UNIVERSITY OF INSUBRIA, VARESE

Department of Biotechnology and Molecular Sciences

Ph.D. School:
Biological and Medical Sciences

Ph.D. Program in:
"ANALYSIS, PROTECTION AND MANAGEMENT OF BIODIVERSITY"
- XXIII Course -

Biodiversity in eastern Mediterranean marine aquaculture: an approach to new species

Dr George Anastasiades

Guide Professor:
Marco Saroglia

December, 2010

Abstract

Aquaculture is an important agro-food activity to ensure the amount of EPA and DHA indicated by WOS for human health, on the top of precious proteins. In spite that in the Mediterranean area only few marine species (sea bass and sea bream) cover the 92.4% of the market, an increased biodiversity in the production would improve the nutritional quality, as well as the resource for the market and the possibility to better exploit natural resources. This research work is focused on the evaluation of biological and physiological functions of new species, such as rabbitfish (*Siganus rivulatus*) and common pandora (*Pagellus erythrinus*), in respect to their capacity for aquaculture production. Morphometric measurements on wild rabbitfish revealed that the TL-TW relationship is described by the equation y=0.068x+11.044. Condition factor (K) was 1.65±0.17, HSI was 1.4±0.4 and GSI was found to reach maximum values near the full moon during summer months. Respirometry on individual cultured rabbitfish was measured at 17, 20, 23 and 26°C. Oxygen consumption rate increased with water temperature and it was varied between 2.4×10^{-3} mg O_2/g BW/min at 17°C and 1.1×10^{-2} mg O_2/g BW/min at 26°C. Hypoxia-inducible factor-1a, which responds to changes in available oxygen in the cellular environment, was identified in brain and liver samples. Growth experiment on rabbitfish that lasted for 1 year and tested two feeds with different protein content, 35 and 44%, showed no significant difference between the two treatments. Experiment on the effect of vitamin C and astaxanthin on the reproduction performance and quality of eggs and larvae in common pandora, showed that vitamin C improves the reproductive capacity of broodstock and the quality of offsprings. Sodium-dependent vitamin C transporter (SVCT-1) was identified in samples of liver of common pandora. The increase of biodiversity in eastern Mediterranean marine aquaculture is a feasible task, but further work should be done on new species that constitute possible candidates for aquaculture.

Aknowlegments

I would like to thank my promotor Prof. Marco Saroglia, who was always next to me to supervise, support and guide my experimental work. His supervision and direction during my thesis writing were also essential for the completion of this work. Thanks Prof. Saroglia for guiding me throughout the last three years and teaching me aquaculture.

I would like to thank Dr. Genciana Terova for her guidance and help during the genomic work.

Special thanks also go to all the staff and students of the Unit of Animal Biotechnology and Aquaculture.

Last but not least, I would like to thank the staff of Meneou Marine Aquaculture Research Station for their help during the experimental work.

Table of Contents

List of Figures

List of Tables

1. Introduction

1.1. Marine Biodiversity

Diving into literature, someone can find several definitions for biodiversity; some of them complex and some others too simple to describe the magnitude of this word. The magnitude of biodiversity can be better attributed by the fact that "without biodiversity human would not be able to survive". According to the Convention on Biological Diversity (CBD) of the United Nations, biodiversity is defined as:

> "The variability among living organisms from all sources including, *inter alia*, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems" (CBD, 1994)

Biodiversity exists as a complex web of life forms that interact with each other in an ecosystem, in a region or globally. Biodiversity drives the functioning of ecosystems through countless reciprocal interactions with the physical and chemical components of the environment.

Up to now, scientists have described 1.7-1.8 million species of plant and animal and it is estimated that another 10-100 million species are remaining to be discovered (around 1,300-1,500 new species are described every year). Due to the fact that aquatic environment covers 71% of the earth's surface and contains more than 99% of the space for life (taking depth into account), it could be assumed that the majority of the species described are coming from the sea. However, in reality less than 250,000 species have been described from the sea, which represents less than 15% of the world total (Bouchet, 2006). Out of the total number of marine species described, 93,000 species or 37% are coming from the coral reefs. In the sea, the three most diverse phyla or subphyla are the mollusks (± 52,525 species), the crustaceans (± 44,950 species) and the pisces (fish) (± 16,475 species) (Table 1). In Mediterranean Sea, a review of several relevant studies estimated that more than 8500 species of macroscopic marine organisms should exist (Bianchi & Morri, 2000).

Although the magnitude of biodiversity in our lives have been stated in the start, it is worth mentioning again that human life is ultimately dependent upon myriads of other organisms with which we all share the same planet. However, the important role of biodiversity, and in this case marine biodiversity, in the production of goods and services (Table 2) is usually ignored or underestimated by humans. It is enough to consider that all food is directly or indirectly obtained from animals, plants and other photosynthetic organisms, in order to give to biodiversity the appropriate importance. Apart from food, marine biodiversity is involved in the production of many other goods, such as seaweed

for industry and fertilizer, fishmeal for aquaculture and farming, pharmaceuticals, ornamental goods and even land (the carbonate platforms that make up the Bahamas).

