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UNIVERSITÀ DEGLI STUDI DI TORINO

CURRENT STATUS AND FUTURE PERSPECTIVES OF ITALIAN FINFISH AQUACULTURE

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CURRENT STATUS AND FUTURE PERSPECTIVES OF ITALIAN FINFISH AQUACULTURE

Giuliana Parisi^{1,2*}, Genciana Terova^{1,3}, Laura Gasco^{1,4}, Giovanni Piccolo^{1,5}, Alessandra Roncarati^{1,6}, Vittorio Maria Moretti^{1,7},
Gerardo Centoducati^{1,8}, Pier Paolo Gatta^{1,9}, Antonio Pais^{1,10}

¹ASPA (Association for Science and Animal Production) Commission “Aquaculture”, Viterbo, Italy.

Website address: <http://aspa.altervista.org/>

²Department of Agri-Food Production and Environmental Sciences, Section of Animal Sciences, University of Florence,
Florence, Italy

³Department of Biotechnology and Life Sciences, University of Insubria, Varese, Italy

⁴Department of Agricultural, Forestry, and Food Sciences, University of Studies of Turin, Grugliasco (Torino), Italy

⁵Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy

⁶Department of the School of Veterinary Medical Sciences, University of Camerino, Matelica (Macerata), Italy

⁷Department of Veterinary Sciences for Health, Animal Production and Food Safety, University of Milan, Milan, Italy

⁸Department of Animal Production, University of Bari, Valenzano (Bari), Italy

⁹Department of Veterinary Medical Sciences, University of Bologna, Ozzano Emilia (Bologna), Italy

¹⁰Section of Animal Sciences, Department of Agriculture, University of Sassari, Sassari, Italy

*Corresponding author: Prof. Giuliana Parisi, Department of Agri-Food Production and Environmental Sciences, Section of
Animal Sciences, University of Florence,

Via delle Cascine, 5 – 50144 Firenze, Italy. Tel. +39 055 3288265 - Fax: +39 055 321216

E-mail: giuliana.parisi@unifi.it

Abstract

Currently available data show that shellfish and finfish production in Italy, derived both from fisheries and aquaculture activities, is on the order of 474,000 tons, each activity representing 50% of the total amount. In this context, the finfish aquaculture industry contributes on average 31 % to the national aquaculture production and on average 59 % of its value, giving a total amount of 72,000 tons and a value of around 351 million € (2010).

According to FEAP statistics, Italy is the fourth largest finfish producer in EU27, after the UK, Greece, and Spain, while it is also one of the six largest finfish producers among the non-EU and EU member countries, together with Norway, UK, Greece, Turkey, and Spain. Presently, fish culture activities are mainly focused on rainbow trout (*Oncorhynchus mykiss*, 55.5 %), followed by European sea bass (*Dicentrarchus labrax*, 13.6 %), gilthead sea bream (*Sparus aurata*, 12.2 %), gray mullet (*Mugil cephalus*, 5.3 %), sturgeon (*Acipenser* spp., 2 %), and European eel (*Anguilla anguilla*, 1.7 %).

Over the last 20 years, freshwater fish production and aquaculture (trout, carp, and eel) have decreased in Italy, with the exception of sturgeon. In contrast, marine fish production has significantly increased during the same period, and the two leading species, European sea bass and gilthead sea bream, presently contribute 25.8 % of the finfish production. From 1,900 tons in 1990, production reached 19,000 tons in 2010, with a 900 % increase, at an average percentage of 4.5 %. In addition,

new marine fish species were successfully cultured over the same period.

This review outlines the past and present situation of finfish culture in Italy and discusses future developments and priorities, with particular emphasis on new, emerging aquaculture species.

Key words: Italian emerging aquaculture species, production systems, reproduction and larval rearing, feeding and quality.

1. Introduction

1.1 Current status of Italian finfish aquaculture

Currently available data show that the production of fish, molluscs, and crustaceans in Italy, derived both from fisheries and aquaculture activities, is on the order of 474,000 tons (ISMEA 2012). In this context, the aquaculture industry contributes on average 49 % to the national production and 33 % of the revenue, providing a total amount of 232,000 tons and a value of around 600 million €.

Italy is among the four largest finfish producers in EU27, together with the UK, Greece, and Spain. According to FAO (2012e; 2012f), Italy is also one of the six largest finfish producer among the non-EU and EU members, together with Norway, the UK, Turkey, Greece, and Spain.

It is worth mentioning that, in addition to its national production, Italy needs to import more than 900,000 tons of fish and fish products yearly to meet the domestic demand. The average per capita consumption of fish and seafood products is 21 kg year⁻¹, and the self-supply grade of the aquaculture and fisheries chain is the lowest among the animal food chains, around 38 % (Table 1).

Rainbow trout is the most important species (55.5 %), followed by European sea bass (13.6 %), gilthead sea bream (12.2 %), gray mullet (5.3 %), and sturgeon (2 %). Marine fish production has increased significantly over the last 20 years, and the two leading species are presently contributing 25.8 % of the total finfish production.

Aquaculture production in Italy is presented in Table 2. The most important freshwater species cultured, some of which only for restocking purposes, are:

rainbow trout - *Oncorhynchus mykiss* (Walbaum, 1792)

brown trout - *Salmo trutta trutta* Linnaeus, 1758

marble trout - *Salmo marmoratus* Cuvier, 1829

Arctic charr - *Salvelinus alpinus* (Linnaeus, 1758)

Siberian sturgeon - *Acipenser baerii* Brandt, 1869

Russian sturgeon - *Acipenser gueldenstaedtii* Brandt & Ratzeburg, 1833

Adriatic sturgeon - *Acipenser naccarii* Bonaparte, 1836

white sturgeon - *Acipenser transmontanus* Richardson, 1836

79 black bullhead - *Ameiurus melas* (Rafinesque, 1820)

80 channel catfish - *Ictalurus punctatus* (Rafinesque, 1818)

81 common carp - *Cyprinus carpio carpio* Linnaeus, 1758 and several other carps (*Cyprinus* spp.)

82 tench - *Tinca tinca* (Linnaeus, 1758)

83 hybrid striped bass - *Morone saxatilis* (Walbaum, 1792) \times *Morone chrysops* (Rafinesque, 1820).

84

85 Of the aforementioned species, the traditionally farmed species trout, sturgeon, catfish, and carp and the new, potential species

86 tench represent more of 97 % of the total cultured freshwater species.

87 For the hybrid striped bass (*Morone saxatilis* \times *Morone chrysops*), originating from the USA and reared in northern Italy,

88 favorable results have been achieved in the last few years, with production surpassing 400 tons annually. On-growing takes

89 place in outdoor basins where hybrid striped bass are cultured and reared for 16-18 months to a large, marketable size (800-900

90 g). Indeed, the Italian Ministry of Agriculture has recognized the importance of this fish and has now included it in the updated

91 list of commercialized fish products and assigned to it the common name of “*persico-spigola*” (G.U. 2008).

92

93 As regards cultured euryhaline species, we can distinguish between traditional and new species. Traditional euryhaline species

94 include:

95 European sea bass - *Dicentrarchus labrax* (Linnaeus, 1758)

96 gilthead sea bream - *Sparus aurata* Linnaeus, 1758

97 European eel - *Anguilla anguilla* (Linnaeus, 1758)

98 thicklip gray mullet - *Chelon labrosus* (Risso, 1827)

99 golden gray mullet - *Liza aurata* (Risso, 1810)

100 thinlip mullet - *Liza ramada* (Risso, 1827)

101 leaping mullet - *Liza saliens* (Risso, 1810).

102

103 Over the past 20 years, new species have been successfully cultivated. However, it is worth noting that, while some of these

104 species are presently cultured close to commercial scale, the great majority of them are still in the experimental or pilot stage.

105 New, emerging aquaculture species are seen as providing possible opportunities for further diversification of Italian

106 aquaculture (Quémener et al. 2003). Unfortunately, the production of new finfish species is still currently restricted in relation

107 to market demand (meagre, *Argyrosomus regius*), competition with traditional species, and the lack of standardized

108 reproduction (Atlantic bluefin tuna, *Thunnus thynnus*), weaning, feed (greater amberjack, *Seriola dumerili*), and/or on-growing

109 techniques. Furthermore, research program coordination was transferred from the national level to the regional level and thus

110 the technology required for many research projects could not always be transferred to a wider range of practical users.

Reliable reproduction techniques have been developed for sharpsnout sea bream (*Diplodus puntazzo*) (Faranda et al. 1985), common dentex (*Dentex dentex*) (Rueda and Martinez 2001), white sea bream (*Diplodus sargus*), blackspot sea bream (*Pagellus bogaraveo*), common pandora (*Pagellus erythrinus*), red porgy (*Pagrus pagrus*), shi drum (*Umbrina cirrosa*), meagre (*Argyrosomus regius*) (Poli et al. 2003; Piccolo et al. 2008), common sole (*Solea solea*) (Bonaldo et al. 2011a), turbot (*Scophthalmus maximus*), and brill (*Scophthalmus rhombus*).

1.2 Production systems

Aquaculture in Italy can be divided into three different types of farming systems: extensive farming (land-based farms), semi-intensive farming (land-based farms), and intensive farming (land-based and offshore plants). According to the Consensus Platform (European Commission 2005), aquaculture activities can be classified into five segments, which are based on the combination of driving technical forces and controlling environmental conditions. According to these criteria, the following main production systems are found in Italian aquaculture:

1. *Flow-through systems*. The flow-through systems are almost exclusively land-based fish farming facilities, where water from a river is pumped through the production unit. The other water sources include springwater, pumped groundwater, cooling waters, or coastal waters. After running through ponds or tanks where the fish are reared, the water flows back into the river or sea. Some freshwater production systems utilize cooling water from steel or power plants. Some species, such as eel and sturgeon, can be reared in such systems.
2. *Extensive systems*. Euryhaline species are traditionally cultivated in extensive systems, contributing to wetland resource management and water use in coastal lagoon systems, which in Italy is called “*vallicultura*”. Large areas of brackish water are exploited for lagoon farming of commercially valuable species such as mullet, European eel, European sea bass, and gilthead sea bream.
3. *Coastal and offshore finfish systems* are used for marine species, including European sea bass, gilthead sea bream, sharpsnout sea bream, and tuna. For the cages, a broad range of technology, shapes, and sizes is available, and the cages are made of different materials. There is no specific design that is suitable for all locations and types of management.
4. *Recirculating aquaculture systems* (RAS) are used in marine hatcheries for land-based culture of euryhaline species. These systems are water-saving, with a strict control of water quality, low environmental impact, and a high level of technology. More than 80 % of Italian hatcheries producing fingerlings of Mediterranean species are based on RAS technology because of its environment-buffering capacity. Nevertheless, this technology is not relevant for on-growing stages. Indeed, a few such farms for on-growing fish are operating at the experimental or pilot scale in Italy, but none of them is of real commercial interest.

1.3 Key aspects and priorities

During the past 10 years, fish prices and, hence, profits have decreased in the Italian aquaculture sector due to the growth of the fish industry in other countries and to a market volume increase. In 2009, the import flow of fresh products consisted of 30,672 tons of mussels, 19,613 tons of salmon, 19,068 tons of gilthead sea bream, and 18,358 tons of European sea bass, accounting for 52.3 % of total imports of fresh fish (192,579 tons). Imports to Italy come mainly from Greece (19.3 %), Spain (17.3 %), and France (15.7 %) (ISMEA 2010).

Aquaculture products imported from other countries at low prices are very competitive as compared to the national products. This strong foreign competition has pushed the market prices towards their lowest level, with a consequent decline in profit for farmers. The farmers have reacted by diversifying their production towards gutted and filleted products and new processing and packaging techniques, with more integration among the production, processing, and marketing sectors. Furthermore, a strategy based on quality improvements and nutritional properties of aquaculture products has been promoted.

Aquaculture has been one of the fastest growing animal food sectors in the past few decades, but aquaculture is not developing at the same rate in Europe as in the rest of the world. According to a recent FAO report on the aquaculture situation in 15 countries of the Mediterranean area (Barazi-Yeroulanos 2010), the future of aquaculture growth in the Mediterranean greatly depends upon resolving some problems common to all Mediterranean countries. Competition for space with other users on the coastlines (tourism), simplifying licensing procedures, simplifying legislation so as to improve the industry's competitiveness and productivity, and high costs associated with compliance to strict environmental and health and animal welfare regulations constitute the main problems limiting the future of aquaculture growth.

In addition, as mentioned above, aquaculture is facing some market challenges, including a fall in market prices due to high global competition, increases in the fish supply, and fluctuations in demand. High production costs reduce the competitive advantage in relation to other global suppliers. The liberalization and expansion of the market and, in particular cases, an overproduction has saturated the market, resulting in price reductions and consequently lower profit margins for the producers. A wide range of external factors may affect the future development of Italian aquaculture. In order to strengthen the competitiveness and long-term sustainability of the fish-farming industry, therefore, Italian players and the State should consider focusing on the following key aspects:

- a) Environmental factors, with particular reference to climatic change, location of farms, and effects of industrial pollution. In this respect, a certification system for responsible and sustainable aquaculture practices should be adopted.
- b) Biological and technical aspects, mainly concerning disease issues and vaccine availability, but also including biodiversity, seasonality of the production, uncompleted life cycles for certain species, and optimization of feed in order to reduce the environmental impact.
- c) Market constraints, such as price fluctuation, image of aquaculture products, changes in trade policy and tariffs, changes in consumer preferences, and improving and extending distribution systems. Many consumers have a very limited understanding

of the aquaculture process. Efforts should be made to explain the rearing techniques to consumers, in order to change their perceptions and attitudes to farmed fish. Furthermore, more attention should be paid to consumer concerns regarding the quality of farmed fish and its safety, as these factors are becoming increasingly important and influence purchasing decisions. Attention should also be paid to production ethics with respect to fish welfare, environment, and sustainability.

d) Competitive factors, such as new, emerging species, new product forms (providing new processing and packaging solutions), and new producers. In particular, there is an increasing demand for certified products that address food safety, fish welfare, and environmental concerns, or a combination thereof.

e) Variations in inputs to the sector, mainly referring to wild seed recruitment, fish meal, and fish oil availability and costs, energy costs and labor costs.

f) Access to coastal areas, i.e., scarcity of potential sites for new aquaculture farms, and competition with other coastal users (urbanization, tourism, navigation, wildlife park projects, harbors, and maritime traffic).

g) Government policy and regulatory frameworks.

h) Financial factors such as investments, interest rates, taxation level, and insurance premiums.

i) Global and regional economic crises that have led to changes in consumer purchasing power.

In the following sections a detailed overview of traditionally farmed species [rainbow trout (*Oncorhynchus mykiss*), European sea bass (*Dicentrarchus labrax*), gilthead sea bream (*Sparus aurata*), sturgeon (*Acipenser* spp.), European eel (*Anguilla anguilla*), catfish such as black bullhead (*Ameiurus melas*) and channel catfish (*Ictalurus punctatus*), and cyprinids such as common carp (*Cyprinus carpio carpio*) and goldfish (*Carassius auratus*)] is given. Then a selection of the new, emerging aquaculture species [i.e., tench (*Tinca tinca*), Atlantic bluefin tuna (*Thunnus thynnus*), greater amberjack (*Seriola dumerili*), blackspot sea bream (*Pagellus bogaraveo*), sharpsnout sea bream (*Diplodus puntazzo*), common sole (*Solea solea*), turbot (*Scophthalmus maximus*) and brill (*Scophthalmus rhombus*), and meagre (*Argyrosomus regius*)] is examined in detail, considering different aspects of the rearing chain (such as reproduction, larval rearing and weaning, rearing, on-growing, quality, and market) with the objective of presenting current knowledge and of highlighting the most relevant critical points for each of them. The new, emerging aquaculture species have been selected by focusing attention on those with more promising perspectives concerning: i) importance for the market, ii) proximity and imminence of passing from the experimental scale to small-scale aquaculture and to the productive scale, and iii) involvement of some Italian researchers in finalizing culture techniques. In summary, the way forward for Italian aquaculture in the near future will proceed from and through these species and, in the short term, a new scenario is expected for this productive sector.

2. Traditionally farmed species

2.1 RAINBOW TROUT

207 A number of species of freshwater fish belonging to the genera *Oncorhynchus*, *Salmo*, and *Salvelinus*, all of the subfamily
208 Salmoninae of the family Salmonidae, are called trout. The rainbow trout, *Oncorhynchus mykiss*, originally named by Walbaum
209 in 1792, is native to the cold water rivers and lakes of the Pacific coasts of North America and Asia. One specimen of this
210 species was named *Salmo gairdneri* in 1836 and, in 1855, *Salmo iridia*, later corrected to *Salmo irideus* (Behnke 1966). In
211 1992, following DNA studies which showed that rainbow trout is genetically closer to Pacific salmon (*Oncorhynchus* species)
212 than to brown trout (*Salmo trutta*) or Atlantic salmon (*Salmo salar*), and the steelhead trout, *Salmo gairdneri* of the Salmonidae
213 family, was reclassified in the genus *Oncorhynchus* (“hooked nose”) and *mykiss* (a Siberian word for the species) (Behnke and
214 Williams 2007). Although they are the same species, the common name of the freshwater *Oncorhynchus mykiss* is rainbow
215 trout, whereas the sea-run rainbow trout (anadromous), which usually returns to freshwater to spawn after two to three years at
216 sea, is known as steelhead (Canada and the United States) or ocean trout (Australia) (Gall and Crandell 1992; Behnke and
217 Williams 2007).

218 Rainbow trout possesses the well-known streamlined (salmonid form) body shape. An adipose fin is present, which usually has
219 a continuous outline of black surrounded by a clear window. Body coloration is blue-green to olive above a reddish-pink band
220 along the lateral line and silver, fading to pure white at the lower parts. Back, sides, head, and fins are covered with small black
221 spots (Gall and Crandell 1992; Behnke and Williams 2007). However, body shape and coloration vary widely and reflect
222 habitat, age, sex, and degree of maturity; stream residents and spawners tend to be darker with more intense color, whereas lake
223 residents are brighter and more silvery (FAO 2012d).

224 *Oncorhynchus mykiss* is a fast-growing fish and can tolerate a wide range of environments and handling. It is capable of
225 occupying many different habitats such as gravel-bottomed, fast-flowing, well-oxygenated rivers and streams, cold headwaters,
226 creeks, and lakes (Coombs 1999). The species can withstand vast ranges of temperature variation (0-27 °C), but the temperature
227 range for spawning and growth is narrower (9-14 °C). The optimum water temperature for rainbow trout culture is below 21 °C.
228 It is usually not found in water reaching summer temperatures above 25 °C or ponds with very low oxygen concentrations.

229 Rainbow trouts are predators with a varied diet, which depends on the age and size of fish, on the size of the food item, and on
230 the habitat. The natural food items most frequently consumed by this species are aquatic and terrestrial invertebrates and small
231 fish. Terrestrial insects such as adult beetles (Coleoptera), flies (Diptera), ants (Formicidae), and larvae of Lepidoptera (moths
232 and butterflies) are also eaten when they fall into the water (Montgomery and Bernstein 2008). In the sea, rainbow trout preys
233 on fish and cephalopods. Some lake-dwelling strains may become planktonic feeders. In flowing waters populated with
234 salmonids, trout eats various fish eggs, including those of salmon and the eggs of other rainbow trouts, alevin, fry, smolt, and
235 even leftover carcasses (Gall and Crandell 1992). The most important food is freshwater shrimp, which contains the carotenoid
236 pigments responsible for the orange-pink color in the flesh. In aquaculture, this pink coloration can be produced by including
237 the synthetic pigments astaxanthin and canthaxanthin in aquafeeds (where desired) (FAO 2012d).

238 A normal adult rainbow trout weighs about 2-3 kg, while its maximum length and weight are 120 cm and 25.4 kg, respectively.

The maximum recorded life-span for a rainbow trout is 11 years (Froese and Pauly 2009). The anadromous strain is known for its rapid growth, achieving 7-10 kg within 3 years, whereas the freshwater strain can only attain 4.5 kg in the same period.

2.1.1 Reproduction

In rainbow trout, the onset of sexual maturity varies markedly between individuals as it is controlled by complex interactions between genetics and environmental conditions (water temperature, food availability, population density, and productivity of the aquatic environment). In small streams, rainbow trout females mature at 2 or 3 years of age and males often mature a year before females. Once maturity is reached, trout may spawn annually or skip a year or two before spawning again. Rainbow trouts have been known to spawn up to age 11 (Froese and Pauly 2009).

Spawning takes place in the autumn-spring, from November through April, as daily water temperatures reach 6-10 °C. Females are able to produce up to 2,000 eggs kg⁻¹ of body weight. Eggs are relatively large in diameter (3-7 mm). Before spawning, he adult rainbow trouts usually seek out the shallow gravel riffles or a suitable clear water stream. The female uses her tail to prepare a nest, 10 to 30 cm deep and 25 to 38 cm in diameter. From 200 to 8,000 eggs are deposited in the nest, fertilized by a male, and covered with gravel. Hatching normally takes place from a few weeks (4-7) to as much as 4 months after spawning, depending upon the water temperature; for example, eggs will hatch within 30 days at 10 °C. A few more weeks may be required for the tiny fry to emerge from the gravel (Lucas and Southgate 2012).

In aquaculture systems, trout do not spawn naturally; hence, eggs are artificially spawned from high-quality brood fish when fully mature (ripe). Either the wet or dry method can be used to fertilize the eggs. The most common approach is the dry method of fertilization, without admixture of water. In this case, eggs are removed manually from ripe females by applying pressure from the pelvic fins towards caudal direction or by air spawning, causing less stress to the fish and producing cleaner, healthier eggs. Inserting a hypodermic needle about 10 mm into the body cavity near the pelvic fins and air pressure (2 psi) expels the eggs. The air is then removed from the body cavity by massaging the sides of the fish. An anesthetic is used to calm the fish to reduce stress, minimize any potential handling injury, and avoid damaging the eggs (Hinshaw et al. 2004). Eggs are collected in a dry pan and kept dry, improving fertilization. Males are stripped in the same way as females, collecting milt in a bowl, avoiding water and urine contamination. To ensure good fertilization and to reduce inbreeding, milt from more than one male is mixed with the eggs, usually mixing milt from three or four males prior to fertilization.

