm) (Millipore Co., Billerica, MA, USA) seawater and the gonadal products (orange-red eggs from females and white emulsion from males) are emitted from the genital pores. After cleavage, the larval period can last differently, and metamorphosis may be delayed due to environmental features. In fact, the ciliary movement of the larvae can be negatively affected by several factors and, consequently, may influence their feeding ability. Generally, however, the *echinopluteus* stage is reached within 48 hours and if the *echinoplutei* are properly fed, they can undergo the metamorphosis within 3 weeks (Yokota *et al.*, 2002). Subsequently, the newly formed juveniles can settle in the substrate and their benthic phase begins.

Aquacultural activities

In case of severe depletion of *Paracentrotus lividus* wild stocks, aquacultural practices could represent a valid alternative to fishing (Fernandez and Pergent, 1998). Actually, as several recent studies demonstrated the possibility of improving cultivated edible sea urchin gonadal quality (Spirlet *et al.*, 2000; Shpigel *et al.*, 2005), local and tourism roe market demand could be supplied by industrial applications of similar techniques. Furthermore, the optimisation of *P. lividus* gametogenesis (Shpigel *et al.*, 2004; Luis *et al.*, 2005), if aimed at obtaining fertilised eggs, larvae and juveniles, could be useful to test restocking practises in the most severely exploited areas.

As described above, *P. lividus* artificial reproduction is a very easy practice. In contrast, the subsequent rearing phases are to some extent more difficult to carry out. *Post*-metamorphic juveniles can be fed using different algal species (Cellario and Fenaux, 1990; Grosjean *et al.*, 1996, 1998) until their mean individual size reaches 3 to 5 mm. However, since the growth of the juveniles is not homogeneous, sea urchins are graded regularly, and those with a diameter larger than 5 mm are transferred into *pre*-growing nurseries. Subsequently, the subadults (*i.e.*, specimens whose size exceeds 10 mm, but it is below the minimum marketable size of 40 to 50 mm) are positioned in rearing baskets with all sides made out of mesh. These rearing baskets are suspended in tanks to allow a good seawater circulation around and inside them, and an effective removal of solid wastes produced by the sea urchins. In general, during the growing phase sea urchins are fed *ad libitum* with fresh algae (or, in alternative, with artificial diets) twice a week. The cleaning of the baskets and tanks is regularly done and dead specimens are daily removed. Furthermore, sorting of sea urchins is done to divide the batches into different size classes. During the entire production cycle, a photoperiod of 12h/12h light/dark is usually used.

Rearing in Europe

In the past decades, several studies carried out in Europe have pointed out the possibility of successfully rearing this Echinoid. Indeed, much research has been done to reproduce the entire life cycle of *Paracentrotus lividus*, since aquaculture techniques have the potential for production of this species for both human consumption and for its use as model in research in developmental biology. Therefore, a number of studies have been conducted on the feeding preferences of juvenile and adult sea urchins and, as these echinoderms are herbivorous, they have focused on the use of several algal species (Le Gall, 1989; Frantzis and Grémare, 1992; Boudouresque *et al.*, 1996; Lemée *et al.*, 1996; Grosjean *et al.*, 1996, 1998; Cook and Kelly, 2007a; Cook *et al.*, 2007). On the other hand, the use of different artificial diets has been tested in trials aimed to improve gonadal quality and somatic growth of the sea urchins (Fernandez, 1997; Basuyaux and Blin, 1998; Fernandez and Pergent, 1998; Spirlet *et al.*, 1998b, 2001; Fernandez and Boudouresque, 2000; Pantazis, 2009; Fabbrocini *et al.*, 2011). In addition, a number of polyculture systems have been tried out in small-scale rearing experiments in order to enhance the production performances of *P. lividus* (Schuenhoff *et al.*, 2003; Cook and Kelly, 2007b, 2009).

At present, however, despite all the above mentioned farming experiences, no rearing activities of commercial importance are present in Europe. The only exception is represented by the Dunmanus Seafood Ltd. (Durrus, Ireland), in which hatchery-reared juveniles of this species are grown to market size mainly by ranching and are then seeded to rock pools or subtidal areas (Kelly and Chamberlain, 2010).

Conclusions

Shellfish culture can be regarded as a positive example of aquaculture activity. In particular, bivalve culture can be looked at as a paradigmatic example of sustainable economy safeguarding the environment. Indeed, it is at a low trophic level, it does not require the provision of feed input, it can help to reduce the level of water eutrophication, and it is suitable for integrated forms of aquaculture [*i.e.*, integrated multi-trophic aquaculture (IMTA)].

Nevertheless, we cannot hide or leave out some criticism on this activity, such as the environmental consequences due to the translocation of non native species which in turn are vectors of coastwise introduction of non-indigenous species (protists, algae, macroalgae, invertebrates, *etc*.), the impact generated on benthic communities (when the intensiveness of the culture is very high), or due the tools utilised for harvesting (as in the case of burrowing species).

The two sides of the same coin dictate more careful choices than those made in the past. These new choices should favour the cultivation of native species and avoiding the translocation of seed, juveniles and adults. This approach can be an economic driver, stimulating the development of a complete production chain, enhancing the creation of hatchery for spat production and reducing the potential dependence from other countries for seed supply. Although significant improvements could be achieved through specific actions on the species traditionally cultured, the way forward for the future is the culture of new species. The above survey, focused on the perspectives offered by some mollusc native species and the echinoderm *Paracentrotus lividus*, shows that we can make a virtue of necessity. This can be done thanks to research and experimentations carried out primarily in other countries that insist on the Mediterranean, but also in Italy where the transition from the experimental trials to the effective culture could be immediate for some species (*i.e.*, flat oyster and grooved carpet shell), or less immediate but certainly feasible for other species (*i.e.*, razor clams, octopus and purple sea urchin) for which we are still in a more or less advanced development of the whole culture process.

Therefore, if we move from theory to practice, enormous economic and environmental benefits can be achieved through the creation of new activities, the demand for new jobs, an expanded product offer for the market and, simultaneously, a reduced impact on natural resources that the traditional fishery for shellfish species of commercial importance notoriously produces.

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