Table 1. Global numbers of marine species, by taxa (Bouchet, 2006)

Taxa	Number of species	Taxa	Number of species
Bacteria	4,800	Acanthocephala	600
Cyanophyta	1,000	Cycliophora	1
Chlorophyta	2,500	Entoprocta	165-170
Phaeophyta	1,600	Nematoda	12,000
Rhodophyta	6,200	Nematomorpha	5
other Protoctista		Ectoprocta	5,700
Bacillariophyta	5,000	Phoronida	10
Euglenophyta	250	Brachiopoda	550
Chrysophyceae	500	Mollusca	52,525
Sporozoa	?	Priapulida	8
Dinomastigota	4,000	Sipuncula	144
Ciliophora	?	Echiura	176
Radiolaria	550	Annelida	12,000
Foraminifera	10,000	Tardigrada	212
Porifera	5,500	Chelicerata	2,267
Cnidaria	9,795	Crustacea	44,950
Ctenophora	166	Pogonophora	148
Platyhelminthes	15,000	Echinodermata	7,000
Nemertina	1,180-1,230	Chaetognatha	121
Gnathostomulida	97	Hemichordata	106
Rhombozoa	82	Urochordata	4,900
Orthonectida	24	Cephalochordata	32
Gastrotricha	390-400	Pisces	16,475
Rotifera	50	Mammalia	110
Kinorhyncha	130	Fungi	500
Loricifera	18	**TOTAL**	**229,602**

The contribution of marine biodiversity to human life is not restricted only to the production of goods, but it also comprises many services, such as the production and mineralization of organic material, the storage of carbon, the storage of pollutants and waste products from land, the buffering of the climate and of climate change, coastal protection (mangroves, dune-beach systems, coral reefs), water purification, soil formation, flood prevention, nutrient cycling and aesthetic and cultural impact.

The economic valuation of goods and services, which are directly or indirectly produced from biodiversity, might not be an appropriate way to emphasize the importance of the myriads of other organisms in our lives, but it presents a solid fact or figure for those who give a monetary value to everything. A review of more than one hundred studies attempting to value ecosystem goods and services have estimated their aggregated annual value to lie in the range of about $20 trillion to $60 trillion, around a rough average of $40 trillion (Costanza *et al.*, 1997). These figures are of similar size to the total gross national product of the world (GNP). In a more recent study, it is estimated that 40% of the global economy is based on biological products and processes (Packer, 2002). Although such estimates should be interpreted with caution, they nevertheless indicate the potential magnitude of the global ecological goods and services.

Although, the last few decades, humans have achieved huge advances in technology, such as in informatics, automation, biotechnology, nanotechnology, robotics and satellites, replacement of complex biological functions with technology has not be implemented. Therefore, humans have no other choice than the conservation of biodiversity and ecosystems in order to sustain the production of goods and services through natural biological processes. The necessity for conservation and the growing environmental concerns have attracted the attention and guided international and national bodies to form strategies and promote legislation for the conservation of biodiversity. In December 1993, the United Nations took into force the treaty on the Convention on Biological Diversity (CBD), which has been ratified by over 186 countries. The CBD has three main objectives: i) the conservation of biological diversity, ii) the sustainable use of the components of biological diversity, and iii) the fair and equitable sharing of benefits arising from the utilization of genetic resources. At a European level, two strategies have been introduced, the Pan-European Biological and Landscape Diversity Strategy (PEBLDS) and the European Community Biodiversity Strategy (EC 1998). The first aims in strengthening existing European conventions and the latter introduces four sectoral Action Plans, namely Conservation of Natural Resources, Agriculture, Fisheries and Economic Co-operation and Development. In addition, The Natura 2000 programme, which is supported legislatively by the European Birds (EEC 1979) and Habitats Directives (EEC 1992), outlines habitats and species for the protection and has been an important guide in creating priority conservation areas within Europe.

Table 2. An overview of goods and services provided by marine biodiversity (Beaumont et al., 2008)

Good / Services	Definition	Examples
Production Services		
Food provision	Plants and animals taken from the marine environment for human consumption	Fish, seaweed and shellfish
Raw materials	The extraction of marine organisms for all purposes, except human consumption	Seaweed for industry and fertilizer, fishmeal for aquaculture and farming, pharmaceuticals and ornamental goods such as shells
Regulation Services		
Gas and climate regulation	The balance and maintenance of the chemical composition of the atmosphere and oceans by marine living organisms	Regulation of volatile organic halides, ozone, oxygen and dimethyl sulphide, and exchange and regulation of carbon
Disturbance prevention and alleviation	The dampening of environmental disturbances by biogenic structures	Dampening and prevention of tidal, storm and flood damages, and alleviation through binding and stabilization of sediments and creation of natural sea defences
Bioremediation of waste	Removal of pollutants through storage, dilution, transformation and burial	Assimilation and chemical de and re-composition of wastes, such as oil, sewage and shipwrecks
Cultural Services		
Cultural heritage and identity	The cultural value associated with the marine environment, e.g. for religion, folk lore, painting, cultural and spiritual traditions	Ancient monuments and shipwrecks, development and sustenance of societies (e.g. fishing communities), and inspiration for arts, local customs, tradition, crafts and skills, language and dialect
Cognitive values	Cognitive development, including education and research, resulting from marine organisms	Technology, medicine, environmental resilience and stress, climate change and bio-indicators
Leisure and recreational	The refreshment and stimulation of the human body and mind through the perusal and engagement with, living marine organisms in their natural environment	Seabird watching, rock pooling, beachcombing, sport fishing, recreational diving and whale watching
Non-use values – bequest and existence	Value which we derive from marine organisms without using them	The value the current generation places on ensuring the availability of biodiversity and ecosystem functioning to future generations and the value placed on simply knowing marine biodiversity
Option Use Value		
Option use value	Currently unknown potential future uses of the marine environment	Value associated with retaining the option of exploration
Supporting Services		
Nutrient cycling	The storage, cycling and maintenance of availability of nutrients mediated by living marine organism	Nitrogen, phosphorus, sulphur, silicon and metals
Resilience and resistance	The extent to which ecosystems can absorb recurrent natural and human perturbations and continue to regenerate without slowly degrading or unexpectedly flipping to alternate species	Respond of a range of species to various environmental perturbations and anthropogenic pressures, such as overfishing and pollution
Biological mediated habitat	Habitat which is provided by living marine organisms	Provision of living, breeding, nursery, feeding and hiding space for plants and animals