Water is added to activate the sperm and increase the size of the fertilized eggs by about 20 % by filling the perivitelline space between the shell and yolk; this process is known as "water-hardening" (FAO 2012d). Water-hardened eggs can be transported from 1 to 48 hours after fertilization, but after 48 hours the eggs are very sensitive to any movement and should not be moved until they reach the eyed stage (when the eye pigment is clearly visible), after 180-200 day degrees (i.e., 26-29 days after fertilization at 7 °C). At the eyed stage, eggs are robust and can be transferred to a container to flush away debris and dead eggs. Rainbow trout hatcheries may be based on flow-through systems, but an increasing number of Italian farmers are using

recirculation technology of varying designs. Both systems are equipped with several incubators, each with a number of hatching trays. The temperature of the hatching water is usually kept constant at about 7 °C and, to prevent infective agents from developing, the water may be sterilized by UV light.

2.1.2 Larval rearing and weaning

Rainbow trout eggs hatch after about 300-370 day degrees (i.e., about 45 days after fertilization at 7 °C, 100 days at 3.9 °C, and 21 days at 14.4 °C) (Hinshaw et al. 2004; Jokumsen and Svendsen 2010). Typically, 95 % of rainbow trouts hatch with a reserve of nutrients in a yolk sac; hence, they are referred to as yolk-sac fry or alevins. Hatching rate depends on the water temperature, but is usually completed within 2-4 days. The developing embryos and fry feed from the yolk sac and receive oxygen through the entire body surface (Woynarovich et al. 2011). When the yolk has been depleted after about 120 day degrees (about 14–20 days at 7 °C) posthatching and the mouth is fully developed, the fry swim up to the water surface to inflate the swimbladder and start feeding exogenously (Jokumsen and Svendsen 2010). At this time, weaning with very fine dry feed (powder) can be initiated.

Fry are traditionally reared in fiberglass or concrete tanks, preferably circular in shape, to maintain a regular current and uniform distribution of the fry. Water is delivered to the side of the tank using an elbow pipe or a spray bar to create water circulation (Woynarovich et al. 2011). The fry facility may also be a flow-through system with concrete raceways as the fish-holding tanks. The water supply may be a spring, a well, or a nearby watercourse. However, water supplied from a spring or a bore well is preferred due to the lower risk of pathogens, the constant temperature, and the more stable water supply and quality. Some fry producers use recirculation technology to improve the production efficiency. The benefits of using recirculation technology include the possibility of higher rearing temperature and securing high and constant water quality, which give better growth potential and fish health (Jokumsen and Svendsen 2010; Woynarovich et al. 2011).

Fry (with a total length of 5 cm and average body weight of 2 g) are fed specially prepared starter feeds when approximately 50 % have reached the swim-up stage. The feed is administered in excess to make sure that all fish are offered feed. As the fish grows, the pellet size and daily feed amount is adjusted accordingly (Hinshaw et al. 2004).

After starting external feeding, the actual duration of development in the different age groups depends not only on the temperature and oxygen content of water but also on the quality and quantity of consumed feed. In trout that are adequately fed with commercial feeds, the fry stage lasts approximately 500 day degrees (i.e., about 10 weeks at 7 °C); by the end of this stage, the fish fry may have reached about 5 g each. At this stage the young fish are called fingerlings. During the following 2-3 months the fingerlings may grow to be about 50 g each at about 7 °C (Jokumsen and Svendsen 2010). The feed pellets provide nutritional balance, encouraging growth and product quality, and are formulated to contain approximately 50 % protein, 12-15 % fat, vitamins, minerals, and possibly a pigment to achieve pink flesh (where desirable) (FAO 2012d).

The fingerlings are moved from the fry and fingerling-producing systems to production farms for on-growing to marketable

size.

2.1.3 Rearing

Monoculture is the most common practice for cultivating rainbow trout in Italy, and intensive systems are considered necessary in most situations to make the operation economically attractive. A variety of grow-out facilities are used, ranging from flow-through systems to cages in lakes. The majority of Italian trout farms use flow-through systems which consist of raceways or concrete tanks with continuously flowing water. The dimensions of raceways can vary widely, depending on the size and shape of the area available to the farmer. Water is pumped into one end of the raceway, flows down the raceway, and is removed at the outlet end by gravity or a pump; water quality can be improved by increasing flow rates. Although the raceways provide well-oxygenated water, the stock is vulnerable to external water quality, and ambient water temperatures significantly influence growth rates (Hardy et al. 2000). Fish are grown on to marketable size (30-40 cm), usually within 9 months, although some fish are grown on to larger sizes over 20 months.

Alternative on-growing systems for trout include cage culture production systems, where fish are held in floating cages, ensuring a good water supply and sufficient dissolved oxygen. This method is technically simple as it uses existing water bodies at a lower capital cost than flow-through systems; however, stocks are vulnerable to external water quality problems and fish-eating predators (birds), and growth rates depend on ambient temperature (FAO 2012d).

As previously noted, one of the basic, vital factors influencing the growth of rainbow trout is the quality of water. Rainbow trout is a typical cold water fish: the appetite of rainbow trout is optimal in the water temperature range of about 7-18 °C; above 18 °C, fish appetite sharply decreases and feed intake stops. The water temperature in which the trout make the best growth out of the consumed feed varies from 13 to 15 °C (Molony 2001). Rainbow trout needs clear water as keen eye sight is crucial for efficient feeding in this species. The water should sustain dissolved oxygen in high concentrations (near saturation, 7-10 mg l⁻¹), in order to ensure smooth respiration; water should be clean, i.e., free of harmful solid and harmful gaseous waste materials produced during metabolism and respiration. As for the pH of water, both the optimal and acceptable ranges for age groups from swim-up fry to table fish are from 6 to 8.5 (Woynarovich et al. 2011).

In order to ensure that used water is replaced in the rearing devices, a continuous supply of fresh, clean, and oxygen-rich water is essential. A rule of thumb is that a year-round supply of about 10 l sec⁻¹ (600 l min⁻¹) high-quality water should be calculated for each ton of rainbow trout produced (Edwards 1990; Hardy et al. 2000).

In general, both cold surface and underground waters are good for trout farming. Therefore, trout farming is an ideal option for sustainable use of water resources in Italy's mountainous regions because both surface and underground waters are suitable for this purpose there. Groundwater can be used where pumping is not required but aeration may be necessary in some cases. Under certain conditions, well water may become supersaturated with one or more gases, most frequently with nitrogen. In this situation, fish can develop gas emboli, which prevents circulation and causes a condition known as gas-bubble disease.

Alternatively, river water can be used but temperature and flow fluctuations alter production capacity. Feeding is the most expensive part of producing trout (40 to 60 % of the cost of production) (Kaushik 2000). Feeds for rainbow trout have been modified over the years and cooking-extrusion processing of foods now provides compact, nutritious, pelleted diets for all life stages. The previously developed high-protein feeds with a feed-conversion ratio (FCR) of between 2 and 3 have been definitively replaced in the modern trout-farming industry by very efficient pelleted dry feeds (0.6-1.1 FCR). Feed formulations for rainbow trout use fish meal, fish oil, and other ingredients, but the amount of fish meal has been reduced to less than 50 % in recent years by using alternative protein sources (FAO 2012d).

2.1.4 Main issues

Trout farms inevitably impact upon the environment. Escapee trout from farms can have negative impacts, potentially displacing endemic species (especially brown trout) and exhibiting aggressive behavior that alters fish community structure. There are also problems with the transmission of diseases from farmed stock to vulnerable wild populations. Impacts from flow-through systems largely involve disease treatment chemicals, uneaten feed, and fish excreta, which can alter water and sediment chemistry downstream of the farm (Bergero et al. 2001). When properly managed, flow-through systems employ means to capture solid wastes and dispose of them in an appropriate manner. Output restrictions in Italy require farms to have settling areas to remove solid wastes (Trincanato 2006).

2.1.5 Quality and market

Two aspects must be considered in assessing the quality of rainbow trout: its nutritional value as a source of n-3 PUFA (polyunsaturated fatty acids) and sensory attributes (Kiessling et al. 2001).

Salmonids are usually considered to be medium-fat fish, with muscle lipid content in the range of 5-10% (Jobling 2001). An average protein content of 20 %, fat content of 3 %, and mineral (particularly calcium, phosphorus, magnesium, and potassium) content of 1.2 % make the rainbow trout a lean fish, recommended as ideal food for human consumption. Trout fillet is also appreciated for its tenderness, juiciness, and flavor (Vranić et al. 2011). Clinical studies have shown that there is a connection between cholesterol introduced by food, cholesterol in blood plasma, and atherosclerosis; thus, the relatively low amount of cholesterol and the PUFA composition of trout make it a very suitable type of fish for human nutrition. Studies by Cahu et al. (2004) and Lichtenstein et al. (2006) indicate that freshwater fish can be a good source of n-3 PUFA because these fish desaturate fatty acids and form long-chain PUFA, EPA (C20:5n-3, eicosapentaenoic acid) and DHA (C22:6n-3, docosahexaenoic acid), better than sea fish.

It is a known fact that freshwater fish caught under natural conditions contains less fat and higher amounts of EPA and DHA than farmed fish of the same species, when these values are expressed as percentage of total fatty acids. However, fish from aquaculture contain a higher percentage of total fat and, when values for PUFA are expressed per 100 g of fish, intake of EPA

and DHA in the human organism is higher when farmed fish is consumed than for the same fish species caught in nature (Vranić et al. 2011).

Weaver et al. (2008) carried out a study to investigate the fatty acid profile of fish from aquaculture in comparison to that of the same fish species caught in nature. They concluded that, of 30 fish species from aquaculture and free-catching, the highest amounts of n-3 PUFA were found in farmed trout and salmon (above 4 g/100 g). The highest variations in content of n-3 fatty acids were established in trout, as a consequence of different rearing methods and feeding systems, and under different aquaculture conditions.

With regard to the nutritional value, the quality of fish lipids is determined by PUFA/SFA and n-3/n-6 ratios (Ahlgren et al. 1996) as, in addition to optimal quantities of essential fatty acids, the n-3 and n-6 intake ratio is important. Healthy ratios of n-3/n-6, according to some authors, range from 1:1 to 1:4 (an individual needs more n-3 than n-6 fatty acids) (Lands 2005). Other authors believe that a ratio of 1:4 (when the amount of n-6 is 4 times greater than that of n-3) is already healthy (Simopoulos and Artemis 2002; Simopoulos 2003). Henderson and Tocher (1987) reported n-3/n-6 value of 0.5-3.8 for freshwater and 4.7-14.4 for marine fish. Higher amounts of n-3 in marketable size trout fillets and lower amounts of n-6 PUFA give a very favorable n-3/n-6 ratio (1.60) compared to, for instance, carp, in which this ratio is less favorable (0.08) due to the higher amount of n-6 and lower content of n-3 fatty acids.

In farmed salmonids, flesh color is another important quality trait, together with freshness and nutritional value of the product (Anderson 2000). Synthetic astaxanthin and canthaxanthin still represent the most important sources of pigments used in the feeding of farmed salmonids because of their high pigmentation ability for both skin and flesh.

The trout-farming sector has always been the most popular one in Italy, practically the only option for rearing freshwater species. Almost 70% of the approximately 360 intensive production sites are located in northern Italy, between Friuli-Venezia Giulia, Lombardia, Veneto, Trentino, and Tuscany (Schiavo 2008).

As regards rainbow trout production in the Mediterranean area, Italy ranked at the top in Europe until the late 1990s, with more than 51,000 tons in 1997 (API 1999). After that time, difficulties due to the significant competition with Atlantic salmon and other fish species such as European sea bass and gilthead sea bream, together with the dioxin scandal in Belgium, the incidence of transmissible bovine spongiform encephalopathy (BSE), and the prohibition of feeds that contained terrestrial animals in 2001 increased production costs and decreased production, which dropped to 40,000 tons in 2010.

In order to reduce costs and enhance the sustainability of trout aquaculture, the most important feed manufacturers began to substitute the fish meal and fish oil contained in trout feeds with less expensive protein and lipids from vegetable sources (Kaushik et al. 1995; Romarheim et al. 2006; Turchini et al. 2009; Mustapha et al. 2013). An analysis of potential trout consumers carried out in different European countries more than 10 years ago ascertained that, in Italy, rainbow trout is perceived as a traditional product, with over two-thirds of trout eaten in the form of the whole fish (Cookson 2001). However, the panorama is changing. The increase in the value of production over the last decade - despite a decrease in volume - is

mainly due to the value added in processing, including filleting, precooked, marinated, smoked, and minced products, and new ready-to-eat preparations. Furthermore, the red-pink flesh coloration, produced by depositing relatively large amounts of carotenoids such as astaxanthin obtained from the diet in muscle, is becoming decisive for diversification of trout as a product. The presence of one of the most important European trout companies in Italy, with 14 fish farms located in different regions of the country and one in Belgium, has contributed highly to this effort and improved marketing and promotion of the national product and significantly enhanced the food quality. In the last few years, a general awareness of the importance of the traceability and labeling of trout products has increased, in accordance with the Codex Alimentarius Commission (2003) and the Code of Responsible Aquaculture published by the Italian Association of Fish Farmers (API 2003) and based on the Federation of European Aquaculture Producers guidelines. As reported by the Federation of European Aquaculture Producers (FEAP 2012), Turkey is still the largest trout producer in the Mediterranean area with 85,165 tons, followed by Italy with 41,000 tons. The future of rainbow trout cultivation will probably be linked to reducing the environmental impact, improving efficiency in preventing diseases, and obtaining a product of high quality, as described in a recent paper (Trincanato 2006). The production of rainbow trout for sport fishing does not have any perspective to expand as these fish are only introduced in private waters; it seems to continue to absorb 13,000 tons destined for recreational ponds and artificial reservoirs for fishing competitions. An interesting opportunity is offered by the rearing of other salmonid species, such as brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*), which are valued both for sport fishing and meat quality. As for the Italian market prices, in March 2013, the price for whole rainbow trout ranged from a minimum of 2.50 € kg⁻¹ to a maximum of 3.60 € kg⁻¹, whereas prices for rainbow trout fillet ranged from a minimum of 2.50 € kg⁻¹ to a maximum of 3.50 € kg⁻¹, with an average price of 3.00 € kg⁻¹.

2.2. EUROPEAN SEA BASS

The European (or common) sea bass, *Dicentrarchus labrax* (Linnaeus, 1758), one of six members of the Moronidae family, belongs to the order Perciformes (perch-like fish), which is the largest order of vertebrates, containing about 40 % of all bony fish. The European sea bass is primarily a marine fish, but it is sometimes found in brackish and fresh water; its habitats include coastal waters, estuaries, lagoons, and rivers (Pickett and Pawson 1994). It is found in coastal waters of the Atlantic Ocean from South of Norway (60°N) to Western Sahara (30°N) and throughout the Mediterranean and the Black Sea (Wheeler 1975). It has also been introduced for culture purposes in Israel, and more recently in Oman and the United Arab Emirates. The European sea bass has a silvery, elongated body (depth of body is 22-27 % of total length), with two clearly differentiated dorsal fins, and a rather high tail (Pickett and Pawson 1994). The body is covered by large, regular scales and its color is dark gray on the back, passing to gray-silver on the sides, while it is white-silver or pale yellow on the abdomen. Specimens from

the sea show a much clearer color than fish from lagoons and estuarine environments. The maximum length of European sea bass is over 1 m and weight is over 12 kg.

The European sea bass is a highly eurythermal (2-32 °C) and euryhaline (0.5 to 36 ‰ salinity) fish. The influence of temperature or the availability of food may be the main motivating factors for postlarval and older bass to enter low-salinity waters. However, it is quite possible for them to adapt to freshwater under cultivation conditions (Pickett and Pawson 1994).

The commercial value is high for both for fish captured from wild stocks and from aquaculture production. In Italy, European sea bass was historically cultivated in coastal lagoons and tidal reservoirs before the race to develop the mass-production of juveniles started in the late 1960s (Ravagnan 1992). Fish culture was initially associated with salt production in coastal evaporation pans and marshes. During the late 1960s, France and Italy competed to develop reliable mass-production techniques for juvenile European sea bass and, by the late 1970s, these techniques were well enough developed in most Mediterranean countries to provide hundreds of thousands of larvae. *Dicentrarchus labrax* was the first marine nonsalmonid species to be commercially cultured in Europe. At present it is the most important commercial fish that is widely cultured in the Mediterranean area, with Greece, Turkey, Italy, Spain, Croatia, and Egypt being the largest producers (FAO 2012c).

2.2.1 Reproduction

The European sea bass is a gonochoric species: the female shows a deeper body with a longer pointed head and greater predorsal and preanal lengths. Sexual dimorphism for growth exists and is characterized by a growth advantage of females over males. Stronger at early stages (70 %), the growth difference tends to stabilize at 20-30 % after the second year (Saillant et al. 2006). Sex can only be confirmed with certainty, however, during the spawning season by observing the protrusion of the anus and genital papilla in the females and checking for the presence of sperm by gently squeezing the males (Moretti et al. 1999). Sex determination in European sea bass has been the object of much research activity (see review of Piferrer et al. 2005) because a heavily skewed sex ratio appears in the species under culture conditions, with between 70 and 99 % of offspring typically being male. The results of these studies showed that sex in European sea bass is determined by both genetic and environmental factors, the main environmental factor being temperature (Piferrer et al. 2005).

The European sea bass is a seasonal breeder, spawning in winter and early spring, with some differences in timing according to the specific location. There is only one reproductive season per year, which takes place in winter in the Mediterranean population (December to March) and up to June in the Atlantic populations (Moretti et al. 1999). Sexual maturation ensues earlier in males and earlier in southern populations. Precocious males mature soon after sexual differentiation during the first year (average of 50 g) and the male population as a whole completes maturation during the second year. Females reach puberty between the second and third year (Chavanne et al. 2008).

The European sea bass is a fractional spawner (3-4 depositions over the reproductive season). Male semen is characterized by a high sperm concentration (up to 60×10^9 spz. ml⁻¹) and a short duration of sperm motility (<1 min). Fertilization takes place

externally: females deposit unfertilized eggs and males swim by and fertilize them. Fertilized egg diameter ranges from 1.2 to 1.5 mm and female fecundity averages 200,000 eggs per kg body weight (Chavanne et al. 2008). As fecundity and egg quality improve after the first spawning, the optimal parent age for female European sea bass is between 5 and 8 years, whereas for males this range is lower at 2-4 years (Moretti et al. 1999; Chavanne et al. 2008).

Fertilized European sea bass eggs float in full seawater (35 to 37 ‰ of salinity) and hatch between 4 and 9 days after fertilization, depending on the sea temperature. During the following 2-3 months, the growing larvae drift from the open sea inshore towards the coast, and eventually into creeks, backwaters, and estuaries. Juveniles occupy these sheltered habitats for the next 4-5 years, before they mature and adopt the migratory movements of adults (Pickett and Pawson 1994).

In aquaculture, sperm release and egg deposition can be enhanced by hormonal induction. By using different hormone delivery systems egg deposition can be synchronized in females three days after treatment or milt production in males and multiple spawns in females can be enhanced over the longer term (Forniés et al. 2001), but none of these systems are officially registered in the EU.

2.2.2 Larval rearing and weaning

European sea bass larvae are not completely formed immediately after hatching. At that time, they have a total length of around 4 mm and a large yolk sac (almost 1/2 of the whole body length), their eyes are not functional, and the mouth is closed (Barnabé 1990; Moretti et al. 1999). Within the following three to six days, the length of larvae reaches 5 mm, the eyes became completely pigmented, the volume of the yolk sac decreases by more than 60 %, the mouth opens, and the digestive tract, though still primitive, can now assimilate food. Then, larval swimming behavior becomes active and the animal is thus able to maintain a horizontal position. At the end of this period, the postlarval stage begins and the young fish starts feeding on live preys, such as rotifers or brine shrimp nauplii.

As no artificial larval diet can completely fulfill larval nutritional requirements at present, successful rearing still depends on an adequate supply of high-quality live feeds, usually in the form of rotifers (the rotifer *Brachionus plicatilis* fed on unicellular algae) and brine shrimp (*Artemia salina*). Due to the larger size of its mouth at first feeding, European sea bass fry can accept *Artemia* as the first prey; thus, a supply of rotifer is not compulsory. Therefore, in farms only dealing with European sea bass, live feed production is often limited to *Artemia* nauplii, which are hatched after incubating the dry resting eggs (cysts) (Saroglia and Ingle 1992; Sola et al. 1998; Moretti et al. 1999).

Feeding on live prey usually lasts 40 to 50 days, according to water temperature, and the rearing protocol. Then fish are switched to an artificial diet (weaning). The timing of the first dry food supply has been continuously moved forward to an earlier date in recent years due to new, more elaborate artificial diets, including vitamins and immunostimulants, which better fit the larval requirements in terms of composition, size, buoyancy, and flavor (Moretti et al. 1999). The young fish actually receive the first feeding with inert feed at the very early age of 17-19 days, but dry, compounded feed does not become their

only source of nutrition until much later. Young fish are weaned in a dedicated section of the hatchery where the metamorphosed fish (about 45 days old) can grow to a size of 2-3 g. At this stage, they are called fingerlings or juveniles and must have assumed the adult appearance. When a size of 2-5 g is reached, weaned fry leave the hatchery and are stocked in the on-growing/fattening facilities (Moretti et al. 1999). The majority of Italian hatcheries are located in Veneto, followed by Apulia and Tuscany. The two major hatcheries, “Valle Ca’ Zuliani” in Veneto and “Panittica Pugliese” in Apulia, produce approximately 65 % of the country’s fingerling supply. Almost 95 million juveniles were produced in 2002, of which 50 million were European sea bass. Approximately 20 million European sea bass juveniles were exported in 2002, mainly to Greece. In 2007, fingerling production exceeded the national demand (Cardia and Lovatelli 2007).