Despite the above measures and the growing concerns regarding world biodiversity loss, little has been done to assess the biodiversity and the losses of biodiversity within the world's oceans. There are two basic explanations for this gap in knowledge of marine biodiversity. First, the oceans are difficult for humans to explore and second, until recently humans tended to view the oceans as too vast for humans to affect much. The deterioration of marine environment and the loss of biodiversity are mainly attributed to the numerous catastrophic activities of people. Although, traditionally both scientists and policymakers have focused on marine pollution as the most important problem affecting marine biodiversity, today it has been proved that man-made modification or destruction of habitats, overfishing and inadvertent transport of alien species from one ecosystem to another are also included among the major causes of biodiversity decline in the oceans. The majority of marine pollution, which accounts for 77% of total marine pollution, comes from land-based sources. Out of this, 44% is coming from land-based water pollutant and 33% from land-based air pollution (Craig, 2004). Land-based air pollution can arise from both natural events, such as dust storms, and human-caused events, such as fires and industrial air pollution. This pollution can acidify ocean waters, increase the concentration of heavy metals and other toxic pollutants, increase sedimentation of the oceans, etc. Land-based water pollution can also carry toxics and sediment into the seas, as well as nutrients and pesticides especially from agriculture. Excess nutrients can result in algal blooms that, in turn, can cause contamination of fish and shellfish (in case of Harmful Algal Blooms, HABs). Other causes of marine pollution include oil spills and ocean dumping (waste disposal). Marine pollution constitutes also a cause of marine habitat modification and destruction. Due to the fact that marine environment is more stable that the terrestrial, any environmental disturbance is more conspicuous and specific diversity of organisms tends to decrease according to the intensity of disturbance (Cognetti & Curini-Galletti, 1993). Species able to adapt easily and withstand a wide range of variation of environmental parameters increase in number, but genetic variability of the disturbed habitat decreases. Other causes of habitat modification or disturbance are alteration of coastal areas for recreational purposes (e.g. transport of sand for creation of beach, construction of water breaks, etc.), modification of seabed (e.g. by trawling), war episodes and bleaching events. Overfishing contributes to the loss of marine biodiversity, not only by removing the target species, but also through by-catch (incidental catch) of non-target species and destruction of habitats. UN Food and Agriculture Organization (FAO) estimates that 70% of fish stocks worldwide are now overfished, depleted, or rebuilding after previous overfishing. The same organization estimates that each year 27 million tons of fish, about 25% of all reported commercial marine landings, are caught as by-catch and discarded. Destruction of habitats from fishing is mainly caused by bottom trawling. Dragging of trawling gear over huge areas of seabed alters seabed complexity, removes, damages or kill biota and reduces benthic production, leading to substantial changes in benthic community structure and habitat. Another major threat to marine biodiversity that has also profound ecological and economic impacts is the marine invasive species (also

known as “alien species”). Competition for space and food between the invasive and endemic species can result to the decrease or even extinction of endemic species. Once invasive species become established in marine habitats, it can be nearly impossible to eliminate them (Thresher & Kuris, 2004). As far as Mediterranean Sea concerns, the opening of Suez Canal in 1869 allowed an important influx of Red Sea species into the Aegean Sea, a phenomenon known as ‘Lessepsian migration’. Besides this phenomenon, species are intentionally or accidentally introduced into the Mediterranean via ship fouling, ballast waters, aquaculture, trade of living bait, wrapping of fresh seafood with living algae, aquarium trade and scientific research (Bianchi & Morri, 2000).

1.2. Biodiversity and Aquaculture

According to FAO, aquaculture is the farming of aquatic organisms including fish, mollusks, crustaceans and aquatic plants. Farming implies some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding, protection from predators, etc. The potential of aquaculture to enhance local food security, alleviate poverty and improve rural livelihoods has been firstly recognized by the Kyoto Declaration on Aquaculture, which was adopted by the Kyoto Conference (FAO, 1976). Since then, aquaculture is the most rapidly growing animal food-producing sector of the last few decades. The stabilization of capture fisheries and the increase in world population enforced aquaculture to develop and increase its production fast in order to cope with the demand for fish consumption and protein uptake. Worldwide aquaculture sector has grown at an average rate of 8.8% per year since 1970, compared with only 1.2% for capture fisheries and 2.8% for terrestrial farmed meat production systems over the same period (FAO, 2007). The need for rapid production growth and the low experience level at the beginning of aquaculture development has led to some hazardous environmental impacts. A representative example is the destruction of large areas of mangrove forests for the creation of ponds for shrimp culture. However, as experience with aquaculture grows worldwide, the concept of sustainable aquaculture is increasingly recognized to incorporate both spatial and temporal dimensions of environmental, economic, and social parameters. The necessity of ensuring a sustainable development was already recognized in 1995 with the adoption of the FAO “Code of Conduct for Responsible Fisheries”. According to Article 6.19, “States should consider aquaculture, including culture-based fisheries, as a means to promote diversification of income and diet. In so doing, States should ensure that resources are used responsibly and adverse impacts on the environment and on local communities are minimized”. Later on, in 2000, the further expansion of aquaculture production and the shift towards industrialization was encouraged by the Bangkok Declaration and Strategy (Network of Aquaculture Centres in Asia-Pacific (NACA) and FAO, 2000) that emphasized the need for the aquaculture sector to continue development towards its full potential, making a net

contribution to global food availability, economic growth, trade and improved living standards.

In Mediterranean region most of marine aquaculture production is done by means of sea cages (Fig. 1) at a distance from the shore (off-shore). This distance is between few hundred of meters and a couple of kilometers and the sea depth, where cages are set, is between 20 and 65 meters. Therefore, it is obvious that marine aquaculture in Mediterranean region has a direct influence to the sea and vice versa. Taking into account the advantages of biodiversity mentioned in Paragraph 1.1, it is observed that some of these advantages are also valid in the case of aquaculture. In particular, the benefits of a healthy and large biodiversity to aquaculture include:

- The supply of raw materials, such as fish meal and fish oil for the production of fish feeds.
- The supply of genetic material through the different organisms, such as plankton, fish, crustaceans and mollusks, used in aquaculture. Inbreeding, selection and hybridization in aquaculture can lead to the increase, decrease or lost of specific genes. Therefore, natural environment functions as a reservoir of genetic resources for aquaculture.
- The supply of species for the diversification of marine aquaculture production.
- The balance and maintenance of the chemical composition of the sea, which represents the culture media, by marine living organisms.

Figure 1. Off-shore sea cages of sea bass and sea bream in Cyprus.

The application of bad practices and the bad management of aquaculture units in the past created a rather infamous picture for aquaculture. It was seen as a highly hazardous practice to the environment, even when compared with more hazardous sources of pollution (e.g. nutrient land runoffs), mainly due to the fact that aquaculture facilities could be seen and accused easily. However, the development of scientific research in aquaculture and the growth in experience have improved the picture of aquaculture and promoted its benefits. Some of the benefits of aquaculture that are directly or indirectly support the conservation of the environment and biodiversity are listed below:

- Increase household food supply and improve nutrition.
- Increase household economy through diversification of income and food sources.
- Strengthen marginal economies by increasing employment and reducing food prices.
- Improve water resource and nutrient management at household or community levels.
- Preserve aquatic biodiversity through re-stocking and recovering of protected species.
- Reduce pressure on fishery resources if done in a sustainable manner.
- Improving/enhancing habitats.
- Stimulates research and technology development.
- Increase education and environmental awareness.

It is estimated that until 2020 aquaculture will account for 60% of total fisheries production (FAO, 2009). However, the continuous demand from aquaculture to increase production in order to cope with capture fisheries stability in production, growing world population and increasing per capita consumption of fish poses some risks for the environment and marine biodiversity. Although these risks and impacts already exist on a certain level (depending on the farmed species, technology and management), their severity may increase if aquaculture development is not implemented under sustainable standards. Some of the risks and impacts of aquaculture that affect the environment and biodiversity include:

- Habitat destruction to create ponds, e.g. destruction of many hectares of mangrove forests to construct shrimp ponds.
- Pollution of local water by intensive production, e.g. release of organic matter coming from fish wastes and fish feed.
- Effects of antibiotics and other chemical treatments on local microfauna and microfauna.
- Intensive collection of wild seed and fish, e.g. mussel spat in Netherlands, milkfish (*Chanos chanos*) fry in Philippines, bluefin tuna (*Thunnus thynnus*) in Mediterranean.
- Competition by endemic fauna by escaped exotics.

- Introduction of pathogens and parasites, e.g. sea lice on cultured Atlantic salmon (*Salmo salar*)
- Genetic introgression with local fauna by introduction of populations, species and transgenics.

The goal of aquaculture industry today is to increase in production to meet the demand for fish consumption, and on the same time to be as much environmentally friendly as possible. Therefore, sustainable aquaculture development must be advanced in a manner that is environmentally sustainable and that protects the quality of the environment for other users, while it is equally important for society to protect the quality of the environment for aquaculture. Some recommendations towards this direction are listed below:

- Forming integrated coastal and rural/community management plans.
- Emphasize use of processed feeds and not fresh feeds such as low valued marine fish.
- Setting suitability standards and indicators for aquaculture (e.g. benthic fauna as bio-indicators).
- Setting monitoring and evaluation mechanisms.
- Establishing quality standards (certifications) for environmentally friendly practice, processing and sale in the aquaculture industry.
- Expanding finfish aquaculture industry should farm low trophic level fish (e.g. rabbitfish) and preferably in extensive, more responsible practices.
- Developing polyculture and environmentally friendly practices (e.g. culture of seaweeds and crustaceans near finfish cage culture units).
- Eliminating government subsidies for ecologically unsound practices and establish enforced regulatory measure for protection of coastal and aquatic ecosystems.

1.3. Context of aquaculture in the Mediterranean

Aquaculture in the Mediterranean region is an activity which started many centuries ago. It is possible to find records of aquaculture activities during the ancient Egyptian civilization. The tomb of Aktihep (2500 BC) shows what appears to be men removing tilapia from a fish pond. In the Etruscan culture (Italy) the earliest extensive marine farms date back to the 6th century BC. Growing of molluscan shellfish was practiced in the 5th century BC by the Greeks. In the ancient Roman civilization sea bass, sea bream, mullets and oysters were cultivated or simply kept alive off the Italian coast in enclosed facilities.

The end of the Roman Empire led to the disappearance of this type of aquaculture, and it was not until the 12th century AD that a resurgence of freshwater aquaculture was seen, starting in central Europe. It was only in the 15th century AD that

extensive, large-scale aquaculture was seen in the lagoons of the northen Adriatic, referred today as "vallicultura". Thereafter, in the 19th century AD, the culture of shellfish once again became common practice, particularly in the western Mediterranean and along the northern shores of the Adriatic Sea. This origin strongly promoted the initiation of the modern marine Mediterranean aquaculture, which effectively started about 30 years ago.

1.3.1. World aquaculture

According to FAO statistics (FAO, 2009), total world fisheries production (which includes capture fisheries and aquaculture) was 143.6 million tons in 2006 (Fig. 2). Among these, 110.4 million tons were food fish, providing an apparent per capita supply of 16.7 Kg, which is among the highest on record. Out of this total, aquaculture accounted for 47% (51.7 million tons).