2.2.3 Rearing

Rearing European sea bass has a long tradition in Italian aquaculture. Fish were allowed to enter appropriate lagoon sites, after which the entrance was closed off, trapping them inside. The trapped sea bass were fed naturally until they were harvested. This constitutes the principle of Italian *vallicultura* (Ravagnan 1992), which is still operational today. In such an extensive system of rearing, sea bass reach a commercial size of 400-500 g in 37 months, with a total lagoon production of 50-150 kg ha⁻¹ year⁻¹ (FAO 2012c). The drawback of this system is the natural feeding behavior of the sea bass because, as predators, they may drastically reduce the natural resources of the lagoon ecosystem.

As an alternative project to improve the system, a semi-intensive lagoon system has been developed which is artificially enriched with fry and then fertilized. Specialist fishermen collect the fry from coastal waters during May and June; then fry are transported in oxygenated tanks for a first stage of growing in special ponds until they are large enough to survive in the lagoon. The production is higher than in the extensive system and amounts to 500-700 kg ha⁻¹ yr⁻¹.

In the 1960s, the increasing scarcity of young fish and the example of salmon farming in northern Europe prompted scientists in the Mediterranean countries to develop an intensive rearing process based on a complex hatchery technique and the production of specific feeds. Extensive scientific research programs were established in the 1960s and 1970s to investigate the complex process of rearing sea bass for intensive breeding. As a result European sea bass farming was initiated in the Mediterranean in the 1980s (FAO 2012c).

In intensive systems, the production units yield more fish by using higher levels of technology and a greater degree of management. Here, on-growing or fattening units are supplied with fry from hatcheries. Juveniles are sold to farmers as on-growing stock at a size of 1.5-2.5 g. The on-growing juveniles reach 400-450 g in 18-24 months. A controlled diet is provided and distributed by automatic feeders. Fish are graded into homogeneous size groups at least twice or three times per cycle in order to avoid growth differentiation and cannibalism. Fattening/on-growing can proceed in water-based systems such as floating cages or land-based systems such as ponds, tanks, and recirculating systems. The level of technology at Italian aquaculture farms is high for both land-based plants and sea cages (Barazi-Yeroulanos 2010).

All Mediterranean countries produce European sea bass and also gilthead sea bream in cages. The leading countries, according to production volume in 2004, were Greece, Turkey, Spain, Italy, Croatia, and France. Altogether these countries accounted for more than 90 % of the total cage production of these two species (Cardia and Lovatelli 2007). The first sea cage-based farms in Italy were established at the late 1990s (Barazi-Yeroulanos 2010). The Italian shoreline has limited sheltered sites and this represents a constraint for expanding the sector. Furthermore, tourism (a major economic sector) often competes for the use of sea and shore resources. Approximately 60 % of marine cage farms are currently located in semi-offshore or offshore sites, which entails higher production costs and requires that different technological solutions in terms of cage models and mooring systems are adopted. Compared to other countries in the Mediterranean, Italy operates a large number of cages specifically designed for offshore sites and several submergible models (Cardia and Lovatelli 2007). The regions of Italy with the most European sea bass/gilthead sea bream farms in floating cages are, in order, Sardinia, Sicily, Apulia, Lazio, Friuli, Liguria, Campania, Tuscany, and Veneto.

Tanks for European sea bass rearing are land-based systems usually supplied with seawater (38 ‰) maintained in a continuous flow-through system under ambient temperature. Alternatively, brackish water (30 ‰) pumped from adjacent lagoons may be used. Stocking densities are high (20-35 kg m⁻³); thus, it is essential to accurately control the water quality and carefully monitor fish health. The most common tank designs in standard use in European sea bass aquaculture systems are round tanks and raceways.

Recirculation systems to control water temperature (between 13-18 °C) are used during autumn/winter, frequently full-time in hatcheries and during the prefattening phase of the production cycle. Such a system is also used for fattening at high-technology farms. This practice improves growth but can be very expensive due to the technology required for water quality control (filtering, air stripping, UV treatment, and catabolite removal) (FAO 2012c).

2.2.4 Critical points

The variability in fry quality remains one of the main problems. Other important issues are related to the species' slow growth, its susceptibility to viral diseases in warm waters, and to its poor conversion efficiency (>1.6 in large size fish). Some of these critical aspects can be linked to the fact that, in farming conditions, there is an excess of males (70 to 95 %) in which sexual maturation is precocious (<100 g) and growth slower (Haffray et al. 2006).

2.2.5 Quality and market

Owing to the taste of European sea bass, it is one of the most economically important cultured fish in the Mediterranean area (Saglik et al. 2003), which also explains why it is one of the most studied fish in aquaculture. In the last decade, several studies focused on the fatty acid and mineral composition of seabass flesh (Saglik et al. 2003; Periago et al. 2005; Erdem et al. 2009). Bhouri et al. (2010) evaluated the nutritional quality of *D. labrax* by analyzing the total lipid (TL) content and the fatty acid

compositions of dorsal and ventral muscles in wild and farmed fish. The farmed European sea bass showed a considerably higher TL content than the wild fish, with ventral muscle being richer in TL than dorsal muscle in both types of fish. A high TL content in farmed sea bass was also reported by Alasalvar et al. (2002) and Saglik et al. (2003).

In muscle of both wild and farmed fish, concentrations of PUFA were higher than those of saturated (SFA) and monounsaturated (MUFA) fatty acids. The concentrations of n-3 and n-6 PUFA in dorsal and ventral muscles were similar in wild and farmed sea bass, revealing lower values in the n-6 PUFA than in the n-3 PUFA fraction. Docosahexaenoic acid (C22:6n-3) was the prominent PUFA in muscles of both types of fish, followed by arachidonic acid (C20:4n-6) and eicosapentaenoic acid (C20:5n-3).

It has been reported that assimilation patterns of dietary fatty acids in fish muscle reflect the content of the dietary lipid sources (Pirini et al. 2000; Izquierdo et al. 2003). Generally, the high content of SFA sometimes observed in farmed European sea bass is due to the FA composition of the manufactured diets, which may contain high levels of SFA and MUFA, but are deficient in n-3 PUFA (Alasalvar et al. 2002; Orban et al. 2002; Periago et al. 2005).

However, in intensive fish farming the quality of the entire production process can be controlled and the final product quality obtained is very close to that of wild fish. In particular, the lipid profile of fish fillet can be modulated, within certain limits, through the formulation of feeds with high levels of n-3 PUFA, which are known to be responsible for the health-promoting effects of fish lipids (Orban et al. 2003).

Orban et al. (2002) compared the nutritional quality of free-living European sea bass in the lagoon of Orbetello (Tuscany, Italy), one of the areas with the highest catch of euryhaline species in Central Italy, with European sea bass cultured intensively in tanks supplied with water from the lagoon. Fish of comparable size were analyzed for mineral content and fatty acid profiles of total, neutral, and polar lipids. The macromineral content of fish muscle was not affected by the fish-growing conditions, while zinc and iron, added as integrators to the formulated feed, were found to be higher in European sea bass from intensive cultures. As regards the total percentages of n-3 and n-6 PUFA and the n-3/n-6 ratio, total lipids of free-living and farmed European sea bass did not differ significantly. This observation, together with the evaluation of the fatty acid profiles of farmed fish lipid fractions, suggests that the formulation of the diet administered to sea bass in the tanks was optimal and met their n-3 PUFA requirements (Orban et al. 2002).

Muscle cellularity and quality parameters of the flesh were studied in specimens of wild and farmed *D. labrax* of approximately commercial size (Periago et al. 2005). Muscle fiber density was higher for wild specimens. In agreement with previous reports, the total fat did not show significant differences between wild and farmed fish and SFA and MUFAs were significantly higher in farmed than in wild European sea bass, whereas wild fish showed a higher content of PUFAs. No significant differences were found in the total content of n-3 fatty acids between the two groups. All textural properties were significantly higher in wild than in farmed fish, and all of them showed a positive and significant correlation with muscle fiber density, pH, hydroxyproline, and collagen contents. Changes in these parameters determined marked differences in the flesh

quality of wild and farmed European sea bass, whereas no relationship was found between muscle cellularity and nutritional composition of the fillets. According to these results, genetic factors as well as the influence of extrinsic factors such as feeding regimes and/or exercise may determine significant variations in some structural and flesh quality parameters of the European sea bass (Periago et al. 2005).

As reported by the Federation of European Aquaculture Producers (FEAP 2012), European sea bass aquaculture production reached 117,075 tons in 2011 in the Mediterranean area, with Turkey producing 43,200 tons, followed by Greece (43,000 t), Italy (8,700 t), Spain (14,370 t), and France (3,000 t).

Currently the market clearly distinguishes between wild and farmed products. Prices for European sea bass caught in the wild command a premium in the market over the same size category of farm-raised fish, in part because their quality is perceived to be higher but also because of the relative scarcity of large-sized European sea bass. The same can be said for gilthead sea bream. In March 2013, the Italian market price for European sea bass of 400-600 g was 5.70-6.00 € kg⁻¹, whereas for specimens of 800 g, the price ranged from a minimum of 9.50 € kg⁻¹ to a maximum of 11.50 € kg⁻¹. It is, however, highly probable that a substantial quantity of large-sized (800 g and above) European sea bass sold on the market as wild are actually the product of aquaculture (Barazi-Yeroulanos 2010). Compared to many other species of farmed fish, such as salmon or trout, European sea bass in Italy is mainly marketed as a whole fish (the most common market size range is between 300-500 g) and fresh, with only limited volumes being processed in any form or value being added. One major reason is the conservatism of Italian consumers, who are accustomed to seeing the fish whole when sold retail.

In very recent years the demand for European sea bass has been heavily impacted by the ongoing economic crisis since much of this species is consumed in restaurants, a sector which, of course, is sensitive to changes in consumer confidence and perceptions about future purchasing power. On the other hand, a lack of adequate alternative species in the fresh sector may still enable producers to market their product throughout the year. Farmed salmon prices, for example, are on a rising trend and higher salmon prices should benefit bass sales over the coming months (FAO Globefish 2013).

2.3 GILTHEAD SEA BREEM

Gilthead sea bream (*Sparus aurata* L.) represents the most intensively cultivated fish species in the Mediterranean region. In 2011, the Italian production of gilthead sea bream at market size was estimated at 9,200 t (FEAP 2012). In the 1980s and early 1990s, Italian research was mostly oriented towards studies concerning the first phases of the production cycle, as biological aspects aiming to elucidate the biological aspects of gametogenesis (Carnevali et al. 1992) and optimize working recirculation aquaculture systems (RAS) (Melotti et al. 1990) and reproductive protocols to be adopted in hatcheries to obtain viable eggs and juveniles (Barbaro et al. 1991). At the beginning of the 1980s, the first hatcheries for European sea bass and gilthead sea bream faced several similar problems with broodstock maturation, spawning, and larval culture. Good success was obtained first at a small scale, but large-scale production of juveniles was only possible a few years later, in the mid-1980s, when these

problems were solved. The availability of controlled reproduction techniques generated a production scheme based on a constant and reliable supply of large quantities of good-quality fry. This development opened the door to the industrialization of marine aquaculture in Italy and our country thus increased its production. In 2007, Italy was the third largest producer of juveniles in Europe, accounting for 9.3 % of the production of European sea bass (55 million) and gilthead sea bream (52 million) juveniles, after Greece and Turkey. In 2009-2010 production was estimated at around 100 million fingerlings obtained from 13 different hatcheries (Barazi-Yeroulanos 2010), the majority of them being located in Veneto, Apulia, and Tuscany and involved in production of fingerling of both species.

In subsequent years, other kinds of studies on feeding to assure higher growth performances started to be carried out (Lanari et al. 1999) that also took into account the possibility of substituting fish meal and oil with vegetable components (de Francesco et al. 2007).

Initially, only a very few Italian genetic studies were conducted and compared performance differences, based on body weight, between Atlantic and Mediterranean strains (Aleandri and Galli 2002). Nowadays, Italian research groups are contributing to fill the knowledge gap in different fields, such as nutrition and pathology. Genes and amino acid sequences, in particular the expression and regulation of intestinal oligopeptide transporter (PepT)-1, were studied using vegetable sources as a substitute for fish meal in the diet of gilthead sea bream (Terova et al. 2013). In pathology, Italian research is involved in trying to solve health problems due to photobacteriosis, which seems to be associated with immune suppression in juveniles. According to a recent study, changes in the expression of selected immune genes, validated with qPCR and demonstrated by up-regulation of lectins and antibacterial proteins, are characteristic for alternatively activated macrophages that do not develop acute inflammatory responses (Pellizzari et al. 2013).

2.3.1 Reproduction

The gilthead sea bream is characterized by protandrous hermaphroditism, asynchronous ovarian development, and multiple daily spawning with fluctuating numbers of eggs spawned per day, for up to 3 months in captivity (Francescon et al. 1994).

To meet the increasing fry demand from intensive farms, induced spawning technologies were established by the end of the 1980s using different kind of hormones that could be adopted to trigger the last phases in egg maturation and induce spawning (Colombo et al. 1989); the most common of these is an analogue of the luteinizing hormone-releasing hormone (LH-RHa) and human chorionic gonadotropin or synthetic agonists of gonadotropin-releasing hormone (GnRHa). However, egg quality in the initial and subsequent ovulatory cycles may be adversely affected by high hormonal dosages (Francescon et al. 1994). Internationally, the moderate effectiveness of acute GnRHa treatments was explained by the short half-life of the peptide, despite its proteolytic resistance, and the resulting limited duration of the endogenous gonadotropin (GtH) peak, which was likely to be too short to activate the internal mechanisms for long-term spawning (Zohar 1989).

At present, photoperiod and thermoperiod manipulation is often planned to shift reproductive cycles so that eggs and larvae

can be obtained all year around (Moretti et al. 2005) and meet market demands. Broodstock are stocked in spawning tanks (water salinity required = 35 ‰) at the sex ratio of one female per two males, after ascertaining that the maturation stage is correct (ovarian biopsy for females and spontaneous or stripping sperm releasing for males). In hatchery systems, new males must be added to the broodstock. Whenever possible, wild fish are taken for renewal (5 to 20 % per year) although farmers often select the males that are among their best performing specimens (Sola et al. 2006). Usually, females of 1.5 kg (>3 years) and males over 350 g (2-3 years) are used for artificial reproduction.

As in captivity, sex reversal can be influenced by hormonal and social factors, and care should be taken when establishing groups of broodstock (Moretti et al. 2005). Broodstock should be fed balanced diets rich in vitamins and polyunsaturated fatty acids (n-3 PUFA) in order to maximize reproductive performance.

The spherical, transparent eggs are small (less than 1 mm) and pelagic and present a single large oil droplet. The fertilization rate is 90-95 %; fertility and egg quality are strictly related to a calm environment and a balanced diet (Sola et al. 2006). As fertilized eggs float on water having 35-37 ‰ salinity, they are automatically collected in special collector devices (airlift or overflow collectors) and placed in dedicated incubation cylindroconical tanks (100 to 250 l capacity) made of either plastic or fiberglass.

2.3.2 Larval rearing and weaning

Hatching starts around 48 hours after spawning at 16-17 °C and the eggs hatch small larvae (approximately 3 mm). The rearing system consists of circular fiberglass tanks (3-6 m³) located in a specific unit of the hatchery where environmental parameters are carefully controlled (temperature: 18-20 °C, with maximum fluctuation of 0.5 °C in 24h; dissolved oxygen: 80 to 100 % saturation; photoperiod: 16 h light, 8 h dark; light intensity: 1000 to 3000 lux up to age 25 days, thereafter 500 to 1000 lux; salinity: 35-38 ‰ = full seawater; bottom aeration: from 0.1 to 0.6 l min⁻¹; total ammonia nitrogen: < 0.5 ppm). Optimization and control of the major environmental parameters were the first steps taken to improve fry production (Moretti et al. 2005; Sola et al. 2006).

Larvae are stocked in rearing tanks at densities ranging between 150 and 250 fish l⁻¹. At hatching, fish larvae do not have an active swimming behavior and are not completely formed. They rely on their yolk sac as the only food source. The feeding protocol includes an initial administration of enriched rotifers, gradually substituted by enriched *Artemia* nauplii and inert diets of different size (200-300, 300-500, 500-700 µm). The administration of natural enriched feed starts with different microalgae [*Isochrysis* sp., *Nannochloropsis* sp., *Nannochloropsis oculata* (Droop) Hibberd and *Nannochloris atomus* Butcher], maintaining a concentration of $500 \pm 100 \times 10^3$ cells cm⁻³ in the rearing tanks. Concerning microalgae characterization, Italian studies reported that fatty acid profiles vary in relation to the culture phase and production method (Roncarati et al. 2004) and described technologies for cultivating microalgae in tubular reactors (Chini-Zitelli et al. 1999).

As soon as larvae develop an active swimming behavior (from 3 to 6 days of age, depending on water temperature), daily

checks should be performed to detect and correct as soon as possible any problems that could strongly influence later growth performance, among them swimbladder development (Moretti et al. 2005; Sola et al. 2006). It has been shown that growth and survival rates can be improved in several species by including phospholipids in microdiets, which also has a preventive effect against skeletal deformities. In the past two decades, the rate of malformations in larvae from hatcheries was very high, especially due to axial deviations, operculum atrophies, and cranial abnormalities; hatchery management requires frequent manual sorting and if not correctly applied, the performance of hatchery-reared fish is reduced, for example, swimming ability, conversion index, growth rate, and susceptibility to stress, pathogens, and bacteria. Features of morphological alterations such as the definition of the overall larval quality of a farm production cycle were investigated by Boglione et al. (2001), who performed a meristic count analysis while examining fish for malformations. The larval quality should be quantified in hatcheries within 50-100 days from hatching.

Probiotics have also been investigated in Italian scientific research; they are considered to be a valid alternative to antibiotics in aquaculture, particularly in sea bream larviculture, to prevent high mortality and to improve welfare and promote growth (Avella et al. 2010).

When fry reaches the size of 2-5 g, they leave the hatchery to be stocked into fattening and rearing units, either in floating cages or tanks.

2.3.3 Rearing

Until the 1990s, most fish came from land-based farms. Successively, with the development of mariculture techniques, first with floating cages in sheltered areas and later with submersible cages in open sea areas, the products coming from offshore facilities represented more than half of the total Italian fish production. Italian offshore fish farms are characterized by low to medium production capacities, with individual productions from 100-200 t year⁻¹ to a maximum of 800-1000 t year⁻¹. Except for a few farms, most companies use medium-sized, circular-shaped cages of about 1000-2000 m³ that are equipped with a polyethylene collar. In exposed areas, submersible cages are used more frequently. The development of offshore facilities, compared with land-based farms where energy and oxygen consumption negatively impacts final costs, was sustained by lower production costs and began to be suitable when cage technology was able to ensure a minor risk level and when 5- to 6-g fry became available on the market. Moreover, products from open-sea facilities presented a higher growth rate and a better flesh quality than those reared at a high loading charge (30-40 kg m⁻³) in land-based farms (Roncarati and Melotti 2007).

Juveniles destined to be reared in floating sea cages are prefattened in land-based basins for 2-3 months; then they are transferred to a sea plant where they are farmed for a period of 12-15 months where, at favorable water temperature (18-26 °C), they reach the marketable size of 400 g.

In this phase, farmed animal welfare becomes a relevant and significant topic because both consumer awareness and ethical reasons make this issue a top priority for animal farming conditions. A wide range of physical, physiological, and behavioral

measures and indicators are used to assess fish welfare and different studies have taken either a classical or innovative approach to accurately evaluate these parameters. In the first case, hematochemical parameters of nutrition were monitored during the rearing cycle (Roncarati et al. 2006) and during preslaughter and killing phases (Bagni et al. 2007). More recently, veterinary and metabolic molecular indicators of chronic stress using comparative proteomics are being considered (Alves et al. 2010; Fazio et al. 2012).

Gilthead sea bream is reared with feeds containing high protein content (40-45 %), mainly from fish meal. Interesting studies have been carried out with the aim to use vegetable sources as substitutes for fish meal and also fish oil. Substitution of fish meal or fish oil in gilthead sea bream diets has been studied with respect to growth, composition, and sensory characteristics (de Francesco et al. 2007) or taking into account the target to balance rapid fish growth and the cost-effective use of the nutrients. According to recent trials, this aim could be achieved by using diets containing an adequate energy level at a moderately restricted feeding rate (Bonaldo et al. 2010). Italian research is also considering the importance of employing vegetable feedstuffs not contaminated by aflatoxins. Chronic aflatoxicosis is of great concern in aquaculture systems since it was found to be implicated in the decline in health of reared fish and in decreased stock quality (Santacroce et al. 2008).

2.3.4 Quality and market

The market for gilthead sea bream can be broadly divided into two principal areas: foodservice (or catering) and the retail sector. The catering markets are of particular importance in Italy. Many retailer chains increasingly dominate the market, with the market share of traditional fishmongers diminished and decreasing. Demand has traditionally been mainly for smaller fish, especially in the catering market, which is the biggest sector in Italy. In the past, therefore, production was targeted at smaller sizes, but there is an increasing trend towards bigger fish.

However, changes in consumers' habits (a growing proportion of the population is reluctant to buy whole fish), the need for product diversification (value-added fillets), and the higher prices that whole, large-sized (>800 g) fish attain in the market prompted several producers to expand the on-growing period for up to 40 months or more to harvest larger fish. The gilthead sea bream is commercialized as fresh, refrigerated, and frozen fish.

Studies have investigated the effects of modifying fatty acid profiles in the tissues of cultured fish species. Such studies often involve altering tissue composition to increase the growth, survival, or marketability of cultured species. The fatty acid composition found in the fish muscle mirrors the fatty acid composition of the respective diets. National research is moving towards applying new tools such as proteomics to distinguish wild from farmed sea bream using isotope ratio mass spectrometry (CF-IRMS) (Moreno Rojas et al. 2007); furthermore, a muscle proteomic map seems to represent a good and reliable "fingerprint" of the fish species (Addis et al. 2010).