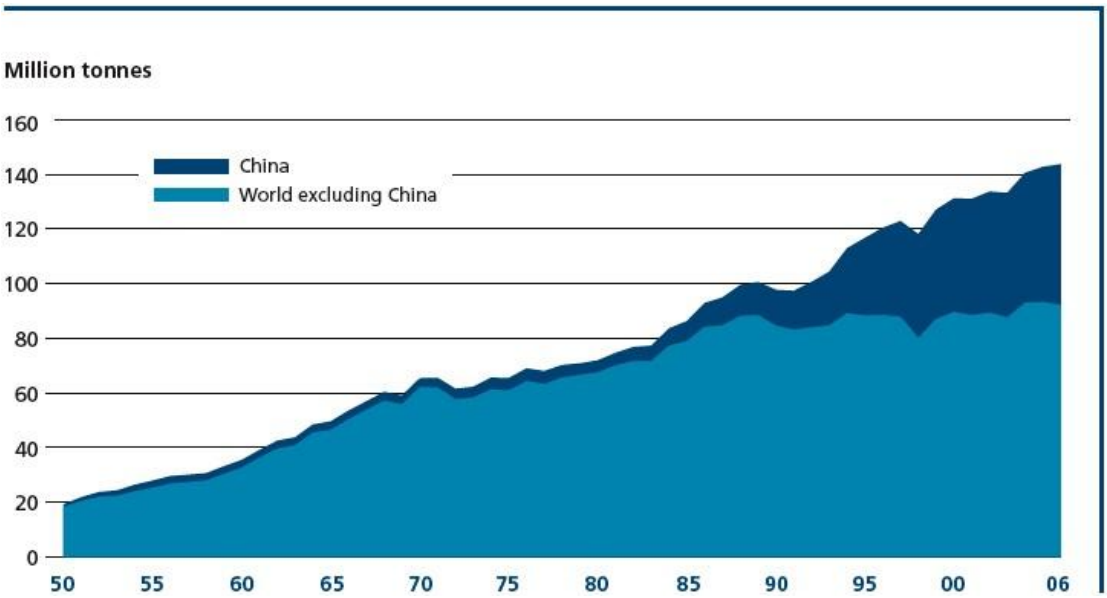

Figure 2. World capture and aquaculture production (FAO, 2009).

The contribution of aquaculture to global supplies of fish, crustaceans, mollusks and other aquatic animals has continued to grow, increasing from 3.9% of total production by weight in 1970 to 36% in 2006. The growth of supply from aquaculture not only offset the effect of static capture fishery production and the rise in world population but it also increased the per capita supply. Specifically, per capita supply from aquaculture increased from 0.7 Kg in 1970 to 7.8 Kg in 2006, attaining an average annual growth rate of 7%.

Aquaculture is the most rapidly growing animal food-producing sector of the last few decades. From a production of less than 1 million tones in the early 1950s, production in 2006 was reported to have risen to 51.7 million tones, with a value of US$ 78.8 billion. While capture fisheries production stopped growing in around mid-1980, the aquaculture sector has maintained an average annual growth rate of 8.7% worldwide (excluding China with 6.5%) since 1970. Annual growth rates in world aquaculture production between 2004 and 2006 were 6.1% in volume terms and 11% in value terms.

In 2006, countries in Asia and the Pacific regions accounted for 89% of production by quantity and 77% of value. Of the world total, China is reported to produce 67% of the total quantity and 49% of the total value of aquaculture production (Fig. 3)

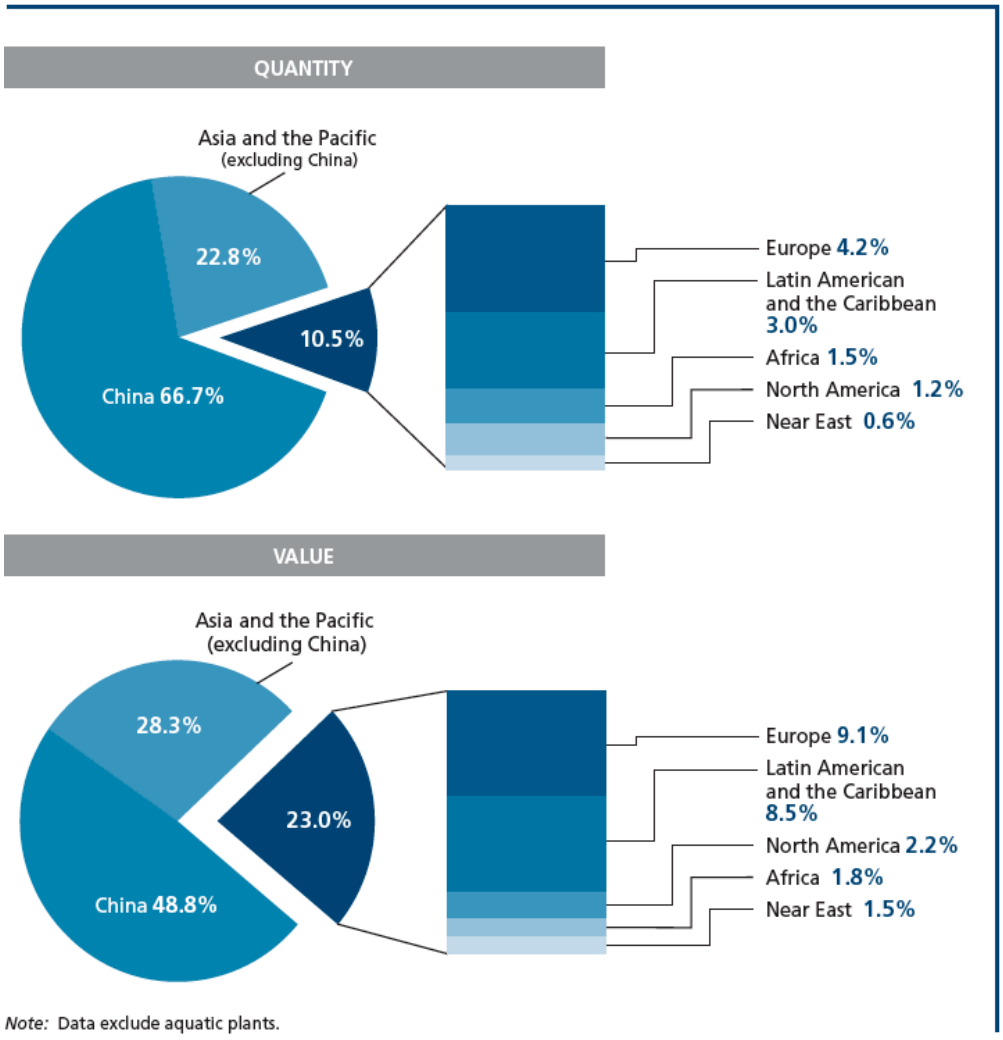

Figure 3. Aquaculture production by region in 2006 (FAO, 2009).

Most aquaculture production of fish, crustaceans and mollusks continues to come from inland waters (61% by quantity and 53% by value). An allocation of aquaculture

production by aquatic environments shows that the freshwater environment contributes 58% by quantity and 48% by value, whereas marine environment contributes 34% by quantity and 36% by value. In 2006, more than half (27.8 million tons or 54%) of global aquaculture production was freshwater finfish, while the second-largest share was obtained by mollusks with 14.1 million tons (27% of total production). Marine fish production accounted for 3% of quantity and 8% of value (Fig. 4).

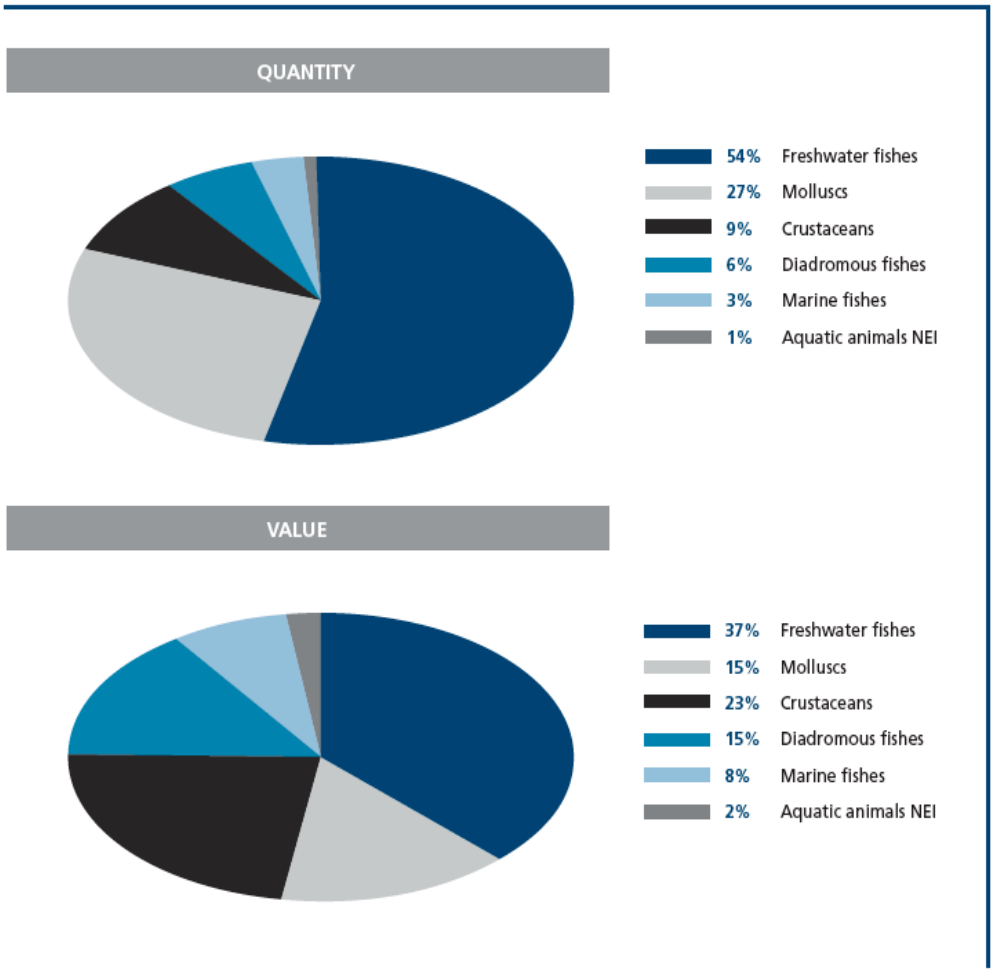

Figure 4. World aquaculture production: major species group in 2006 (FAO, 2009).

It is worth mentioning that overall fish provided more than 2.9 billion people with at least 15% of their average per capita animal protein intake. The share of fish proteins in total world animal protein supplies grew from 14.9% in 1992 to a peak of 16% in 1996, declining to about 15.3 percent in 2005 (Fig. 5).