2.3.5 Limiting factors – Critical points

Larval malformations and pathology represent the most important key factors for the future development of gilthead sea bream farming . Many problems concerning morphological anomalies in gilthead sea bream have already been solved (Boglione and Costa 2011) but the abnormal development of the opercular bone is still particularly common. This abnormality develops at an early larval stage (17 days posthatching and earlier). Physical criteria by which the very first deviations in normal skeletal development can be detected are crucial to alleviate its occurrence in rearing facilities, such as the level of hydroxy-apatite mineralization of the opercula; this increased mineralization might be induced by an osteogenic response to increased mechanical loading of the opercle, as a result of this folding (Galeotti et al. 2000).

This fish species is prone to develop different diseases. High losses of cultured sea bream are reported due to bacterial infections such as: *Pasteurella piscicida*, with typical extensive, acute multifocal necroses in the spleen and kidney; the opportunistic pathogen *Flexibacter maritimus*, which provokes hyperplasia and erosion of the gills; or vibriosis caused by *Vibrio* sp., mainly represented by *Vibrio anguillarum*, characterized by external ulcers and internal hemorrhages. High mortality is usually associated with high water temperatures (above 18-20 °C) and important results have been obtained by evaluating the genetic variation of disease resistance and growth in gilthead sea bream experimentally infected with a highly virulent strain (Antonello et al. 2009). Winter disease (*Pseudomonas anguilliseptica*) outbreaks affect both juvenile and adult sea bream when the temperature drops below 12-13 °C and can induce high mortality as a result of hemorrhagic septicemia. Among the most recent Italian research, a study carried out in cultured and wild sea bream observed that the type of rearing system (floating cages, land-based tanks) and different environmental characteristics may affect the presence or absence of parasites belonging to *Contracaecum* spp. (Salati et al. 2013).

2.4 STURGEON

Sturgeons belong to the class of Actinopterygii, subclass Chondrostei (primarily cartilaginous fish showing some ossification) and are classified in the order Acipenseriformes, family Acipenseridae. Being anadromous fish, almost their entire life is spent in marine water, entering rivers for reproduction and swimming upstream to rocky dead areas for spawning. Historically, three sturgeon species were found in Italy: the common sturgeon (*Acipenser sturio* Linnaeus, 1758), the beluga sturgeon [*Huso huso* (Linnaeus, 1758)], and the endemic Adriatic sturgeon or Italian sturgeon (*Acipenser naccarii* Bonaparte, 1836). Due to excessive fishery and dam constructions along Italian rivers, sturgeons have been classified as an endangered species and various laws were thus established to protect the species, first in marine waters in 1982 with a law by the Ministry of the Merchant Marine prohibiting any capture. This law was subsequently adopted by the Emilia Romagna Region and the Veneto Region for freshwaters. Other regional restrictions have been successively adopted together with some restocking programs using sturgeons from hatcheries.

The first experimental sturgeon farming began in 1977 with sturgeon caught in the Po basin in Italy and kept in captivity.

At the commercial level, sturgeon farming in Italy started towards the middle of the 1980s with the weaning of white Pacific

sturgeon (*Acipenser transmontanus* Richardson, 1836) larvae. This species was imported from California in the mid-1980s (Muratori 1985). The Siberian sturgeon (*Acipenser baeri* Brandt, 1869) began to be cultivated at the beginning of the 1990s. Among the native Italian species of sturgeons, controlled reproduction has only been carried out in Adriatic sturgeon *A. naccarii*, artificially reproduced for the first time in 1988. Since then, it has been used for aquaculture and restocking purposes. Intensive sturgeon farming has been actively developed in Italy in the last 20 years. Currently, about 1,380 tons of sturgeon are produced annually (Bronzi et al. 2011). Sturgeon farms are mainly based in northern Italy, in the Treviso, Brescia, Cremona, Milan, and Novara provinces. Each farm produces more than one species but includes American (or white) sturgeon (*A. transmontanus*), Russian (or diamond) sturgeon (*A. gueldenstaedtii*), Siberian sturgeon (*A. baerii*), Adriatic (or cobice) sturgeon (*A. naccarii*), beluga sturgeon (*Huso huso*), or Siberian sturgeon (*A. stellatus*) as the main product. Moreover, hybrid species showing good zootechnical performance and caviar production are farmed as well (Bronzi et al. 1999). Among them, Adriatic sturgeon has been cross-bred with Siberian sturgeon (*Acipenser naccarii* female \times *Acipenser baerii* male) (Arlati et al. 1999; Bronzi et al. 1999; Pazzaglia and Giovannini 2009), the latter recognized as a commercial species among the Acipenseriformes in Italy (G.U. 2008). In terms of volume, the most representative cultured species is white sturgeon (*Acipenser trasmontanus*) that, under intensive conditions, grows and matures faster than under natural conditions. A few companies are specialized in farming this species. After sexing at 3 years of age, males are sold for meat consumption (fresh or smoked) and females are cultured to sexual maturity.

2.4.1 Reproduction

Sexual maturity occurs quite late in sturgeons in comparison with other fish species and, as is characteristic for each species, may be strongly affected by environmental factors, among them water temperature and feeding. For instance, male Russian sturgeon (*A. guldenstaedtii*) grown in nature reaches sexual maturity at ages 5-7; females begin developing eggs at ages 9-12 and first spawn at age 15. Maintained in captivity, males mature at an early age (3-4 years), vitellogenesis starts at the age of 6 years in females, and first maturity is found at age 7. While in other species sexual maturation occurs earlier, as in *A. ruthenus* (3-4 years), even longer maturation times have been observed in other species in captivity (*A. transmontanus*), even up to 14 years.

2.4.2 Larval rearing and weaning

Ontogenetic development in sturgeons is very different from that in teleosts (Balon 1986; 1999), exhibiting three distinct periods: first, a lecithotrophic period in which they feed exclusively from their yolk reserves, coinciding with embryo and eleuthero-embryo stages; second, a lecithoexotrophic period in which the animals consume yolk and food of exogenous origin during the transition from eleuthero-embryo to metamorphosing juvenile; and finally, an exotrophic stage.

As in teleosts, culturing late larvae is considered to be one of the most critical and difficult stages in intensive sturgeon

farming, often associated with high mortality during and after yolk sac absorption (Gisbert and Williot 2002). Adequate feeding plays a major role in the survival of farmed sturgeon during early life stages, especially when the digestive system is not completely differentiated and the stomach is not yet fully functional.

Sturgeon larvae, in contrast to larvae of other fish species, cannot feed on commercial feed immediately after the yolk sac has been absorbed. The minimum time for their digestive system to develop is 3-4 days after absorbing the yolk sac, but the minimum time to feed with commercial feed is 30 days, at an average weight of 2 g (Ebrahimi 2006).

2.4.3 On-growing

For carnivorous species, on-growing diets generally contain 40-45 % crude proteins, 19-20 MJ kg⁻¹, whereby fish meal is partially substituted by soybean protein concentrate, rapeseed meal, and other vegetable proteins (Memis et al. 2006; Mazurkiewicz et al. 2009). Alive, frozen fish, and shellfish alike are provided by some farmers as a feed supplement, in particular during the final ovarian growth period. Other nutritional needs are comparable with those of other freshwater carnivorous species. The vitamin C requirement in sturgeon has been a matter of discussion in the literature; nevertheless, a vitamin C supplement is needed, as reported by Papp et al. (1999). Sturgeon performance is species-specific and changes with age; for example, juvenile *Acipenser ruthenus* at 18.5-24.5 °C has been reported to show a specific growth rate (SGR) of 2.84-3.03, a FCR of 1.32-1.41, and a protein efficiency ratio (PER) of 1.78-1.90 (Przybył et al. 2006).

Farming technologies for sturgeons in Italy have mainly been established in freshwater ponds and raceways, with the natural water temperature ranging from 11 to 26 °C. Nevertheless, heated effluents from power plants of iron-steel factories are being used with great success. Other technologies have been implemented successfully elsewhere, such as water recirculation plants and pen and cage facilities.

2.4.4 Quality and market

The main products derived from sturgeon are meat and caviar. Meat is usually obtained from: (i) undifferentiated animals, (ii) males slaughtered after gender identification around 4-5 years of age (depending on the species), or (iii) females slaughtered at sexual maturity for caviar preparation. Unfortunately, in western European countries the market for sturgeon meat is not consolidated and is often considered as a by-product of caviar production. The ex-farm prices for sturgeon meat fluctuate in the range of 2.5-3.2 € kg⁻¹, having been sensibly reduced in the course of the last 10 years. No differences exist between male and female meat; nevertheless, as the female is larger, the meat may possibly be processed in more convenient ways, offering sliced, smoked, or other preparations to the market. Meat color and composition may vary in complex ways, depending on the sturgeon species and on feeding strategies (Badiani et al. 1996; 1997).

Caviar ex-farm prices, after being maintained at a steady rate for the last 3 years, are increasing, now on the order of 350-550 € kg⁻¹, depending on the species and quality. Consumers might find it at retail shops for up to 3,200 € kg⁻¹ or more.

Caviar represents the most important commodity in sturgeon aquaculture, and thus early sex identification is of paramount importance for this industry, males being slaughtered at 3-4 years of age for meat consumption. With the exception of males farmed as breeders, females are only farmed to sexual maturity for egg production, which requires 8-14 years, depending on both the species and the water temperature.

Sex identification is difficult in sturgeons, owing to the absence of phenotypic differences between females and males before sexual maturity, the gonad maturation cycle, and the anatomical characteristics of the reproductive system. Diagnostic methods, including quantification of plasma vitellogenin and sexual steroids, are widely used for sex and reproductive stage identification in sturgeons, but do not satisfy the need for an early sex assessment. For this purpose, gonad biopsy is largely performed in 3- to 4-year-old sturgeons, in spite of being quite an invasive and traumatic approach.

A scalpel is used to make a small abdominal cut (<1 cm) on the sedated fish, reaching the peritoneal cavity near the gonads. The operation is carried out by highly specialized personnel under extremely clean conditions, using specialized instruments and according to criteria similar to that for a veterinary operating environment. The tips of surgical pincers (Hartman's pincer) are introduced into the incision and a small quantity of gonadal tissue is removed. Expert technicians can immediately identify the sex of the animal by examining such fresh biological samples. As the resulting wound is very small, it does not require suturing and scars over in a matter of days. Appropriate measures are also taken to avoid infection.

From biopsies carried out on sturgeon, the nature of the testicle or ovary can be clearly differentiated at different stages of development by histological examination.

The ovarian tissue is then categorized according to the level of maturity that has been reached. The eggs are classified according to their stage of development as follows:

- black eggs, of rather large dimensions, corresponding to mature caviar
- small eggs, black or gray, corresponding to a product which requires a further 6-10 months of maturation
- small, white eggs, corresponding to a product which requires at least a further 12 months of maturation
- ovaries of "immature females," corresponding to a product which requires a further 2-5 years of maturation.

A secondary phase of "staging" may then be carried out, with the aim of creating a detailed production program.

In order to avoid an invasive biopsy, at least for sex identification, ultrasound technology has been developed (Colombo et al. 2004); however, the reliability of this procedure is lower than for biopsy. Recently, an associated approach including ultrasound together with plasma hormone assessment was proposed by Petochi et al. (2011) to improve reliability.

2.4.5 Caviar

Over the past few decades, pushed by an ever-increasing demand for caviar, the wild sturgeon stocks have been overexploited with the resulting dramatic decrease in wild product availability and supply. Thus, in 1997 the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) decided to limit caviar trade by listing all sturgeon species in

Annex II of the convention (Raymakers 2006).

The lack of supply and, simultaneously, high demand for this luxury product has greatly increased the feasibility of caviar production from aquaculture. Consequently, in recent years, high-quality, environmentally friendly, and intensively farmed alternatives are increasingly becoming available on the market. In 2008, the estimated world production of farmed caviar for all species was on the order of 110-120 tons, originating from 16 countries (Bronzi et al. 2011). The most commonly used species is the Siberian sturgeon (*Acipenser baerii*), followed by the Russian sturgeon (*A. gueldensteadii*), the sterlet (*A. ruthenus*), and the stellate sturgeon (*A. stellatus*). Although the white sturgeon (*A. transmontanus*) is the one predominantly farmed for caviar production in Italy, other species and some hybrids are farmed as well. The Italian production of caviar from farmed sturgeon has recently increased, almost exponentially, from 22 tons reported for the year 2008 by Bronzi et al. (2011) to reach over 40 tons in 2011 (farmer interview, unpublished data), hence representing the world's largest farmed caviar production. Considering the variety of fish species used to prepare "caviar-like" products, a standard for caviar was recently adopted by the Codex Alimentarius Commission (2010). Caviar is defined as "the product made from fish eggs of the Acipenseridae family (four genera *Acipenser*, *Huso*, *Pseudoscaphirhynchus* and *Scaphirhynchus* and hybrid species of these genera) by treating with "food grade salt" with a final concentration in the end product of between 3 and 5 g/100 g. Furthermore, the Codex standard describes the essential steps of the production process, storage temperatures, permitted packaging materials, permissible food additives (possibly), essential composition, and main quality factors. The term caviar on each tin may be completed with the usual species name such as Beluga (*Huso huso*), Ossetra (*Acipenser gueldensteadii* and *A. persicus*), or Sevruga (*A. stellatus*). Caviar from sturgeon hybrids needs to be identified by the term "hybrid" combined with the parent name according to the annex A of the Codex standard.

The successful caviar production from aquacultured sturgeon has recently attracted the interest of research aiming to evaluate the quality of the final product. The changes in quality among species can be improved by using nutritional and feeding strategies and by accurately controlling the environmental conditions and further egg preparation protocols, crunchiness as well as flavor and taste being among the main quality goals.

Chemical assays together with sensory studies have been carried out with the goal of determining whether differences might be observed between caviar from farmed and wild sturgeon (Gessner et al. 2008) or of determining the chemical and biochemical composition of caviar from different species and origins (Wirth et al. 2000). To this end, different analytical procedures were applied such as determining fatty acid profiles and analyzing the composition (Caprino et al. 2008) and conducting a sensory evaluation (Cardinal et al. 2002). More advanced techniques such as scanning electron microscopy (SEM) and magnetic resonance imaging (MRI) have been used to study structural properties and conservation states of caviar during storage (Gussoni et al. 2006).

Commodities similar to caviar can be produced from other fish, but have a lower commercial value. From salmon eggs so-called "red caviar" is produced, whereas for lump eggs (*Cyclopterus lumpus*) artificially colored either red or black, the

commercial label must indicate: “*succedaneo del caviale*” according to Italian legislation. In the USA, salted fish roe is produced from paddlefish (*Polyodon spathula*) and is equivocally promoted and commercialized worldwide as “paddlefish caviar”.

To produce caviar, the ovaries are withdrawn from the opened abdominal cavity and delicately massaged to release the eggs, which then undergo a brief washing stage followed by dry salting with food grade salt. For packing caviar a special machine, called a chamber sealer, must be employed to draw out the air. This machine uses a high-performance vacuum pump, a floating-bottom vacuum chamber, and a control panel.

The long biological cycle, survival at the larval/first feeding stages, early sex identification, and caviar quality are currently considered the main bottlenecks in sturgeon aquaculture.

2.5 EUROPEAN EEL

The European eel, *Anguilla anguilla* (Linnaeus, 1758), belongs to the family of Anguillidae, which exclusively includes one genus, *Anguilla*, with 16 species, widely distributed in temperate and tropical areas (Deelder 1984). Among these, the European eel, together with the American eel [*Anguilla rostrata* (Lesueur, 1817)], in North America, and the Japanese eel [*Anguilla japonica* Temminck & Schlegel, 1846], in the Far East, is considered the most commercially valuable species.

The European eel has an elongated, snake-like body that is slightly compressed especially near the caudal fin, reaching 100 cm total length and about 2 kg of weight in females. Males generally do not exceed 50 cm in length and 200 g of weight. The mouth-slit, extending to about the level of the middle of eye, has jaws and vomer with a series of teeth. The pectoral fin has 14-18 rays; the dorsal fin is very long, originating at about half the distance between the top of the pectoral fins and the anus; the long anal fin has its origin in front of the middle of the body length. Dorsal and anal fins become higher near the tail, where they form one rounded fin. The lateral line is quite distinct. Small elliptical scales are embedded deeply in the skin. Body color varies in relation to age and environment: the dorsal side varies from grayish-brown, olive-brown or yellowish to black, whereas the ventral side is yellowish.

It is a catadromous species that enters into the rivers from the sea at the so-called “glass” eel stage, being transparent and mostly unpigmented. In the rivers, glass eels grow into the elver stage and pass through several stages of metamorphosis for 3 to 9 years, up to the yellow stage, when sex differentiation occurs. After about 3-6 more years, they reach the adult silver stage and remain in the river for a variable period of time. At the end of the growth season in autumn, a part of the population ceases feeding, becomes restless, starts to mature, and changes into migrant silver eels. Drastic changes occur during silvering, with the enlargement of the eyes discriminating the yellow and silver phase. Once an eel displays a white silver belly well separated from a black dorsal region by the lateral line, then it is considered to be at the “silver stage” and implicitly a migrant. Furthermore, the pectoral fins become longer and shape changes are evident (Tesch 1991). Based on modifications in length, weight, eye diameter, and pectoral fin length, an index has been proposed to estimate the proportion of silver eels that are true

migrants (Durif et al. 2005). They return to the spawning area in the Sargasso Sea where they reproduce and die. At hatching, clear leptocephali then passively drift with the Gulf Stream towards the European coasts, where they metamorphose into glass eels and begin their migration into coastal lagoons and estuaries (brackish environments), or even further inland up to the lakes and streams (freshwater environments).

In the last few decades, captures from the wild have progressively declined; the number of juveniles coming from the Atlantic has dropped steadily to around 5 % of its average level in the 1970s. At present, due to low availability of young eels and high interest in their intensive culture, the demand for glass eels is increasing worldwide and, consequently, eels are overexploited (EIFAC/ICES, 2006). For these reasons, the European eel is included on the Red List of the International Union for Conservation of Nature (IUCN) as a Critically Endangered Species. Consequently, it is protected by imposing a short fishing season, minimum size for capture, protection of larvae, and careful regulation of the fish trade.

2.5.1 Reproduction

In the wild, European eel spawning has not yet been observed due to the difficulty of catching eels in the sea; it takes place at 600 m depth in darkness and under high pressures between March and July, as demonstrated by the appearance of eggs in plankton samples (Tesch 1991). In captivity, gonads of European eel do not mature spontaneously and therefore many studies have been performed in order to obtain knowledge about aspects of reproduction and the early life history of the eel.

The first gonad development was observed in a male specimen by Fontaine (1936). This was followed by a detailed study on experimental induction of spermiation by Boëtius and Boëtius (1967), who recommended optimal doses of human chorionic gonadotropin (HCG) for maturation of male European eels. Later, a single female was artificially matured but fertilization was not attempted (Fontaine et al. 1964). More recently, different artificially induced breeding techniques have focused on hormonally induced oocyte maturation and ovulation in females and spermatozoa maturation in sexually immature males. A successful protocol derived from the technique applied in the Japanese species (*Anguilla japonica*), consisting of repeated injections of salmon pituitary extract (SPE) followed by the steroid hormone 17,20 h-dihydroxy-4-pregnen-3-one (DHP), was tested in order to increase body size and to induce final oocyte maturation (Pedersen 2003). At the same time, optimal environmental parameters such as water temperature, salinity, and pressure have been confirmed as the most important factors affecting the sexual maturation process (EELREP 2006).

Currently, the weekly administration of hCG in male specimens under a constant water temperature regimen of 20 °C is thought to be the best hormonal treatment for reproductive performance during induced sexual maturation in European eel males (Gallego et al. 2012). In wild females, the injection of increasing doses of carp pituitary extract (CPE) followed by injection of 17,20 h-dihydroxy-4-pregnen-3-one (DHP) for 16 weeks induced sexual maturation and good reproductive performance (Mordenti et al. 2012).

2.5.2 Weaning

European eel aquaculture production has depended and still depends on glass eels, elvers (“*raganelli*”), and wild yellow juveniles (“*ragani*”). Captured glass eels are manipulated and transported to culture facilities by fishermen using traditional methods, taking great care to preserve fish health. In this phase, the young fish are exposed to high levels of stress; capture, long periods of fasting, hypoxia, and unsuitable water conditions can lead to high mortality after arrival at the farm and to disease development or difficulties in weaning during the acclimation and preweaning phases (Heinsbroek 1991).

Wild juveniles are generally weaned by 2 months. During the weaning phase, the juveniles are gradually switched from a diet of fresh fish (such as sardines) to a moist feed containing fish meal with water and fish oil. Frequently, it is necessary to grade the fish in order to separate the weaned fish from those who refuse feed. For the latter, the weaning cycle must be repeated.

Weaning takes place in 50-100 m³ tanks, supplied with water at temperatures of 18 and 25 °C.

The stocking density has been suggested as one important factor and it is believed that high-density conditions produce a higher proportion of males (Colombo and Grandi 1996; Roncarati et al. 1997; Huertas and Cerdà 2006). It has been observed in both nature and in farms that, given equal space, an increase in the number of individuals corresponds to a sex ratio in favor of male eels, which are generally believed to grow slower than females (Tesch 1991).

2.5.3 On-growing

Extensive production from *vallicultura* was the main source of eel in Italy until 1970 when a parasite (*Argulus giordanii*) dramatically reduced the brackish population (Ravagnan 1978). As a consequence, an important development in intensive farming took place. In the 1980s, Italian eel production was estimated at around 4,000 tons. Starting from weaned young eels of 15-50 g, the male size of “*buratelli*” (120-150 g) is obtained in 8-12 months at plants working with water temperatures above 18 °C for at least 7 months. The female size (300-600 g) requires another 4-6 months of fattening. The initial load ranges from 5 to 20 kg m⁻³ and it can be further increased by increasing the supply of oxygen. Survival rates average around 80 %.