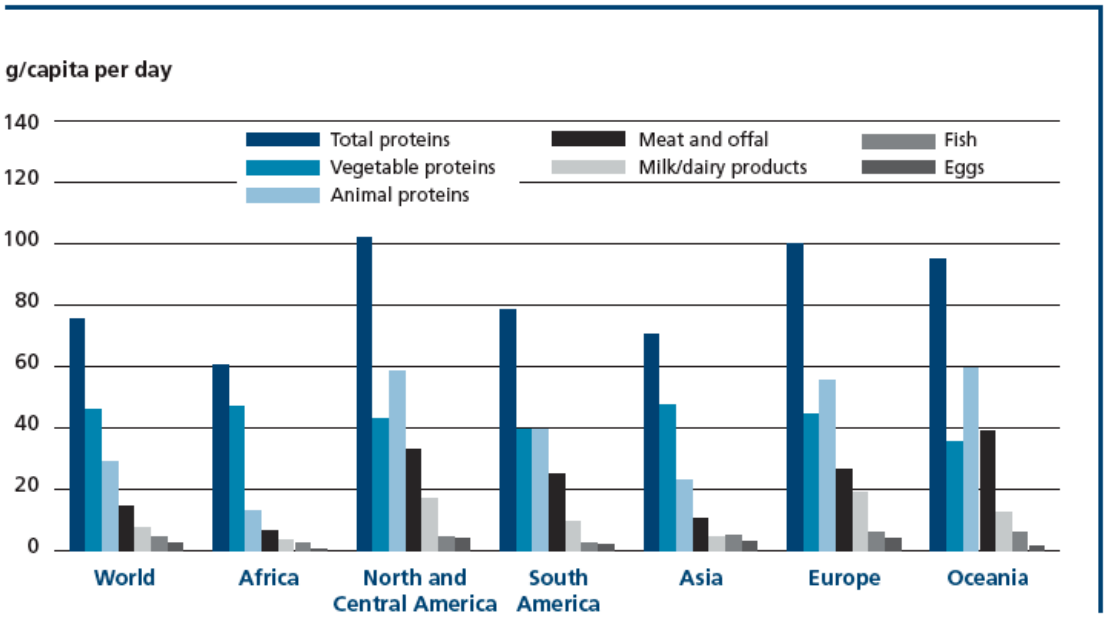

Figure 5. Total protein supply by continent and major food group (2003-05 average) (FAO, 2009).

1.3.2. Aquaculture in Europe

According to FAO statistics (FAO, 2009), total European fisheries production (which includes capture fisheries and aquaculture) was 15.6 million tons in 2006, which represented 10.8% of total world production. Out of this total, 86% (13.4 million tons) was coming from capture fisheries, whereas the rest 14% (2.2 million tons) was coming from aquaculture. Europe's contribution to world aquaculture production was 4.2% in quantity and 9.1% in value (Fig. 3).

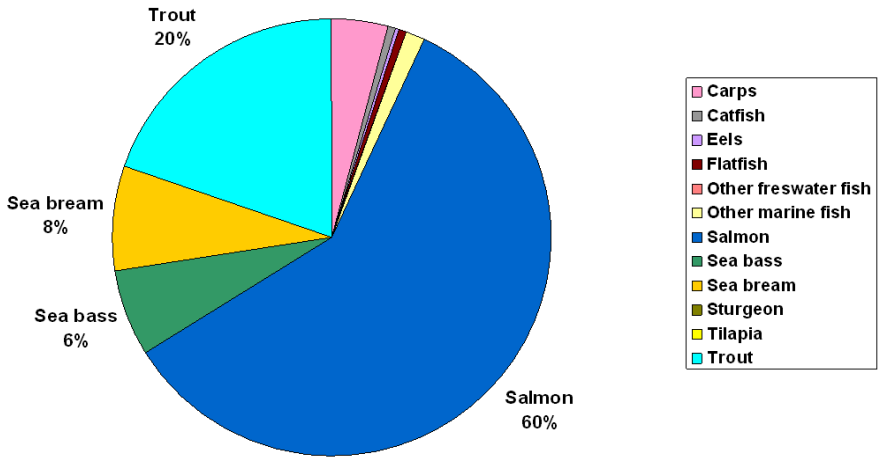

Figure 6. European aquaculture production (in quantity percentage) by species in 2008 (Data from FEAP).

The principal products of European aquaculture are fishes (salmon, trout, sea bass, sea bream) and mollusks (mussels, oysters, clams). In 2008, fish production represented about 77% and mollusks about 23% of the total aquaculture production (FEAP, 2008). Among fishes, salmon was by far the most produced species, which accounted for 59.4% (987,789 tons) of total production. Then, trout accounted for 19.6%, sea bream for 7.8% and sea bass for 6.2% of total production (Fig. 6). The main producer of salmon is Norway, which produced 52.3% of total aquaculture fish production in 2008 (FEAP, 2008). Among the five top European producers were also included United Kingdom, Turkey, Greece and Spain (Fig. 7).

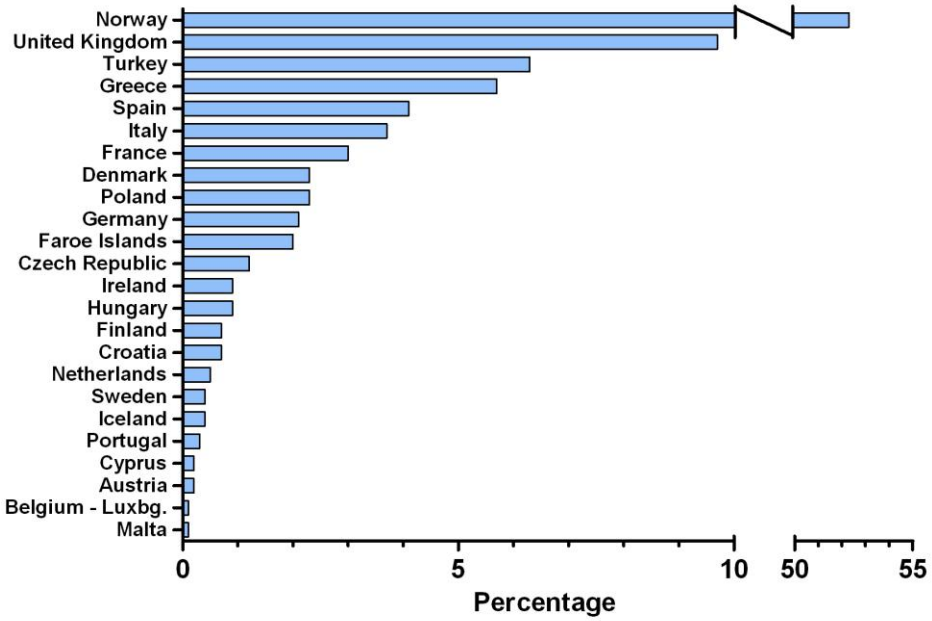

Figure 7. European aquaculture production (in quantity percentage) by country in 2008 (Data from FEAP).