The quality of the young eels and the sex ratio are two key elements of a good management system in which, under normal conditions, at least 60 % of the product will be marketed as female eels. Farming techniques have improved greatly and feed formulations were satisfactory; however, due to the increasing cost of seeding, the profit margin of intensive farms decreased.

In Italy production was progressively reduced, reaching the lowest level of 1,200 tons in 2010, and currently only few plants are working to supply all the products to the national market. At the European level, innovations in intensive aquaculture technologies, such as RAS in which water is reused after undergoing treatment, have been created to increase final production yields. However, the increasing societal pressure to reduce consumption of this endangered species will put constraints on demand and consequently eel production in RAS will decline (Martins et al. 2010).

2.5.4 Quality and market

The demand for this product is always very high during the Christmas holiday period; the quality of eel meat is especially appreciated in different regions of Italy (Sardinia, Lombardia, Veneto, Emilia Romagna, and Campania).

Based on the lipid content of meat, European eel is among the fattier fish; the eels' body-lipid composition generally reflects the lipid diets provided throughout the production cycle. Body lipids can be slightly reduced by improving the quality of lipid content of diets delivered during the later stages of eel production (Garcia-Gallego and Akharbach 1998). The quality traits of the product coming from brackish waters, such as eels from Valli of Comacchio located in the Delta Park of Po river, appear to be more favorable than those fattened in intensive farming (Roncarati et al. 2008a).

As reported previously, due to different factors – including overfishing, habitat reduction, and contamination – the availability of young eels for aquaculture purposes has strongly declined and the consequence has been a very high increase in prices. Glass eels are quoted at around 300 € kg⁻¹, elvers at around 18 € kg⁻¹. The sale prices of “*buratelli*” and female eels are 13-13.50 € kg⁻¹ and 15-15.50 € kg⁻¹, respectively (API 2012).

2.5.5 Critical points

Considering the decline in catches, studies focused on reproduction represent an important step for gaining complete control of the production cycle and deserve to receive grants and support.

In intensive culture, due to the variability of these natural stocks, the growth rates of glass eels during the first months of rearing in captivity are highly heterogeneous, which produces great disparities in weight and length of the populations and favors increased mortality by cannibalism (Heinsbroek 1991).

Diseases represent another critical point. Parasites also have a negative impact on fish culture production, reducing the growth rates and nutritional condition of individuals (Haenen et al. 1994; Madsen et al. 2000). Viruses are known to affect blood-forming tissues in eels and typically become virulent during stress. Rhabdovirus infections are described in the literature as infections with EVEX (Eel-Virus-European-X) in eels during spawning migration, causing a decline in hematocrit levels. These viruses are considered a contributing factor to the worldwide decline of eel (van Ginneken et al. 2005).

3. Other minor species

3.1 CATFISHES

Among the various catfish species, *Ameiurus melas* and *Ictalurus punctatus* are of some importance in Italy.

3.1.1 BLACK BULLHEAD

Black bullhead (*Ameiurus melas*) is an ictalurid catfish native to central and eastern North America (Wang 2010). It was introduced to European countries in the late 19th and early 20th century and has become widespread in Europe (Wheeler 1978;

Copp et al. 2005). It is common in lower sections of small-to medium-sized streams with low gradients plus backwaters, ponds and silty, soft-bottomed areas of lakes and impoundments (Scott and Crossman 1973) as well as shallow diked or flooded wetlands (Braig and Johnson 2003; Cucherousset et al. 2006). The black bullhead can tolerate harsh conditions imposed by small, isolated aquatic systems with poor water quality (e.g., pollutants or extremely high temperatures) (Scott and Crossman 1973; Braig and Johnson 2003; Ribeiro et al. 2008a; Novomeská and Kováč 2009; Novomeská et al. 2013; Rutkayová et al. 2013). In such habitats, it consumes plant material, terrestrial prey, and co-occurring fish species (native or exotic) and thus it could be considered as generalist or opportunistic, foraging on the most abundant and available prey (Leunda et al. 2008). It is considered a multiple spawner with asynchronous oocyte development and indeterminate fecundity. The examination of its life-history traits suggests that this species has the attributes necessary for flexible reproductive responses to new environments (Cucherousset et al. 2006; Novomeská and Kováč 2009). For all these reasons, this species has a great potential to invade new areas and to establish viable populations and is often considered as an undesirable species in European rivers and lakes (Leunda et al. 2008). In many cases, the wild population is subjected to control by provincial or regional agencies with the purpose of limiting its spread. Among the various strategies proposed, overfishing encourages the consumption of fresh or processed (e.g., smoked) fish through a commercial enhancement of this species.

3.1.2 CHANNEL CATFISH

Channel catfish (*Ictalurus punctatus*) is native to the Midwest and eastern parts of North America extending from Canada to Mexico (Page and Burr 1991; Wang 2010). As *A. melas*, it was introduced to Europe in the late 19th and early 20th century. It is an extremely resilient fish that can survive a wide range of environmental variability. This species is reported to live up to 40 years and attain approximately 1 m in total length and nearly 20 kg in body weight (Chapman 2012). Channel catfish tends to be a piscivorous bottom feeder but its diet can vary considerably depending on the region and the specific habitat it is residing in, being omnivorous as needed (Moyle 1976; Boersma et al. 2006). Channel catfish has an elaborate breeding behavior and the male usually incubates the eggs. Several production and management schemes are used to commercially produce channel catfish. Most of the farm-raised catfish are cultured in ponds constructed with levees. Catfish are also raised in watershed ponds and in high-density culture systems that make use of tanks, raceways, and cages. Channel catfish are efficient food converters and will gain between 0.45 and 0.67 g of body weight per gram of food consumed (Chapman 2012). The time required to raise this species to market size is primarily dependent on water temperature, age of fish, fish density, quality of diet, and level of feeding. Estimated time to raise channel catfish from egg to food-size fish is between 15 and 18 months. The channel catfish is the primary species of farm-raised fish in the United States (Chapman 2012), where it is considered an excellent food fish and where modern farming technology and fish product processing plants have made channel catfish culture into a multimillion dollar seafood industry (Wang 2010). In Italy the importance of this species is much more limited.

3.1.3 Catfish exploitation in Italy

Until the 1990s, Italian catfish production was well consolidated with over 3,000 t year⁻¹, especially in Emilia Romagna, Lombardia, and Veneto, where the black bullhead had a tradition as food supply and for sport fishing, whereas consumers have gained a certain interest in the channel catfish, especially as a processed product (fresh and frozen fillet).

Plants involved in catfish production could be classified in the semi-intensive category, with productions ranging between 1.5 and 8 t ha⁻¹ year⁻¹. A balanced diet was widely adopted and the FCR varied between 1.2 and 1.5. The surface area of catfish farms ranges from 5 to 7 ha and the total annual production from 22 to 50 t (API 1990; Melotti et al. 1993).

Since 1994, a drastic and progressive reduction in the number of black bullheads took place, reaching 200 t in 2006. This contraction was caused by a herpesvirus first isolated in two catfish farms in 1994. After that, mass deaths were recorded at the largest Italian plants and most of them were forced to give up farming this species (Melotti et al. 1999). This agent is still particularly dangerous today; vaccines are not available and channel catfish (*Ictalurus punctatus*) cannot be reared to compensate. In fact, channel catfish farming only maintains an annual production of around 400 t since its larger size makes the fish unsuitable for sport fishing (Roncarati and Melotti 2007) and it does not find favor with Italian consumers and thus is not well accepted in our market.

Nowadays, some farms are achieving very satisfactory results using tanks working in closed recirculating water systems. Other farms are also using other catfish species (*Clarias gariepinus*) to dispose of the dead fish, taking advantage of the specific feeding behavior of this species.

3.2 CYPRINIDS

Cyprinidae is a large family of freshwater fish (about 280 genera and more than 1,600 species) and quantitatively forms the most important group of teleost fish reared around the world. Cyprinids are widely cultured in Asian countries and in eastern Europe. Among the many species of cyprinids, only few of them are present or reared in Italy, those mainly bred and introduced for faunistic purposes, ornamental fish trade, and sport fishing. Some species are of some interest for food, although fish belonging to this family are usually considered less valuable because of the abundance of fish bones and the “mud” flavor that Italian consumers often complain of. Among them are: common carp (*Cyprinus carpio*), with its numerous selected varieties or breeds, goldfish (*Carassius auratus*), crucian carp (*Carassius carassius*), common barbel (*Barbus barbus*), brook barbel (*Barbus caninus*), common bream (*Abramis brama*), grass carp (*Ctenopharyngodon idella*), and tench (*Tinca tinca*).

As concerns the perspectives for cyprinid production, one possible way to sustain this area may be to diversify species, considering Chinese carps or common tench (*Tinca tinca*). The latter, subsequently discussed in this review, seems to play an important role in increasing the value of local production, i.e., the “*Tinca gobba dorata del Pianalto di Poirino*” in the Piedmont region (Gasco and Zoccarato 2001), which has recently obtained PDO certification from the EU.

3.2.1 COMMON CARP

Common carp (*Cyprinus carpio*) is certainly the best-known cyprinid and one of the world's most popular freshwater fish. The species generally inhabits lakes, ponds, and the lower sections of rivers (usually with moderately flowing or standing water), but is also known from brackish-water estuaries, backwaters, and bays (Barus et al. 2001). Carps are mainly bottom dwellers but search for food in the middle and upper layers of the water body. Typical "carp ponds" in Europe are shallow, eutrophic ponds with a muddy bottom and dense aquatic vegetation at the dikes. The ecological spectrum of carp is broad. Best growth is obtained when the water temperature ranges between 23 and 30 °C. The fish can survive cold winter periods. Salinity up to about 5 ‰ is tolerated. The optimal pH range is 6.5-9.0. The species can survive low oxygen concentration (0.3-0.5 mg l⁻¹) as well as supersaturation. Carps are omnivorous, with a high tendency towards the consumption of animal food, such as water insects, larvae of insects, worms, molluscs, and zooplankton (FAO 2012a). Intensive breeding may be carried out in spawning ponds or using artificial breeding techniques, with the hypophysation of the females and the incubation of the eggs in Zug jars. The hatched fry are kept in large conical tanks for 1 to 3 days and are usually stocked at the stage of "swim-up" or "feeding fry" into properly prepared ponds. Common carp can be produced in extensive, natural food, and supplementary feed-based monocultural production systems, and in stagnant water ponds. Artificial feed-based intensive monocultural production can also be carried out in cages, irrigation reservoirs, and running water ponds and tanks, or in recirculation systems. The importance of carp culture in Italy has always been modest owing to the absence of suitable areas and the low market value and demand. Initially intended only for human consumption, it has been used for other purposes over time, such as restocking, sport fishing, and ornamental breeding. Common carp farming has been extensively reduced due to the strong competition from Eastern European countries such as Hungary and Croatia, which are the main exporters to Italy. The current production is estimated to be around 700 tons year⁻¹.

3.2.2 GOLDFISH

Not less popular than carp, goldfish (*Carassius auratus*) is an allochthonous species that is abundant in many Italian lakes, showing great adaptability and a high invasive capacity due to its tolerance to extreme environmental conditions (low temperature, high rate of pollution, and cloudy water with a low percentage of oxygen), high fertility, and a wide alimentary regimen. Goldfish commonly hybridizes with the carp (*Cyprinus carpio*), giving rise to individuals that are intermediate in morphology between the two parent species (Lorenzoni et al. 2007). It has been reared in Italy since the end of the 1800s in some local areas of northern Italy (Bologna district) in typical hemp retting ponds, and more recently in raised ponds and intensive farming systems (Melotti 1986). When caught for food, the greatest economic disadvantage of this fish is the large number of fine intermuscular bones in the muscle, which makes it laborious to eat. Thus, this meat is used as food only after it is processed, which improves its economic value. Despite these undesirable characteristics, goldfish fillets are characterized by a high nutritional value and good oxidative stability (Dal Bosco et al. 2012). These traits could represent a good starting point

for the commercial enhancement of the species, encouraging the consumption of fresh or processed goldfish and enhancing the capture of this fish in natural environments as a solution to reduce the number in Italian lakes.

Goldfish is produced for ornamental purposes, providing about 20 million of specimens per year, but this business is also restricted. These plants are often involved in koi carp production, estimated at around 3 million specimens in 2006. Recently, the Koi Herpes Virus (KHV) compromised the expansion of rearing this species, causing high mortality rates in several countries of the world (EU, United States, and Asia) (Ronen et al. 2003).

4. New species for the aquaculture diversification

4.1 TENCH

Tench, *Tinca tinca* (Linnaeus, 1758), is a freshwater fish that belongs to the Cyprinidae family. It has a stocky carp-like shape and olive-green skin, darker above and almost golden below. The mouth is rather narrow and has a very small barbel at each corner. The caudal fin is square while the others are rounded in shape. In adults, a visible sexual dimorphism in ventral fins appears, males having longer ventral fins and with a thicker outer ray than females. The scales are very small and deeply imbedded in the derma.

Tench is a benthophagous, omnivorous fish endemic in central and southern Europe that inhabits stagnant waters in places with a muddy bottom and abundant vegetation (Billard and Flajšhans 1995; Brylinska and Brylinska 2000). It has been cultured together with carp in ponds for many centuries although research has shown that the two species occupy the same spatial position and compete for the ponds' resources. In contrast, in Extremadura (southwest Spain) and in Piedmont (northern Italy), tench has traditionally been reared in extensive monoculture, in ponds of various sizes used as water supply for livestock and culture.

Italian tench production is very low and tench is not in very high demand, except for a specific area of Piedmont (Pianalto di Poirino). Here, it is considered to be a high-value niche product (18 € kg⁻¹) and consumer demand is increasing, creating an important market. Unlike other Italian regions and foreign countries where tench are eaten at over 500 g in weight, in the Pianalto area, tench are consumed at a weight between 80 and 120 g and are in high demand and are highly valued. The same feature is reported in Extremadura.

In Piedmont, tench plays an important role in increasing the value of typical productions (Gasco et al. 2001; Orban et al. 2002) and since February 2008 has been recognized as a Protected Designation of Origin (P.D.O.) with “*Tinca Gobba Dorata del Pianalto di Poirino*” as product name.

European tench production has shown a decreasing trend in the last 10 years (1997-2008) from more than 2,000 to about 1,250 tons (FAO 2012f).

Tench has been introduced in Africa, Australia, Southeast Asia, and North America and more recently into China where it represents a promising new species for aquaculture (Wang et al. 2006). It is valued by consumers and used for angling and as

ornamental fish. Some artificial-bred varieties of tench called the golden, blue, or white tench have been created and are popular as ornamental fish for ponds (Kvasnicka et al. 1998).

As tench is currently seen as a promising new species for aquaculture production, many fish farms have expressed interest in this species and joint projects are being carried out with research institutes with the aim of improving production parameters.

4.1.1 Reproduction

Tench females reach sexual maturity at 3-4 years and males at 2-3 years, although episodes of sexual precocity are frequent. Spawning occurs when the water temperature reaches 20 °C. It therefore takes place from late May to August, depending on the region. The duration of the spawning season is approximately 9 weeks, with a peak in early July (Linhart et al. 2006). A tench female lays her eggs in sequence and each female can breed 4-5 times during the reproductive season. The fecundity of the female is high, being from 140,000 to 230,000 eggs kg⁻¹ of body weight year⁻¹.

The sticky green eggs are very small (about 0.5 mm for nonswollen eggs) and are released on a plant substrate. The sperm has a milky color and is thick, and the total number of spermatozoa/male body weight is about 18.5×10^9 (Linhart and Billard 1995). The incubation period ranges from 60 to 120 day degrees. Upon hatching the larvae are small (3.5 to 4.3 mm long) and absorb the yolk sac 3-6 days after outbreak.

In ponds, several uncontrollable factors can influence reproduction and, in order to increase tench culture, artificial reproduction techniques have been investigated in depth and are now well controlled. Usually, in April tench broodstock are kept sex-separately in ponds or tanks and successively assessed for maturity. Suitable spawners are treated with a hormonal injection; then stripped eggs are fertilized with activated sperm and, after a desticking procedure, incubated in Zug bottles or Wiess jars of 2-10 l (Linhart et al. 2000; Gela et al. 2003; Carral et al. 2006; Linhart et al. 2006).

4.1.2 Larval rearing and weaning

At the age of 2-3 days, the mouth is developed and larvae start searching for external food. Studies carried out on feeding of tench larvae under natural conditions have shown that tiny tench larvae are selective in the uptake of planktonic crustaceans and rotifers (Sestáková et al. 1988). Later, with increasing size, small tench can feed on Cladocera, Copepoda, water mites, and larvae of chironomids.

Initially, under indoor controlled conditions where no natural food was available, the lack of suitable food for very small larvae constituted a serious problem and many authors have studied the possibility of feeding larvae using live food caught in ponds (Sestáková et al. 1988; Hamackova et al. 1995). These authors reported many problems linked to seasonal dependence and the difficulties of handling this food.

Currently, *Artemia* nauplii distribution is the most common indoor practice (Wolnicki and Korwin-Kossakowski 1993; Fleig et al. 2001; Wolnicki et al. 2003; Carral et al. 2006; Celada et al. 2007) with higher survival rates than for maintenance in open-

air ponds. However, cysts are expensive when purchased commercially and these feeding practices are associated with higher labor costs (Celada et al. 2007; Rothbard and Biton 2010). Then, several studies on the possibility of using artificial dry diets as the sole start food were carried out (Wolnicki and Górny 1995; Wolnicki and Myszkowski 1998; Quirós et al. 2003; Wolnicki et al. 2006; Mamcarz et al. 2011), but these diets proved to be unsuitable, showing high mortality and deformity rates.

4.1.3 Rearing

Tench can adapt well to harsh environments, characterized by low levels of oxygen and relatively high temperatures (Wieser 1991; Myszkowski and Kamler 2010). The best growth performance is obtained in water with a pH between 7.5 and 8.2 and temperatures ranging from 25 to 29 °C (Perez-Regardera et al. 1994).

Growth depends on climatic conditions and, in Europe, the growing season is from April to end of September. During the winter season or when the water temperature drops below 10 °C, tench stop feeding and use their body reserves to survive the fasting season (Billard and Flajšhans 1995; Özogul et al. 2007).

Tench aquaculture is currently performed in ponds under extensive or semi-extensive conditions, where it is difficult to control environmental parameters, leading to unpredictable productions, high mortality, and slow growth.

After weaning, tench are cultured in ponds (initial density of 0.5-1.0 million larvae ha⁻¹), where they are fed artificial diets formulated for other fish species and grow until marketable size.

Tench is characterized by a slow growth rate, and the market size (80-120 g in the area of Pianalto) is not reached before the end of the second year of farming (Gasco et al. 2010). At least 3 years are needed to reach a size of 400-500 g (Kocour et al. 2010).

4.1.4 Quality and market

Tench has palatable meat characterized by a fine flavor. The dressing percentage amounts to about 87 % (Gasco et al. 2010). The protein content is around 20 % and the lipid profile shows a good proportion of long-chain fatty acids (Vácha and Tvrzická 1995; Özogul et al. 2007). Tench stores lipids not only subcutaneously, but also as muscle fat (Gasco et al. 2010). Indices and ratios related to human health (Atherogenic Index, Thrombogenic index, PUFA/SFA and n-6/n-3 ratios) are in accordance with values recommended for high-quality food for humans. It is well known that several factors such as environment, type of rearing, and composition of the diet dramatically influence the lipid content and composition of fish and through diets fish can be produced that have a high lipid quality in muscle (Jankowska et al. 2006; Steffens and Wirth 2007; Turchini et al. 2007; Zakęś et al. 2010). In the same way, raw materials used in diet formulations can influence the volatile compound profiles and produce different sensory characteristics of the fish fillet (Grigorakis et al. 2003) with sometimes negative effects due to off-flavor attributes (Turchini et al. 2007).

4.1.5 Critical points

There is currently great interest in increasing the production of tench, a very promising fish for diversifying aquaculture production. Several attempts to intensify tench production in ponds or in recirculation systems have been carried out but no feeds specifically formulated for tench are commercially available. At present, commercial feeds developed for other species are used but they do not meet the nutritional needs of tench, consequently resulting in suboptimal growth, deformities, and high mortality (Wolnicki and Myszkowski 1998; Rennert et al. 2003; Kamler et al. 2006). Deformities cause subsequent, low growth rates and devalue the product (Cahu et al. 2003). Moreover, an unbalanced diet can also result in an excess nutrient loss in effluent waters, which may contribute to a negative environmental impact. San Juan et al. (1999) used different protein contents and their results showed that high initial growth rates and also high mortality were associated with high-protein diets. However, opposite results were presented by Quirós et al. (2003), who obtained high growth rates and low mortality with high-protein diets. The authors highlighted that not only the quantity, but also the type of protein should be considered. Moreover, it is well known that the optimal dietary protein level is affected by several factors, including species, age, dietary protein quality, and energy level, as well as the protein-to-energy balance (Wilson 2002). Ideally, dietary lipids or carbohydrates should be maximized in fish feeds to spare dietary protein from being used for energy.

Concerning lipids, which are important components of fish muscle since they are components of cell biomembrane and provide energy reserves, it is unknown whether linoleic acid (C18:2n-6) or α -linolenic acid (C18:3n-3) is more significant for tench. In the same way, the optimal dietary lipid level has not been assessed. According to both Lall (2002) and Wolnicki et al. (2006), quantity and quality of dietary lipids may play important roles in the appearance of deformities and diets with a low fat content should be preferred.

De Pedro et al. (2001) studied the effect of different protein, lipid, and carbohydrate diets on growth and energy storage in tench. Results indicated that the best growth rates were obtained with the control and protein-enriched diets while the carbohydrate diet produced the worst results, suggesting that it is not advisable to reduce dietary fish protein to below 35 % and that it is not possible to obtain a protein-sparing effect of either lipids or carbohydrates.

The other critical point in tench rearing is the lack of a suitable feeding strategy. Tench are known to be shy and nervous in intensive conditions and are a nocturnal species (Herrero et al. 2005; Owen et al. 2010). A study on the influence of light intensity on spatial disposition and on gregarism of tench has shown that tench are very sensitive to light and gave evidence that tench selected low light intensities (40 lux) (Garcia-Ceballos et al. 1998). Therefore, great attention must be paid to stocking density and light intensity. In this sense, several studies have been carried out with the aim of determining optimal environmental conditions and also to set the best feeding frequency (Wolnicki et al. 2003; Kamler et al. 2006; Celada et al. 2008; Myszkowski and Kamler 2010), but further investigations are needed.

4.2 ATLANTIC BLUEFIN TUNA

The Atlantic bluefin tuna, *Thunnus thynnus* (Linnaeus, 1758), is a highly developed, predatory species (Cort and Liorzou 1991), considered one of the largest, fastest, and most wide-ranging teleost in the oceans. It can reach an impressive size (up to 700 kg of body weight and >3 m of length) and swim at the speed of 90 km per hour during transoceanic migrations (Safina 1993).

The currently accepted classification system is based on morphological features and includes tunas, together with mackerels, in the family of Scombridae (Collette and Nauen 1983; Collette et al. 1984; 2001). *Thunnus* is a monophyletic genus, as it was originally proposed based on morphological analyses, and more recently confirmed by molecular biology studies (Chow and Kishino 1995; Alvarado Bremer et al. 1996).

The bluefin tuna species includes the Atlantic bluefin tuna *Thunnus thynnus*, the Pacific bluefin tuna *Thunnus orientalis* (Temminck & Schlegel 1844), both inhabiting temperate waters, and the southern bluefin tuna *Thunnus maccoyii* (Castelnau, 1872), which is widespread in the southern oceans (Collette et al. 2001).

The Atlantic bluefin tuna is also named *northern bluefin tuna* in English, *thon rouge* in French, *tonno rosso* in Italian, and *atún rojo* in Spanish. It has a fusiform body shape with the dorsal head profile more or less straight and the mouth in a terminal position. The second dorsal fin is higher than the first one, whereas the pectoral fins are very short. The lower sides of the body and the abdomen are silvery. The first dorsal fin is yellow or bluish whereas the second is reddish-brown; the anal fin and the finlets are dark yellow, edged with black, and the median caudal keel is black in adult specimens. The lateral line is uninterrupted and the number of gill rakers varies between 34 and 43. The Atlantic bluefin tuna has 39 vertebrae, two dorsal fins, 8-10 dorsal finlets, and 7-9 ventral finlets. The total number of spiny rays of the dorsal fins is 12-14. The caudal fin is forked and the anal fin has no spiny rays, but only soft rays.

4.2.1 Reproduction

The Atlantic bluefin tuna inhabits the pelagic ecosystem. In the western Atlantic, it is found from Labrador and Newfoundland south to the Gulf of Mexico and the Caribbean Sea and has also been observed off the coasts of Venezuela and Brazil. In the eastern Atlantic, it occurs from the Lofoten Islands of Norway to the Canary Islands and the Mediterranean Sea (Fromentin and Powers 2005; Wilson et al. 2005). Among the tunas, the Atlantic bluefin tuna has the widest geographical distribution and is the only large pelagic fish living permanently in temperate Atlantic waters (Bard et al. 1998; Fromentin and Fonteneau 2001).

The Atlantic bluefin tuna is oviparous and iteroparous like all tuna species (Schaefer 2001). It has an asynchronous oocyte development (Medina et al. 2002; Corriero et al. 2003) and is a multiple batch spawner. Like the majority of fish, egg production appears to be age (or size) dependent: the estimated batch fecundity is about 93 oocytes g⁻¹ of body mass (Medina et al. 2002). Spawning fertilization occurs directly in the water column and these fish hatch without parental care after an

incubation period of about 30 h at 25-26 °C or 32 h at 24 °C (De Metrio et al. 2010).

Larvae (around 3-4 mm) are typically pelagic with a yolk sac and a relatively undeveloped body form. The yolk sac is reabsorbed within a few days and then the larvae start active predation (De Metrio et al. 2010).

It is generally agreed that spawning takes place in warm waters of specific and restricted locations and occurs during a limited temporal window, in contrast to tropical tuna which spawn and feed throughout the year in the tropical and subtropical oceans (Schaefer 2001). The Atlantic bluefin tuna reproduces in different geographical locations: the Gulf of Mexico and Florida Straits from April to July and the Mediterranean Sea from May to July (Susca et al. 2001; Medina et al. 2002; Corriero et al. 2003; Heinisch et al. 2008). In the Mediterranean Sea basin, three spawning grounds have been identified: 1) the waters around the Balearic Islands (Medina et al. 2002; Corriero et al. 2003; Heinisch et al. 2008), 2) the area of the central Mediterranean Sea, which includes Malta and the Aeolian Islands (Heinisch et al. 2008), and 3) the Levantine Sea (Heinisch et al. 2008).

4.2.2 Capture, rearing, and critical points

Atlantic bluefin tuna is captured using a variety of gear types, including purse seines, longlines, traps, handlines, bait boats, and sport fishing (ICCAT 2008). Throughout the Mediterranean, wild Atlantic bluefin tuna have traditionally been caught using traps consisting of long net walls of several kilometers, designed and positioned to guide tuna migrating along the coast into an enclosed area of net (the device is also known as *almadraba* in Spain and *tonnara* in Italy). In Italy, historically, the fishing of bluefin tuna through traps has played a critical role in defining Sardinia's culture and economy of fish and fishing. Since the mid-1990s, purse seines have become the most prevalent fishing method (85-90 % of reported catches) since this method captures the fish destined for farming purposes alive (Mylonas et al. 2010). To detect schools, the purse seine were supported by small aircrafts or even helicopters, leaving little possibility for the schools to escape the capture. This kind of fishery intercepted adult schools swimming on the surface during their migration towards the spawning grounds or when they were already in the spawning grounds, thus seriously damaging the reproductive activity of this species.

The International Commission for the Conservation of Atlantic Tunas (ICCAT) regulates Atlantic bluefin tuna fishery. Due to the overfishing of the Atlantic bluefin tuna stock in the Mediterranean Sea and the reduction in the stock biomass, the ICCAT established a multiannual recovery plan for the eastern bluefin tuna stock and greatly reduced: a) total allowable catches (TACs); b) commercial fish size (minimum catchable fork length = 130 cm, 30 kg body weight); and c) fishing period (ICCAT 2008; 2009).

The Principality of Monaco proposed including Atlantic bluefin tuna in the list of endangered species for which international trade is prohibited, and this was discussed at the CITES (Convention on International Trade in Endangered Species) meeting, held in Qatar in March 2010. However, Japan, Canada, and many developing countries opposed the proposal and it was rejected.

In the last 15 years, the catches of the purse seine fleet in the Mediterranean have been sold in almost their entirety to so-called

“tuna farms”, where the fish are fattened for a few months before slaughtering (Mylonas et al. 2010). Currently, 15 tuna farming plants, mainly located in southern regions (Campania, Calabria, Apulia, and Sicily), are registered in Italy by ICCAT, with a potential production capacity of 13,000 tons (ICCAT 2013). Bluefin tuna farming is then considered a capture-based aquaculture (CBA), as the farming activity is entirely based on stocking individuals caught in the wild (Mylonas et al. 2010) in order to increase their fat content and weight. Following the capture, tuna are kept alive and carefully transferred into a cage and transported from the fishing area to the on-growing or farm site. The transportation speed is very low (1-1.5 knots) and the trip can take several weeks. Upon arrival to the fattening site, the fish are transferred into rearing cages which can range in size from 30 to 90 m in diameter and from 15 to 30 m in depth. The lipid content during the fattening period can reach about 10 g kg⁻¹ week⁻¹, with great variations according to diet and water temperature (Aguado et al. 2004). The majority of fish used in the diet are fat-rich, low-cost species, including sardinella, pilchard, herring, mackerel, horse mackerel, chub mackerel, bogue, and some cephalopods (Vita et al. 2004; Mylonas et al. 2010). Fish are slaughtered a few months after capture, generally in autumn, depending on market demand and prices.

The sustainability of such activity is being discussed due both to wild fish (frozen mackerel, pilchard, and herring or bogue) being used as fresh feed and to the impact of the use of wild young tuna captured from natural populations. The increasing pressure from many countries for this species to be listed on Appendix 1 of the CITES will probably diminish the prospects for tuna farming activities in the Mediterranean, but considerably improve the prospects for necessary research into tuna reproduction, creating a closed life-cycle farming process in the future.

In the last decade, intensive research has been carried out in an effort to convert the tuna-fattening industry to a true, self-sustained aquaculture activity. Following the EU-concerted action DOTT, which laid the foundation for a pan-European scientist and farmer aggregation, the research project REPRO-DOTT was funded within the 5th Framework Programme of the European Commission. During this project, mature gametes were produced from wild-caught, cage-reared adult bluefin tuna, and in an *ad hoc* design, and gonadotropin-releasing hormone (GnRHa) was administered via a sustained release implant (Corriero et al. 2007; Mylonas et al. 2007; Aranda et al. 2011). Using the same technology, large-scale (tens of millions) embryos were produced and collected in 2008, 2009, and 2010 in the South-Tyrrhenian Sea in the framework of the project ALLOTUNA funded by the Government of the Apulia Region (De Metrio et al. 2010). In the project SELFDOTT, funded within the 7th Framework Programme of the European Commission, existing knowledge on Atlantic bluefin tuna reproduction (including reproduction control in captivity) could be further improved and suitable technologies developed for larval rearing and feeding under culture conditions. In 2009 (following GnRHa treatment) and in 2010 and 2011 (spontaneously), a bluefin tuna broodstock reared in Cartagena (Spain) produced a hundred millions eggs with a fertilization rate close to 100 %. The eggs collected in the framework of the ALLOTUNA and SELFDOTT projects were used for shipment experiments to different hatcheries and research laboratories in Europe and Israel. Larval-rearing experiments were performed using the three targeted methods: mesocosm, pseudogreen water, and clear water. At present, about 100 juveniles spawned in the reproductive season

2011 are still alive in a rearing cage of the Spanish Institute of Oceanography (personal communication of Dr. Fernando de la Gándara, coordinator of the SELFDOTT project).

4.3 GREATER AMBERJACK

4.3.1 Classification, morphology, distribution and habitat, and exploitation

Greater amberjack (*Seriola dumerili*) is an ichthyophagous species belonging to the Carangidae family. It is a slender, fusiform jack with a short and pointed head and relatively small eyes and characterized by a moon-shaped caudal fin, efficient for fast swimming in pursuit of prey. It presents a dark amber stripe running from the nose to just in front of the dorsal fin that becomes more defined during feeding activity or when the fish is excited. It presents a dusky “mask” stretching from the upper jaw through the eye to the first dorsal fin (FLMNH 2011). Distribution is circumglobal, occurring in the Indo-West Pacific, Mediterranean Sea, and Western and Eastern Atlantic Oceans (Andaloro and Pipitone 1997; Thompson et al. 1999). It is a pelagic, epibenthic species associated with reefs, rocky outcrops, or wrecks in depths ranging from 18 to 72 m (Manooch and Potts 1997; FLMNH 2011).

World capture fisheries production of this species amounts to about 3,000 tons per year. Italy is the most important producer among European countries with 500 tons per year, representing one sixth of global capture production (FAO 2012f).

According to FAO statistics, aquaculture production for this species is only starting: only 2 tons were reported for Spain in 2011 (FAO 2012e).

4.3.2 Reproduction

Greater amberjack is a gonochoristic species and adult females are larger than males (Swasey 2011). In the western Atlantic Ocean, this species spawns offshore from March through June (FLMNH 2011), while in the Mediterranean the spawning season lasts from late spring to early summer, from May to July (Lazzari and Barbera 1988; 1989). According to Micale et al. (1993), maturity occurs at 3 years of age but functional breeders are 4 and 5 years old for males and females, respectively. *S. dumerili* is a multiple spawning fish, and it may release several batches of eggs during the same spawning season (Ottolenghi et al. 2004).

Estimates of potential annual fecundity range from 25,472,100 to 47,194,300 oocytes for ages 3-7 (Harris et al. 2007).

Reproduction of greater amberjack has proven difficult in culture conditions. Almost all research and commercial culture activities are based on wild-caught juveniles or eggs produced from hormone-induced mature breeders obtained from the wild (Tachihara et al. 1993; Jover et al. 1999; Lazzari et al. 2000; Mazzola et al. 2000; Nakada 2000; Pastor et al. 2000). Successful maturation and spawning has been achieved with the use of gonadotropin-releasing hormone agonist (GnRH_a) loaded into controlled-release devices (Mylonas et al. 2004).

4.3.3 Larval rearing and weaning

A larval rearing technique for this species was proposed by Papandroulakis et al. (2005) using the mesocosm technology. After mouth opening on day 2 posthatching, exogenous feeding with rotifers, *Artemia* nauplii, and inert feed was initiated, while endogenously produced copepods contributed as food for the larvae from day 7 posthatching onwards. Despite the low survival rate achieved (3.5 %) the mesocosm technique could represent a promising technology for the rearing of greater amberjack larvae.

4.3.4 Nutrition and feeding

Greater amberjack is a carnivorous fish species. In the wild, small specimens (9-18.5 cm) have a planktonic diet based on decapods larvae, pelagic amphipods, and gastropods, while larger ones (>20 cm) have an essentially piscivorous diet (Pipitone and Andaloro 1995). Many studies have evaluated its nutrient requirements (Jover et al. 1999; García-Gómez 2000; Talbot et al. 2000). Fingerlings are highly dependent on dietary protein as an energy source while high lipid diets negatively affect feed efficiency and specific growth rate (Takakuwa et al. 2006). Furthermore, some authors reported that this species does not readily accept dry diets, and when comparing moist pellets to extruded pellets, fish on dry pellets were found to produce inferior growth results (García-Gómez 1993; Mazzola et al. 2000). Papadakis et al. (2008) have shown that a gradual reduction in dietary moisture from 40 to 7 % (moisture content of commercial pellets) was beneficial for weaning greater amberjack to dry feed.

4.3.5 Rearing

The greater amberjack adapts easily to a captive environment (Lazzari and Barbera 1989; García-Gómez 1993; García and Díaz 1995; Fronte et al. 2012) and net cages (Boix et al. 1993; Grau et al. 2000; Mazzola et al. 2000) and is characterized by a rapid growth rate (Kentouri et al. 1995; Thompson et al. 1999; Divanach 2002), reaching 6 kg within 2.5 years of culture (Jover et al. 1999; Mazzola et al. 2000; Pastor et al. 2000). Nevertheless, the culture of this species still needs to be considered as a capture-based aquaculture in which the life cycle cannot be managed on a commercial scale and the “seed” materials (i.e., larvae, juveniles, and adults) are predominantly collected from the wild (Ottolenghi et al. 2004; Nakada 2008).

4.3.6 Quality, market, and critical points

Greater amberjack is known for excellent flesh quality (Rodríguez-Barreto 2012; Saito 2012) and high worldwide demand (Nakada 2000). This aspect suggests a great potential of this species for commercial aquaculture. Nevertheless, further efforts should be made to develop and standardize reproduction and larval rearing technologies in culture conditions and to improve the adaptability of the species to dry commercial feeds during the on-growing phases by developing suitable diets.

4.4 BLACKSPOT SEA BREAM

Blackspot sea bream has a strong biological and commercial potential to be reared at the industrial scale (Peleteiro et al. 2000) and the quality of its meat is valued by consumers. Therefore, blackspot (= red) sea bream, *Pagellus bogaraveo* (Brünnich, 1768) is one of the possible candidates to complement the most commonly farmed marine fish species productions in Italy. *Pagellus bogaraveo* is an ichthyophagous species belonging to the Sparidae family. Morphologically, it is characterized by a very large eye, the eye diameter being longer than the length of the mouth, and the presence, in adults, of a large black spot at the origin of the lateral line. Skin color tends to be pinkish/reddish, while the buccal cavity and the gills are orange. It lives on sandy and rocky bottoms and is widespread across the Atlantic and the Mediterranean sea from Gibraltar to the Adriatic, while it is absent in the Black Sea (Ozorio et al. 2009). Blackspot sea bream is a demersal species: juveniles live near the coasts until they reach a weight of about 100 g, after which they move into deeper waters, around 500 m (Krug 1989). It is currently being intensively fished, which has impoverished natural stocks (Ozorio et al. 2009). European capture fishery production of this species has decreased from 2,255 tons in 2005 to 1,475 tons in 2009. The major EU producers with regard to fisheries are, in order, Portugal, Spain, and France with 1,184, 172, and 90 tons in 2009, respectively (FAO 2012f). Currently Spain has almost an exclusive monopoly in breeding blackspot sea bream on a large scale, with productions of about 200 tons year⁻¹ (FAO 2012e). Not surprisingly, early studies of *P. bogaraveo* breeding were carried out in Spain and date back to the early 1990s (Martinez-Tapia et al. 1990; Peleteiro et al. 1994). Subsequently, the species has adapted well to captivity conditions, giving positive feedback with regard to reproduction, larval rearing, prefattening, and fattening. It has also showed good resistance to handling and diseases (Peleteiro et al. 2000).

2.4.1 Reproduction

Studies of the reproductive biology of *Pagellus bogaraveo* are often contradictory. Blackspot sea bream is a protandric hermaphrodite species (Chilari et al. 2006). Sanchez (1983) considered hermaphroditism in this species a transitional phase, incompatible with sexual maturity, and he found it in 75-100 % of the specimens under the size of 35 cm (5 years) and more rarely in larger sizes. Krug (1990) reported in early stages, both in captivity and in the natural environment, different subjects with three different types of gonads: functional males (males, 29 % of the population), nonfunctional ovotestes (hermaphrodites, 32 % of the population), and functional ovary with degenerated testes (females, 39 % of the population). Moreover, this author suggested that some males may not undergo sex reversal and the size of inversion may be extremely variable, too.

Micale et al. (2011) reported that *P. bogaraveo* is characterized by late sexual differentiation (2nd year of life) with undifferentiated gonads up to 22 cm in size. In adult individuals kept in captivity, these authors identified the following gonadal types (and relative percentages): ovotestes with functional testes and quiescent ovary (25.3 %), ovotestes with functional ovary and regressed testes (28.4 %), ovary without male tissue (41 %), and ovotestes with immature ovary and

testes (5.3 %). Functional males were predominant below the 3rd year of life, while above this threshold functional females were predominant, showing a sex reversal that, according to these authors, occurs around the 3rd year of life. First sexual maturity is reached in nature at the length of 27.7 cm (5 years) and 34.6 cm (8 years) for males and females (50 % of sexually mature individuals), respectively (Krug 1990).

However, in wild individuals kept in captivity, the first spawning occurred after the 3rd year of captivity (4th year after hatching) (Fernandez-Pato et al. 1990). Micale et al. (2002) reported that sexual maturity was achieved at 3 and 4 years, respectively, for males and females kept in captivity, thus confirming a higher precocity of reared individuals than in the wild. The spawning season of blackspot sea bream varies naturally according to latitude and longitude. In the Azores, it falls between January and May with a peak of activity between February and May (Krug 1986), while off the coasts of England spawning begins in September and ends in October. In captivity, reproductive activity occurs between March and May in the Bay of Biscay (Fernandez-Pato et al. 1990), from February to May in the Northwest of the Iberian Peninsula (Olmedo et al. 1998).

Broodstock kept in captivity requires large tank volumes, ranging from 10 to 32 m³, to obtain natural spawning and very low stocking density, ranging from 1.7 to 3 kg m⁻³. The use of hormones during reproduction was tested by Maricchiolo et al. (2007). These authors tested a GnRHa administered as slow-release implants (30 µg kg⁻¹) inserted by subcutaneous injection that induced oocytes maturation, ovulation, and spontaneous spawning at the 14th day posttreatment. Females have ovulatory cycles lasting about 48 hours (group synchronous spawning) and each ovulation leads to the emission of 20-30 cm³ of eggs per female (Peleteiro et al. 1997). This strategy ensures a prolonged breeding period of up to 4 months and is typical of the Sparidae family, to which this species belongs. The estimated fecundity of blackspot sea bream varies from 73,000 to 1,500,000 oocytes per female ranging from 29 to 41 cm length. The eggs are spherical with a diameter just over a millimeter and only one lipid droplet. Embryonic development lasts approximately 54 hours at 14 °C. The hatching rate of fertilized and incubated eggs is approximately 25 %. Larvae measure 3.7 mm at hatching and open their mouth after 5 days at 14 °C.

4.4.2 Larval rearing and weaning

During larval rearing, as well as in all other stages of breeding, it is good practice not to reach excessive densities as they are not well tolerated by the species. At this stage, a stocking density of 10 larvae l⁻¹ is considered optimal. Larval rearing techniques, environmental management, and the administration of live feed until weaning do not differ from those implemented for breeding other Sparidae, such as gilthead sea bream, sharpsnout sea bream, etc. Therefore, rotifers, *Artemia* nauplii, and enriched *Artemia* metanauplii are used up to the 50th day, a time when weaning of the larvae is completed. The survival rate in this phase varies from 5 to 10 % (Ribeiro et al. 2008b).

4.4.3 On-growing

Blackspot sea bream is an omnivorous fish, predominantly carnivorous. In the wild, it feeds on benthonic, mesopelagic and pelagic fish, and invertebrates (Thaliacea, Ophiuroidea and pelagic gastropods) (Morato et al. 2001). The morphology of the gastrointestinal tracts is typical of carnivorous fish species with the presence of stomach, pyloric *caeca*, proximal and distal intestine, and those digestive enzymes normally found in the alimentary tract of carnivorous fish (pepsin in the stomach and pancreatic amylase, lipase, protease, etc., in the intestine) (Caruso et al. 2005).

Very few feeding trials have been carried out for this species; thus, its nutrient requirements are still largely unknown. In one trial, Silva et al. (2006) aimed to identify protein requirements and found 40 % crude protein of the diet to be the level at which a better combination of specific growth rate and FCR was obtained (FCR = 2.1). The same authors found the level of protein daily intake for body maintenance to be 4.3 g kg⁻¹ day⁻¹ for sizes between 20 and 60 g.

The results of feeding trials to test the use of alternative protein, lipid, and carbohydrate sources as partial substitutes for fish meals and oils in practical diets for *Pagellus bogaraveo* were quite encouraging as regards the possibility of replacing significant amounts of such ingredients without affecting growth performance and fish quality (Maricchiolo et al. 2007; Palmegiano et al. 2007; Daprà et al. 2009; Valente et al. 2010). In particular, Maricchiolo et al. (2007) reported that alternative lipid sources rich in linolenic acid produced fillets in which the levels of EPA and DHA were not different from those in fish fed control diets, suggesting that this species shows good elongation and effective desaturation activities of n-3 PUFA precursors.

Initial experience involved fattening of wild-caught individuals. Chereguini et al. (1990), starting from 20 g, obtained 300-g individuals in 17 months by using commercial feeds. Similar results were obtained more recently by Olmedo et al. (2000): subjects of 20 and 48 g born in captivity reached 304 and 355 g, respectively, in 18 months. The same growth was obtained by maintaining the water temperature constant at 19 °C or under a regimen of increasing temperature from 13.6 to 19 °C. Silva et al. (2006) reared blackspot sea bream starting from 22 to 60 g in 3 months with temperatures between 21 and 24 °C. The subjects were fed diets containing increasing protein levels. Ozorio et al. (2009) reported an individual average weight gain of 85 g in 138 days at 19 °C, starting from 64 g initial average body weight. In a recent trial, 37-g individuals allocated to a recirculating water tank system reached 94 g in 103 days at 19 °C (Figueiredo-Silva et al. 2010). One of the observed characteristics of the species under rearing conditions is the high whole-body and visceral lipid deposition (Valente et al. 2010). However, this characteristic can be influenced by dietary protein content (Silva et al. 2006), feeding level (Ozorio et al. 2009), and stocking density (Genovese et al. 1998). Valente et al. (2010) reported a possible means of controlling lipid deposition by reducing whole-body and mesenteric fat and choosing appropriate carbohydrates sources and low-fat diets. Furthermore, the low growth rate observed in this species could be attributed to its higher demand of dietary protein energy in favor of lipid synthesis and deposition (Ozorio et al. 2009; Figueiredo-Silva et al. 2010).

4.4.4 Quality and market

From trials carried out on wild individuals, *Pagellus bogaraveo* could be classified as a “low fat species” according to the percentages of lipids in the edible portion, which varied from 3 to 8 % depending on the season and the site of capture (Soriguer et al. 1997; Maricchiolo et al. 2007). Muscle fatty acid profiles showed significant levels of DHA, above 20 % (Soriguer et al. 1997), and a very favorable n-3/n-6 ratio (>7) (Maricchiolo et al. 2007). From the commercial point of view, blackspot sea bream has a high and stable market value throughout the year. It is known for the quality of its meat and can get high retail prices up to 15-25 € kg⁻¹ (market research in Campania region).

4.4.5 Critical points

In general, some aspects related to the reproduction of *Pagellus bogaraveo* in captivity, which is not yet standardized as in other farmed fish species, could represent critical points for breeding. Problems related to broodstock adaptability to captivity conditions with consequent effects on fertility, egg maturation, spawning, spermiation, sperm motility, and asynchrony between sexual maturation of males and females are factors that may limit the reproductive success of *P. bogaraveo*. Furthermore, the depth at which fish live in the wild requires that specific technical solutions and practical measures are adopted (adequate coverage of the tanks, feed sinkability, etc.). Finally, with particular reference to environmental conditions in Italy and as regards fattening, the high temperatures typical of some areas of the Mediterranean Sea may represent a limitation in rearing this species. Areas presenting adequate water depth, with possible recourse to using submerged cages should be preferred. In addition, the possibility of breeding this species on land with the use of water at lower and constant temperatures throughout the year should be considered.

4.5 SHARPSNOUT SEA BREAM

Sharpsnout sea bream, *Diplodus puntazzo* (Walbaum, 1792), is one of the fish species currently farmed in Italy. However, production is much lower than that of other commonly reared marine species and has been in decline in the past few years. Sharpsnout sea bream is a euryhaline species belonging to the Sparidae family. Morphologically, it is characterized by a very pointy head with a protruding snout, while body color is silver-gray with six dark and six thinner and lighter alternating vertical stripes that can fade in older individuals; normal size in the wild ranges from 15 to 30 cm, although it may reach 60 cm and exceed 2-3 kg in weight (Basurco et al. 2011). It is a benthopelagic fish, living in brackish and marine waters on rocky bottoms mixed with vegetation, not far from the coast at an average depth of 50 m. It is a gregarious species only in the early stages of life, then the fish becomes solitary and normally prefers open waters. It is common throughout the Mediterranean basin and along the eastern coasts of the Atlantic from Gibraltar to Sierra Leone. It is rare in the Black Sea and North Atlantic (Gulf of Biscay), while it is widespread in the Canary Islands and Cape Verde (Bauchot and Hureau 1990).

FAO production data on sharpsnout sea bream European captures are not available. With regard to aquaculture productions, Italy is the main producer in Europe, with a declining output of 159, 50, and 48 tons, respectively, in 2007, 2008, and 2009.

According to the FEAP database (FEAP 2011), the production in 2010 was on the order of 250 tons.

4.5.1 Reproduction

Sharpsnout sea bream is a hermaphrodite species (Micale et al. 1996; Pajuelo et al. 2008). Its gonads are bisexual in their initial development, with both sexes present at the same time. After this initial phase, individuals present a gonadal evolution in a gonochorism direction, called late gonochorism or rudimentary hermaphroditism. Fish kept in captivity in the 3rd year of life have both ovarian and testicular tissues and the relative proportion of the two sexual territories within the ovotestis in the resting period, too, may provide a valuable indication of the functional sex of the individuals (Micale et al. 1996). Sex ratio during the 3rd year of life in individuals kept in captivity is reported to be 1:1 (Micale et al. 1996), while in wild subjects caught off the Canary Islands it varied with season from 1:1.86 in the reproductive period to 1:2.26 in the seasonal resting period (Pajuelo et al. 2008). The spawning season varies according to latitude and longitude: off the Canary Islands from September to February with a peak in November; in captivity, in the Mediterranean Sea, spawning occurs between September and December, with a peak of production in October and optimal temperatures decreasing from 21 to 18.5 °C (Micale et al. 1996; Papadaki et al. 2008). Sexual maturity is reached at 2 years of age (Georgiou and Stephanou 1995). Oocyte maturation and thus spawning are asynchronous and continue for 14-20 days per month, over a period of 3--4 months. Spawning may be brought about through water temperature and photoperiod manipulation. In rearing conditions, mean total annual relative fecundity ranges from 2.5 to 5 million eggs kg⁻¹ body weight. Mean monthly fertilization rate ranges from 76 to 81 %. Annual hatching rate is approximately 86 %. Five-day larval survival can vary greatly from 44 to 85 %, according to several parameters (Papadaki et al. 2008).

4.5.2 Larval rearing and weaning

Sharpsnout sea bream larval rearing techniques are similar to those used for gilthead sea bream (Basurco et al. 2011). At hatching the total length is 3 mm. After 2-3 days, depending on temperature, the larval anus and mouth open and, at this stage, the digestive organs begin to differentiate (Boglione et al. 2003; Kamaci et al. 2010). Starting from this phase, larvae are fed with zooplankton of increasing size until weaning: enriched *Brachionus rotundiformis* and *B. plicatilis* in percentages of 70 and 30 %, respectively, between 15 and 25 days dph (days post hatch), *Artemia* nauplii between 15 and 25 dph, and *Artemia* metanauplii between 20 and 50 dph. From day 35, an inert feed commensurate with larval mouth size (about 0.3 mm) is also administered. Weaning is completed in about 50 dph. Survival at the conclusion of the larval rearing phase is 30-35 % at 70 dph (Pastor et al. 2000; Basurco et al. 2011). Papandroulakis et al. (2004) applied the mesocosm semi-intensive technology to sharpsnout sea bream larval rearing with positive results, obtaining 19.6 mm total length and 107.2 mg body weight larvae on day 50 posthatching, with survival of about 54 % and individual production cost of 0.043 €.

4.5.3 On-growing

Sharpsnout sea bream is an omnivorous species and in the wild it feeds on seaweeds, worms, molluscs, and shrimps (Sala and Ballesteros 1997). Digestive enzymatic activities are distributed differently in the different parts of the alimentary tract: in the stomach acid proteases and cellulases are predominant (pH 1-3), while in the rest of the digestive tract neutral and alkaline proteases, lipases, and amylases are the most abundant (pH 6.0-11.0). The enzymatic pattern of *D. puntazzo* justifies its omnivorous habit and suggests a high enzymatic potential for both protein and vegetable polysaccharide digestion (Tramati et al. 2005). The use of plant protein sources as substitutes for fish meal in *D. puntazzo* diets has been studied by various authors with different results. A 40 % substitution rate of fish meal protein with soybean meal did not affect growth performance of fish (Hernández et al. 2007; Piccolo et al. 2011; 2013) although, at a protein substitution rate of 60 %, feeding efficiency and protein utilization of the diet decreased and poorer digestibility was observed (Hernández et al. 2007). Nogales-Merida et al. (2010) reported that sunflower meal can supply up to 30 % of crude protein in diets for feeding sharpsnout sea bream juveniles without any adverse effect on fish growth, liver and gut histology, and amino acid composition and retention. Chatzifotis et al. (2006) reported general negative effects on growth performance by using alfa-alfa protein concentrate in the diet. Furthermore, organoleptic properties of the edible portion may be affected by substituting fish meal with plant protein sources such as soybean meal, as reported by Rondan et al. (2004a). These authors observed an increased fish PUFA content, mainly due to linoleic acid, but a significant decrease in the n-3/n-6 ratio (from 2.21 to 1.66). In contrast, a progressively increased n-3/n-6 ratio in different tissues and organs may be obtained by restricting food intake (Rondan et al. 2004b).

Finally, sharpsnout sea bream requires diets higher in protein to obtain optimal growth performance. The use of a diet with low protein levels (46.7 %) resulted in a decrease in weight gain and food intake compared to a diet with a higher protein level (52.5 %), although protein was utilized better in the low-protein diet (Bonaldo et al. 2004).

Several pre-on-growing and on-growing trials have been carried out by different authors under intensive rearing in tanks, floating cages, monoculture, and polyculture, using special feeds for sharpsnout sea bream and commercial feeds for gilthead sea bream, yielding encouraging similar results in both species (Basurco et al. 2011). In particular, Favaloro et al. (2002) compared growth performance of sharpsnout sea bream with an initial weight of 6 g that were reared for 1 year in floating cages in monoculture or polyculture with *Sparus aurata* in the Gulf of Gaeta (Latina, Italy). The monoculture group reached a higher final weight (174 g) than the polyculture group (130 g), but with a worse FCR (2.02 vs 1.60). Opposite results were obtained by Sarà et al. (1999) in sharpsnout sea bream reared in tanks in monoculture or in polyculture with gilthead sea bream and European sea bass. A reason for this may be that sharpsnout sea bream also feed on the bottom; hence, the specimens in tanks can recover the food that is left uneaten by the sea bream on the bottom of the tank, whereas in cages this will clearly not occur (Favaloro et al. 2002).

On the other hand, in polyculture aggressive behavior towards its conspecifics, typical of this species when reared at high densities, may decrease due to an equal portioning of food between specimens belonging to different species (Favaloro et al.

2002). Indeed, sharpsnout sea bream have also been reported to feed on “fouling” on the nets in addition to the pellets, as reported by Gatland (1995).
García and García (2010) developed an econometric model of viability/profitability of on-growing sharpsnout sea bream in sea cages. Feed (43.85 %), juveniles (25.04 %), and staff (12 %) represented the highest costs and were therefore of greatest economic importance. According to this model the business would only be viable above a selling price of 3.69 € kg⁻¹ and a production minimum of 709,363 kg year⁻¹. The feeding price threshold indicated that the activity was viable up to a cost of 1.85 € kg⁻¹. The authors stressed the great opportunity to reduce the costs of feeding by using plant protein sources, taking advantage of the omnivorous behavior of this species.

4.5.4 Quality and market

Owing to the fatty acid composition of the edible fraction, this species can be included among those with a high nutritional value for human consumption (Rueda et al. 2001; Hernández et al. 2003; Rondan et al. 2004a, 2004b; Piccolo et al. 2007). Furthermore, the results of sensory evaluation studies highlighted the good organoleptic characteristics of the meat of this species. In a study carried out by Hernández et al. (2002), about 60 % of the surveyed population considered buying sharpsnout sea bream at a price similar to that of gilthead sea bream (10 €), and 85 % found it to be pleasing to the eye. The most important appraisal attributes were flavor, texture, juiciness, and fat level. These positive consumer satisfaction results still remained for sharpsnout sea bream fed on diets in which high levels of fish meal were replaced by soy bean meal (Hernández et al. 2007).

4.5.5 Critical points

Widespread *Diplodus puntazzo* rearing was greatly limited in recent years by the onset of an intestinal parasitic disease caused by *Enteromyxum leii*, a histozoic myxosporidium with low levels of host specificity, although sharpsnout sea bream was the most affected species (Fioravanti et al. 2006). This parasite is transmitted orally and probably through injuries or lacerations of the skin, which occurs more readily in high-density rearing conditions (Sitjà-Bobadilla et al. 2007), while it seems that low temperatures (<18 °C) inhibit its expansion. The parasite causes desquamative enteritis, which is often lethal (Beraldo et al. 2011), but in any case results in a cachectic condition and high mortality especially in the 1st year of life, which is associated with a very great economic loss. Infection monitored in healthy fish put into contact with infected subjects progressed very rapidly: the first infected fish were observed on day 5 (prevalence of 20 %); the total prevalence reached 100 % by day 19 (Golomazou et al. 2006). At present, the high pathogenicity of this myxosporidium and the absence of effective treatments for controlling the infection that are currently permissible have, in fact, greatly reduced the production of this species.

4.6 COMMON SOLE

Sole is a flatfish belonging to the order of Pleuronectiformes and to the family of Soleidae that lives in sandy-bottom coastal areas. Three species are present in the Mediterranean, the common sole, Dover sole or black sole, *Solea solea* (Linnaeus, 1758), the Senegal sole (*Solea senegalensis* Kaup, 1858), and the Egyptian sole (*Solea aegyptiaca* Chabanaud, 1927), but only *S. solea* is currently considered autochthonous for Italy. The common sole is a species with high commercial value and high economic importance. This fish – and accordingly activities of local fisheries – is one of those in highest demand on the market (Vallisneri et al. 2000). The current market price in Italy is around 15-25 € kg⁻¹ depending on size (ISMEA 2012). Sole is among the top ten fish species eaten in Italy as fresh, particularly in the North of the Peninsula.

Sole has pinkish-white flesh, characterized by high-meat quality. From a nutritional point of view, it is a lean and easily digestible fish.

In 2010, the total landing of common sole in Europe was 40,153 tons (FAO 2012f), but wild catches are likely to decline in the near future. Over the last three decades, scientific reports have been published on sole cultivation (Howell 1997; Imsland et al. 2003), and several experiments have been carried out at laboratory and pilot scales in recent years for rearing sole in captivity. These reports and experiments provide new and useful knowledge for standardizing the production cycle of this species. In Italy, a few attempts have been made to farm *Solea* spp.; however, production is not covered in the official statistics. Nonetheless, several national farms and research institutes are currently carrying out projects aimed to develop a sustainable sole culture as a tool to preserve wild stocks and diversify the national aquaculture production.

4.6.1 Reproduction

Fertile eggs can be obtained in captivity from natural spawning of a group of adult specimens. Adult fish are collected from the wild and adapted to captivity using large tanks with a flat, sandy bottom, recirculating seawater 35±0.6 g l⁻¹ (Bertotto et al. 2006), low stocking density 0.6-3.0 kg m⁻³ (Devauchelle et al. 1987), light intensity of 20-1,500 lux, and sex ratio of 0.5-3:1 (males/females). Fish are fed *ad libitum* 3 to 5 times a week with semi-moist pellets (Cardinaletti et al. 2009) that are supplemented with mussels and polychaetes during the spawning season (Bertotto et al. 2006). Photoperiod is set up following the annual cycle and water temperature is maintained according to seasonality in the range between 7 and 18 °C. Natural spawning occurs in spring, between February and May, with a daylight duration of 11-16 hours and in the range of water temperature between 8 and 12 °C (Imsland et al. 2003). Floating eggs are collected from the spawning tank and their management is conducted according to the recommendations of several authors (Fonds 1979; Devauchelle et al. 1987; Knutsen 1992).

Currently, spawning standardization of constant and high-quality eggs still represents a bottleneck in farming production.

4.6.2 Larval rearing and weaning

As for the other flatfish, Common sole exhibits a particular larval life stage that strongly influences the hatchery management

of this marine species. Being initially pelagic, larvae gradually settle to the bottom where eye migration takes place between 13 and 25 dph (Palazzi et al. 2006).

Under intensive farming conditions, the larval culturing phase of this species is considered one of the most critical phases mainly due to the increasing mortality at metamorphosis and weaning (Rueda-Jasso et al. 2005). Furthermore, nutritional imbalances, such as high levels of dietary arachidonic acid, at this stage of development can cause malpigmentation (i.e., hypopigmentation - albinism) of the animals (Lund et al. 2007). The lack of a standardized feeding protocol, feed formulations, and appropriate management technique has often increased the cost for juveniles production to unsustainable levels.

In the past, larvae were reared under different live-feed protocols, including *Artemia* nauplii and metanauplii (Howell 1997) or rotifers and *Artemia* (Palazzi et al. 2006). More recently, Bonaldo et al. (2011a) obtained a fairly good larvae growth performance and survival rate by feeding common sole larvae on *Artemia* nauplii from mouth opening and weaning them onto commercial microdiets at 27 dph and using a cofeeding strategy. Furthermore, it was possible to wean larvae at 13 dph, feeding larvae with commercial microdiets. In this study, early weaning affected growth performance but not survival rate and metamorphosis development, and a shorter period of live feeding improved tank hygiene.

4.6.3 On-growing

The on-growing phase in intensive systems is characterized by critical periods. In fact, most companies reported low growth performance at this stage, which temporarily reduces the potential for commercial intensive farming activities. The most critical factors include unknown nutritional requirements of this species, the need for species-specific feeds, the peculiar feeding behavior, susceptibility to disease, and the effects of stocking density on performance (Day et al. 1997; Imsland et al. 2003; Schram et al. 2006; Piccolo et al. 2008).

Dover sole has been intensively farmed at commercial scale in northern Europe in shallow raceways connected at a recirculation system. This farming system is based on the use of very long and shallow tanks and a very high fish density (100-500 m⁻³). The advantage of this farming system would be to save space and water, maximizing production per unit volume. However, this high-technology intensive system showed problems in terms of sustainability, growth performance, and well-being of the animals and the largest farm in Europe located in the Netherlands producing *Solea solea* was closed in 2010 due to problems in production.

Recent studies have shown that the stock density greatly influences growth performance. Schram et al. (2006) reared Dover sole at six different stocking densities between 0.56 and 12.6 kg m⁻² and found a significant relation between stocking density and productivity, with best performance at a stocking density of 7.4 kg m⁻². In another study, Piccolo et al. (2008) stocked soles at 1.3 kg m⁻² and at 2.3 kg m⁻². They showed a significantly higher weight gain and a better FCR at a lower density. In Italy, on-growing trials in intensive or extensive aquaculture systems were carried out mainly in earthen ponds. This encompasses several advantages, particularly with regard to the welfare of animals, which can use the sandy bottom to hide

and find natural food such as worms, molluscs, and crustaceans. The management of this system, however, still needs to be standardized, especially for monitoring the animals and catching at the end of the production cycle. In a study by Palazzi et al. (2006), two growth-rate trials were carried out in northern Italy under both extensive and intensive culture conditions. The extensive rearing began in September by stocking soles of 3.6 g at a density of 1.5 juveniles m⁻² in a rectangular earthen pond, with a surface of about 370 m². Fish were fed on natural diet only. A double-replicate growth-rate trial was carried out in intensive culture conditions, rearing soles of 7 g, aged 200 days after hatching, at a density of 150 juveniles m⁻². Two circular fiberglass tanks of 10 m² surface and about 9 m³ capacity were used. Soles were fed *ad libitum* with commercial, extruded feed formulated for sea fish. Unfortunately, neither trial was successful due to disease outbreaks that occurred during the production cycle. Recently, in Italy, very promising results have emerged for sole production in some commercial farms using two rearing systems. The first involves the seeding of large batches of animals in large, brackish lagoons through extensive farming (*vallicoltura*). The second system involves the use of concrete tanks and warm groundwater under semi-intensive farming. Focusing on the nutrition and feeding of common sole juveniles, some significant steps forward were recently achieved. In a recent experiment conducted by Gatta et al. (2011) on juveniles, the highest SGR and the lowest FCR were achieved with a diet containing 57 % crude protein. In another study by Piccolo et al. (2008), the diet containing 54 % protein and 18 % lipids led to a higher final weight and a better feed utilization than a diet containing 50 % protein and 21 % lipids. On-growing Senegalese sole require considerably lower lipid levels (8 % DM basis) than other marine fish species (Borges et al. 2009) but specific data on *Solea solea* concerning energy and lipid requirements are still lacking. Other recent findings on juveniles of *Solea senegalensis* (Silva et al. 2009) and *Solea aegyptiaca* (Bonaldo et al. 2006) demonstrated the possibility of feeding *Solea* spp. with diets containing vegetable proteins in partial substitution of fish meal without adverse effects on growth performance. Less information about substitution of fish meal by alternative sources is currently available for Common sole (*Solea solea*) and it is inappropriate to rely on data from other sole species because of the differences in growth performance, optimal thermal regimen, broodstock behavior, and natural range (Imsland et al. 2003; Palazzi et al. 2006). With regard to possible alternatives to dietary fish meal and fish oil, we should consider that fish is not present in the natural diet of sole (Reig et al. 2003); therefore, it is of particular interest to evaluate alternative protein and lipid sources, which should have organoleptic characteristics similar to those of sole's natural feed.

4.6.4 Critical points

Common sole is extremely sensitive to diseases. Most of them are well known and are described in other cultured species such as gilthead sea bream, European sea bass, and turbot, but in sole the course of the infection process is often more severe and generally more difficult to control. The most important diseases caused by bacterial agents are: Pausterellosis, Tail rot, Vibrioses, and Black Patch Necroses. All of them have already been described in various publications (Baudin-Laurencin 1986; Bernadet et al. 1990; Baynes and Howell 1993; Zorrilla et al. 1999) and still represent a real problem for intensive sole

farming in Italy. In particular, Pausterellosis caused by *Photobacterium piscicida* is currently the most serious disease that affects both juveniles and adults and that can cause up to 100 % mortality within 1 week (Zarza 2005). Another serious disease which can be transferred from broodstock to juveniles through the eggs is Nodavirus infection, which has been described in common sole by Starkey et al. (2001). Usually, antibiotics or disinfectants can have some positive effects against bacterial agents in acute events, but most of the diseases need to be controlled by preventive measures because of their chronic manifestation that can lead to prolonged mortality and low fish health status. Improving farming systems in terms of water quality control and treatments, optimizing nutrition and feeding management, and minimizing stress are clear steps that can guarantee future disease control of this species.

4.7 TURBOT AND BRILL

Turbot, *Scophthalmus maximus* (Linnaeus, 1758), and brill, *Scophthalmus rhombus* (Linnaeus, 1758), belong to the order Pleuronectiformes and to the family Scophthalmidae, are widely distributed from Norway to the Mediterranean and the Black Sea, and live on sandy and muddy bottoms in the shallower part of the continental shelf down to about 70-80 m. In the Adriatic Sea, they have always been highly valued commercial fish and are caught mainly by bottom and beam trawls but also by fixed gear such as trammel nets and bottom long lines (Caputo et al. 2001). As flatfish, they present an asymmetric and almost round body with eyes on the left side and scaleless skin, but with irregularly distributed bony protuberances, a big mouth, and small eyes. Dorsal and anal fins expand widely over the dorsal and ventral sides. The blind side (right) is whitish in color and the eye side has variable color, generally gray-brownish with dark spots. Being carnivorous, juveniles feed on molluscs and crustaceans and adults mainly prey on fish and cephalopods.

In turbot aquaculture, the rearing techniques developed in France and the UK are based on the possibility of obtaining fertile gametes in the natural reproductive period, or induction deposition by manipulation of photoperiod and water temperature to recreate the seasonal reproductive conditions at a different time with respect to the wild. In Italy, breeding efforts were carried out in facilities where European sea bass and gilthead sea bream juveniles from France were reared under controlled conditions. Unfortunately, they failed due to the high water temperatures in the tanks during the fattening cycle in the summer (Roncarati et al. 2003). At present, the leading European producer is Spain, with most of its turbot plants located in Galicia, where sea water temperatures are ideal to culture this fish; for this reason, most of the farms work in an open-culture system, involving less initial investment and lower running costs.

4.7.1 Reproduction

In the wild, spawning occurs every 2-4 days, usually between February and April in the Mediterranean and between May and July in the Atlantic area. Eggs have a single fat droplet/egg. Larvae are initially symmetric, but by the end of metamorphosis (day 40-50, 25 mm) the right eye has moved to the left, giving rise to asymmetry.

Studies concerning reproduction and breeding have been adequately carried out from the scientific and technical point of view (Mugnier et al. 2000). Cultivated turbot were successfully spawned using sustained-release pellets containing a gonadotropin-releasing hormone analogue (GnRH_a), [D-Ala⁶-Pro⁹-Netx]-luteinizing hormone-releasing hormone. Significant synchronization of females was obtained, reducing the spawning season by about half. The number of spawning females was increased, and all sexually mature females ovulated. The size of eggs from turbot ranges between 0.97 and 1.10 mm. For incubation, cylindrical fiberglass tanks with a conical bottom at the unit volume of 200 l are used. The eggs are incubated in sea water (salinity = 34-36 ‰) at temperatures between 16 and 20 °C. Egg incubation takes place at a density of about 5,000 eggs l⁻¹ with a water exchange of 1-2 l min⁻¹.

The effects of two diets (one natural based on frozen squid and one commercial pellet diet) and origin (wild caught and cultured brill) were studied in terms of growth, survival, reproductive performance, and egg lipid composition. Diets only promoted differences in the months prior to reproduction, with animals fed dry pellets growing larger during these months. Only wild-caught females matured; among these, the ones fed with frozen squid showed higher reproductive potential, with more spawning females for a longer period of time. Neither diet nor animal origin promoted differences in male reproductive potential; the feeds affected egg lipid composition and fatty acid profile, particularly due to DHA content, which was higher in eggs from females that were fed squid (Hachero-Cruzado et al. 2009).

4.7.2 Larval rearing and weaning

Under controlled conditions, hatching rates range between 30 and 50 %. At hatching, the larvae are transferred to larval rearing tanks (around 2 × 2 × 0.60 m) at a density of 20 larvae l⁻¹ and a temperature between 18 and 20 °C. From day 3 after hatching during yolk sac resorption, which requires 6-7 days, feed constituting rotifers enriched with microalgae is given until day 8, at which time rotifers are substituted by dwarf brine shrimp nauplii. On day 14, *Artemia* nauplii are given until day 30 when feed is completely replaced by inert diets. The artificial feed (protein 58 % DM; lipid 17 % DM) used in the weaning phase is administered *ad libitum* by using a device/tank that supplies the feed for 14 hours day⁻¹ (Mugnier et al. 2000).

The most critical phases, in which mortality can be significant, are the early stages of larval rearing and during the change in body symmetry; around day 40, the fingerlings have an average weight of 80±20 mg. After 90-100 days, fingerlings are moved to fattening tanks. At that time, the average weight is 2±0.7 g.

4.7.3 On-growing

During the growth phase turbot are nursed in square or circular tanks (10-30 m³) with open-circuit pumped seawater. Aeration systems are usually used to maintain the water at oxygen saturation. Juveniles are fed with dry pelleted feed, introduced manually or automatically. The weight range varies between 5-10 g and 80-100 g during the prefattening period (duration 4-6 months).

The photoperiod during the grow-out phase is controlled with an electric bulb in order to inhibit sexual maturity. It has been observed that growth is strongly affected by temperature and was higher at 16 °C than at 10 °C; according to Imsland et al (1995), continuous light has a growth-promoting effect on juveniles at 10 °C from mid-December to late March, while at 16 °C this effect was restricted to December and January.

The concrete rearing tanks are shallow and square with a surface area of 36 to 85 m². The water depth is 70 cm. Each corner is cut to enhance water current.

The culture techniques used in France and Spain are strictly based on high stocking density. The stocking density is controlled according to size and bottom coverage rate. Usually, 1 kg body weight of turbot can stock at a density of 60 kg m⁻². Females of this species reach commercial size 4-6 months before males, explaining the interest of the industry in obtaining all-female populations.

The most recent studies concern nutrition and feeding of turbot aimed at substituting fish meal with plant protein. At present, feeds for turbot usually include at least 60 % marine-based ingredients such as fish meal and fish oil, and the level of plant protein is estimated to be low as compared with the diets of other cultured fish such as European sea bass, gilthead sea bream, salmon, and trout. Important results on feeding and growth performance were obtained by substituting a mixture of plant protein for up to 52 % of fish meal. At 39 % substitution, turbot maintained optimal growth rate and nutrient utilization although the food conversion rate worsened. However, it was not associated with a reduced digestibility of ingredients or alterations in gut histology (Bonaldo et al. 2011b). In another study (Yun et al. 2011), the effects of dietary cholesterol supplementation on growth performance and cholesterol metabolism of juvenile turbot fed high plant protein diets were investigated; the results suggested that 1.25 % of dietary cholesterol is helpful for juvenile turbot fed high plant protein diets to achieve a significantly better growth rate without negative effects. More recently, the combination of 1.0 % cholesterol and 1.0 % taurine was shown to promote significantly better growth without negative effects in juvenile turbot fed high plant protein diets (Yun et al. 2012). During the grow-out phase, Leknes et al. (2012) showed in a recent study that the dietary crude protein level (50 %) could be successfully reduced by approximately 10 % compared to current levels in commercial feed without negative effects on growth.

4.7.4 Quality and market

The weight range for the European market is between 0.5 and 4 kg; the size mainly exported from France to Italy is below 1 kg, whereas over 1 kg weight appears to be the optimum market size in France and Spain.

Considering the high presence of the species on the Italian market, studies have been published to investigate the chemical composition and quality traits of meat of reared turbot from a Spanish farm that supplied an important Italian supermarket company in comparison with wild turbot (Roncarati et al. 2008b) and the potential of different analytical tools to distinguish wild fish from farmed fish. In the latter case, an interesting paper demonstrated that isotopic measurements (isotope ratio mass

spectrometry IRMS) are a promising mean of distinguishing between wild and farmed fish and between wild fish of different geographic origin (Busetto et al. 2008).

4.8 MEAGRE

4.8.1 Classification, morphology, distribution and habitat, and exploitation

Meagre (*Argyrosomus regius*) has been found to be a suitable candidate species for the diversification of aquaculture in the Mediterranean region in the past few years.

Meagre belongs to the Sciaenidae family and is a carnivorous species. Morphologically, it has a relatively large head and an elongated body. The mouth is in terminal position without barbels. A lateral line is evident, extending onto the caudal fin. The second dorsal fin is much longer than the first one. Several branched appendices are present in the swimbladder, which can vibrate, producing a deep sound typical of males during the spawning season. The otoliths are very large. Body color is silver-gray, with bronze traits dorsally. The fin base is reddish brown and mouth cavity yellow-gold. It can reach up to 2 m in length and 50 kg in weight (FAO 2012b).

It inhabits the Mediterranean and the Black Sea and also occurs along the Atlantic coast of Europe where it lives in inshore and shelf waters close to the bottom, as well as in surface and midwaters from 15 to about 200 m (Whitehead et al. 1986). World capture fisheries production of meagre is about 6,000 tons per year, mainly concentrated in African countries (Ghana, Mauritania, Egypt) (FAO 2012f). Meagre culture started in France and in Italy in the late 1990s and has developed in the Mediterranean region, jumping from a few tons in 2000 to almost 14,000 tons in 2011. Nevertheless, this increased production is related primarily to Egypt (12,000 t in 2011) while in European countries the production has remained quite constant or has decreased in some cases, Spain, France, and Italy being the major producers with 1,000, 400, and 150 tons, respectively, in 2011 (FAO 2012e).

4.8.2 Reproduction

Natural spawning occurs in the Canary Islands from January to May (Moal 1957), while under culture conditions the spawning seasons last from April to June, with high hatching and survival rates at first feeding (Roo et al. 2010). A 1.2-m female produces about 800,000 eggs; spawning occurs at 17-22 °C. Fertilized eggs measure 990 µm in diameter (FAO 2012b). A few attempts to cultivate meagre have been made by hatcheries in France and Greece, initially succeeding (Poli et al. 2003; Duncan et al. 2008). An active feeding response and the occurrence of sexual maturation to advanced stages was reported in meagre individuals that were captured and acclimatized to captivity and indicated that the fish adapted well to captivity. Spawning was successfully induced with GnRHa hormonal treatments with hatching percentage varying from 45 to 64 % (Duncan et al. 2008).

4.8.3 Larval rearing and weaning

Larval rearing of this species was performed under different feeding sequences and larval densities, while some authors applied feeding protocols in which gilthead sea bream culture techniques were adapted (Estévez et al. 2007; Pastor and Cárdenas 2007; Roo et al. 2007).

Different larval densities and feeding sequences were tested by Roo et al. (2010). These authors applied the green water technique by adding live phytoplankton (*Nannochloropsis* sp.) to the larval tanks, obtaining the best survival rate (56 %) at a larval density of 125 larvae l⁻¹, while a lower larval density (50 larvae l⁻¹) and an early *Artemia* introduction (8 day after hatching, dah), promoted a better larvae growth. The weaning occurred at 30 dph.

4.8.4 Nutrition and feeding

Meagre is a carnivorous fish. Wild juveniles (age 1) eat small demersal fish and crustaceans (mysids and shrimp). When they reach 30-40 cm, they feed on pelagic fish and cephalopods (FAO 2012b). In culture conditions, diets are similar to those used for other Mediterranean marine species. Extruded feeds with 45-48 % protein and up to 20-24 % lipid are generally given (FAO 2012b; Piccolo et al. 2008). However, excess dietary lipid level should be avoided since increasing lipid in the diet from 17 to 21 % was reported to increase fat accretion and impair growth performance (Chatzifotis et al. 2010).

4.8.5 Rearing

Qualitative traits of farmed meagre from intensive production conducted both in land-based tanks and sea cages are influenced by the rearing system (Angelini et al. 2002; Martelli et al. 2013). This fish has the capacity to tolerate wide ranges of temperature and salinity and has a high resilience to stress factors. Nevertheless, the best temperature for its growth is reported to be between 17 and 21 °C, with an acceptable range of 14-23 °C (FAO 2012b). It is a fast-growing species, where a fry of a few grams becomes a fish over 700 g after 12 months and weighs 2-2.5 kg after 24 months (Quéro and Vayne 1997; Quémener 2002, Piccolo et al. 2008). It is characterized by a good FCR. Some authors obtained a FCR ranging from 1.38 to 1.60, depending on the diet (Chatzifotis et al. 2010).

4.8.6 Quality and market

Meagre flesh quality is characterized by high protein levels and low lipid content. Total lipids are characterized by high percentages of PUFA, which are mainly represented by a high proportion of n-3 PUFA and by favorable n-3/n-6 ratio values (Poli et al. 2003; Grigorakis et al. 2011). From a market viewpoint, meagre is endowed with intrinsic values such as attractive fish shape, good processing yield, good nutritional values, low fat content, excellent taste, and firm texture suitable for a large variety of recipes (Monfort 2010). Yet it is not well known to end consumers so that it is presently a niche species with a limited production directed to selected market segments.

Farmed meagre is mainly sold fresh in bulk, traded with the whole head on, and generally gutted, although the large size of this species offers excellent opportunities for processing, with an output yield ranging from 42 to 45 % (according to the size of the fish and the final product) (Monfort 2010).

4.8.7 Critical points

Meagre is reported to have a high adaptation capacity to environmental conditions and a high resilience to stress factors; however, some Italian farmers have reported that the low water temperatures reached in some areas of the Mediterranean Sea in winter season can represent a limit for this species to adapt in such areas.

Meagre demand on the market is still low due to the fact that it is generally not well known to fish consumers in Italy, apart from some local areas. The species does not have a market image, but does have positive intrinsic values. From a marketing point of view this image will have to be created (Monfort 2010). In this regard, it is worth noting that in Italy, especially in the central and southern regions, fish is still dominantly sold in bulk. This aspect linked to a preference of Italian consumers for well-known and recognizable fish species (European sea bass, gilthead sea bream, etc.) makes it difficult to expand new fish products on the market.

5. Conclusions

This review has provided an overview of the status and the perspectives of Italian finfish aquaculture. Currently, the technology level of marine aquaculture is high in Italy for both land-based farms and sea cages and three different farming systems can be distinguished: extensive farming (land-based farms -- *vallicultura*), semi-intensive farming (land-based farms), and intensive farming (freshwater and marine flow-through land-based systems and coastal and offshore systems for finfish marine species).

Until the 1990s, most of the production came from land-based farms. Thereafter, with the development of mariculture techniques first with floating cages in sheltered areas and later with submersible cages in open sea, products coming from offshore facilities came to account for more than half of the total production in the country. Italy is among the four largest finfish producers in EU27, together with the UK, Greece, and Spain, and among the six largest finfish producers of the non-EU and EU member countries with 72,130 tons in 2010. Within this sector, the need to diversify fish production, which has focused on a few species for too long, is increasingly being recognized. Production is, in fact, mainly centered on three species: rainbow trout (55.5 %), followed by European sea bass (13.6 %), and gilthead sea bream (12.2 %). Concerning the latter two species, the sector has been suffering for several years from competition with other countries of the Mediterranean area, primarily Greece and Turkey. Aquaculture products imported from these countries at low prices are very competitive when compared to the national products. This strong foreign competition has pushed the market prices towards their lowest level, with a consequent decline in profit for farmers. To address this problem, Italian farmers have reacted by diversifying their

products towards gutted and filleted products and new processing and packaging techniques, with better integration among the production, processing, and marketing sectors. Furthermore, a strategy based on quality improvements and nutritional properties of aquaculture products has been promoted, up to the creation of “production poles” (Orbetello in Tuscany, for example), whose products are increasingly perceived by consumers as carriers of an intrinsic quality, by virtue of mere geographical origin with which consumers associate a certain environmental quality and *know-how* resulting from decades of experience in the field. However, it appears evident for the future development of this sector that production must be diversified by focusing on new, valuable fish species. To this end, researchers and workers have investigated alternative species. The main aspects of the biology and breeding approaches for these new species in Italy have been presented in this review, with particular emphasis on controlled reproduction, larval rearing and weaning, on-growing techniques, quality, and market, and by highlighting the critical points peculiar to each. The results obtained so far are encouraging but not always univocal. The problems encountered in the breeding of these new species are related, in general, to incomplete knowledge of the nutritive requirements at different growth stages (tuna), to the lack of standardized reproduction (blackspot sea bream, tuna), weaning (common sole), feed (greater amberjack), on-growing techniques, or containment systems that take into account the different biological and behavioral characteristics of different species. For other species, mainly flatfish and sparids, eggs and fry quality and quantities could not sustain industrial productions. In other cases, the limited market demand limits expansion (meagre). For some other species, in contrast, production at the commercial scale has been strongly affected in recent years by the occurrence of viral, bacterial, and parasitic diseases. This applies to sharpsnout sea bream, for which the limiting factor has been represented by *Enteromyxum leei*, an intestinal parasite that causes high mortality in this species, especially when the temperature rises above certain values. For other species successfully reared in other European countries, the limitation is represented by the particular climatic conditions of the Italian coasts, which in the absence of adequate plant solutions have made them not very adaptable to the specific climate condition (blackspot sea bream). In conclusion, for the future development of Italian finfish aquaculture a wide range of external factors will be relevant: environmental factors, biological and technical aspects, market constraints, competitive factors such as new, emerging species and new product forms, competition for space with other users on the coastlines such as tourism, financial factors such as investments and simplification of the licensing procedure, and legislation to improve the industry’s competitiveness and productivity. Moreover, further research aimed to overcome the problems encountered in the breeding of new fish species will need to be implemented in the future. These efforts in turn will need to be supported by promotional policies and marketing strategies in order to improve consumer demand and distribution for such new products.

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CAPTIONS OF THE TABLES

Table 1. Italian fisheries and aquaculture sector in 2010 (ISMEA 2012).

Table 2. Italian aquaculture production (tons): 2000-2010. Data elaborated from API (2012), FEAP (2011) and ISMEA (2012).

Table 3. New aquaculture species in Italy.

Table 1. Italian fisheries and aquaculture sector in 2010 (ISMEA 2012).

| | Quantity (thousand t) | Value (million €) |
|---|--------------------------|----------------------|
| Aquaculture | 232 | 600 |
| Fish | 72 | 351 |
| Molluscs | 160 | 249 |
| Marine capture | 242 | 1,202 |
| Mediterranean capture | 234 | 1,179 |
| Oceanic capture | 8 | 23 |
| Total production | 474 | 1,802 |
| Imports | 913 | 3,565 |
| Exports | 133 | 494 |
| Trade balance | -780 | -3,071 |
| Apparent consumption | 1,254 | |
| Per capita consumption (kg year ⁻¹) | 20.8 | |
| Self-supply (%) | 37.8 | |

Table 2. Italian aquaculture production (tons): 2000-2010. Data elaborated from API (2012), FEAP (2011) and ISMEA (2012).

| | 2000 | 2010 |
|--------------------------|----------------|----------------|
| Fish | | |
| Rainbow trout | 44,500 | 40,000 |
| European sea bass | 8,100 | 9,800 |
| Gilthead sea bream | 6,000 | 8,800 |
| Meagre | - | 300 |
| European eel | 2,700 | 1,200 |
| Mullet | 3,000 | 3,800 |
| Sturgeon | 550 | 1,380 |
| Ictalurids | 550 | 550 |
| Ciprinids | 700 | 700 |
| Other species | 2,500 | 5,600 |
| Total fish | 68,600 | 72,130 |
| Molluscs | 189,000 | 160,000 |
| Mussels | 136,000 | 120,000 |
| Clams | 53,000 | 40,000 |
| Total Aquaculture | 257,600 | 232,130 |

1 Table 3. New aquaculture species in Italy.
2

| Common name | Scientific name | Successful rearing | | Drawbacks |
|-----------------------|----------------------------|--------------------|------------|--|
| | | Reproduction | On-growing | |
| Atlantic bluefin tuna | <i>Thunnus thynnus</i> | X | Y | low sustainability |
| Blackspot sea bream | <i>Pagellus bogaraveo</i> | Y | Y | |
| Common pandora | <i>Pagellus erithrynus</i> | Y | Y | |
| Common sole | <i>Solea solea</i> | Y | Y | susceptible to VER* |
| Common dentex | <i>Dentex dentex</i> | Y | Y | |
| Greater amberjack | <i>Seriola dumerili</i> | Y | Y | |
| Meagre | <i>Argyrosomus regius</i> | Y | Y | susceptible to <i>Enteromyxum leei</i> |
| Red porgy | <i>Pagrus pagrus</i> | Y | Y | |
| Sharpsnout sea bream | <i>Diplodus puntazzo</i> | Y | Y | |
| Shi drum | <i>Umbrina cirrosa</i> | Y | Y | slow growth rate |
| White sea bream | <i>Diplodus sargus</i> | Y | Y | |

3 Modified from Barazi-Yeroulanos (2010).
4 *VER: viral encephalopathy and retinopathy.
5