Although aquaculture is the most rapidly growing animal food-producing sector and demand for fish consumption is increasing, production growth in Europe has slowed substantially to about 1% per year since 2000. On the other hand, production growth in Latin America is 22%, in Africa is 12.7% and in Asia is 5.8%. This gives the trend in aquaculture production in the future, where Europe will mainly depend on importation of fish to satisfy the demand. Already, Europe with 480 million consumers, out of which 370 million live in EU, represents the largest market for fish importation (IUCN, 2004). The per capita fish consumption in Europe is 20.8 Kg and fish provide more than 11% of animal protein available.

1.3.3. Aquaculture in the Mediterranean

Total aquaculture production in Mediterranean region was over 1.4 million tons in 2006, which represented around 3% of world aquaculture production. Out of this 1.4 million tons, 377,984 tons are coming from marine and brackishwater aquaculture production. The average annual growth for total Mediterranean aquaculture production is estimated at 7.1% (Basurco & Lovatelli, 2003). This is compared to capture fishery production, which was about -0.67% between 1985 and 2006. Production of brackishwater and marine finfish showed the fastest growth rate from 5,344 tons in 1985 to 51,730 tons in 1995, reaching 191,673 tons in 2006. This corresponded to an average annual growth rate of 24.9% for the period 1985-1995 and 14.6% for the period 1996-2006.

The top Mediterranean aquaculture producers are Egypt, Greece, Turkey, Spain, Italy, France and Israel, with a combined share of 97% of all production. The main species are carps and tilapia (produced in Egypt) and mollusks, sea bass and sea bream. The production of mollusks had the maximum increase during the decade 1985-1995 (from 85,598 tons to 187,886 tons) with an average annual growth rate of 8.5%. Comparatively, the annual growth rate for the period 1996-2006 was only 1.3%, translating into a stabilized trend from 185,891 tons in 1985 to 184,182 tons in 2006 (Fig. 8).

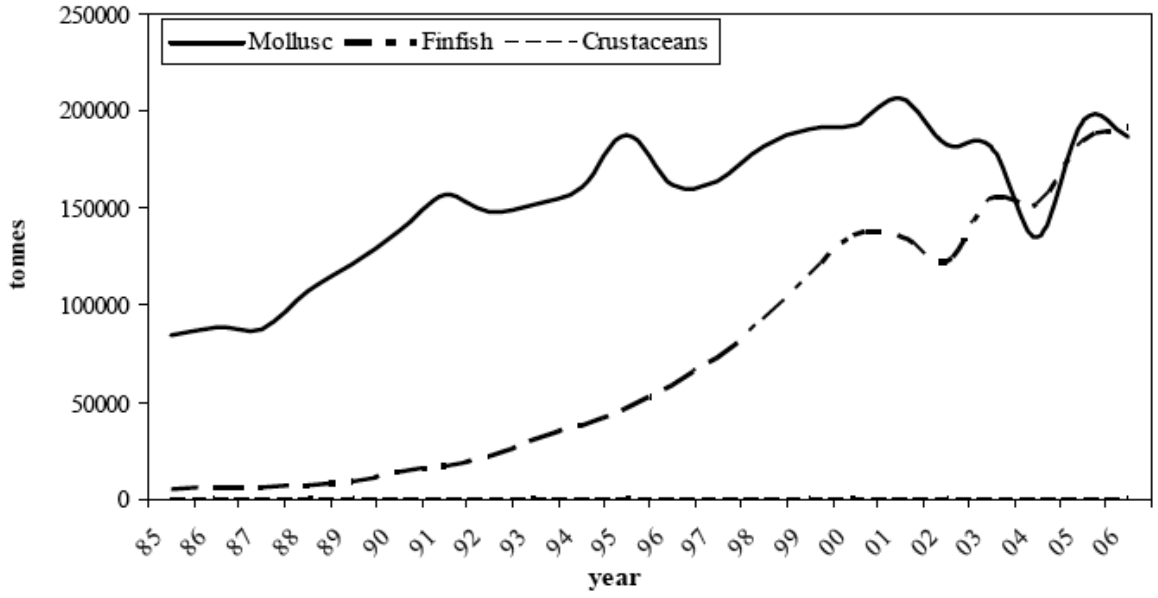

Figure 8. Mediterranean marine aquaculture production: major species group (GFCM, 2008).

In finfish production, seabass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) are accountable for 92.4% (46.2% and 46%, respectively) of total production in

2006 (GFCM, 2008). The average annual growth rate in production of these two species between 1995 and 2008 was 14.8% (Fig. 9) (FEAP, 2008). The main producers of these two species are Greece and Turkey, with a combined share of 71% in sea bass and 67% in sea bream total production. Apart from sea bass and sea bream, bluefin tuna (*Thunnus Thynnus*) was estimated at 9.1% of the production in 2006. However, future prospects for the production of this species are uncertain as domestication has not yet achieved and quotas on capture fisheries tend to decrease due to the depleted stocks of bluefin tuna in Mediterranean.

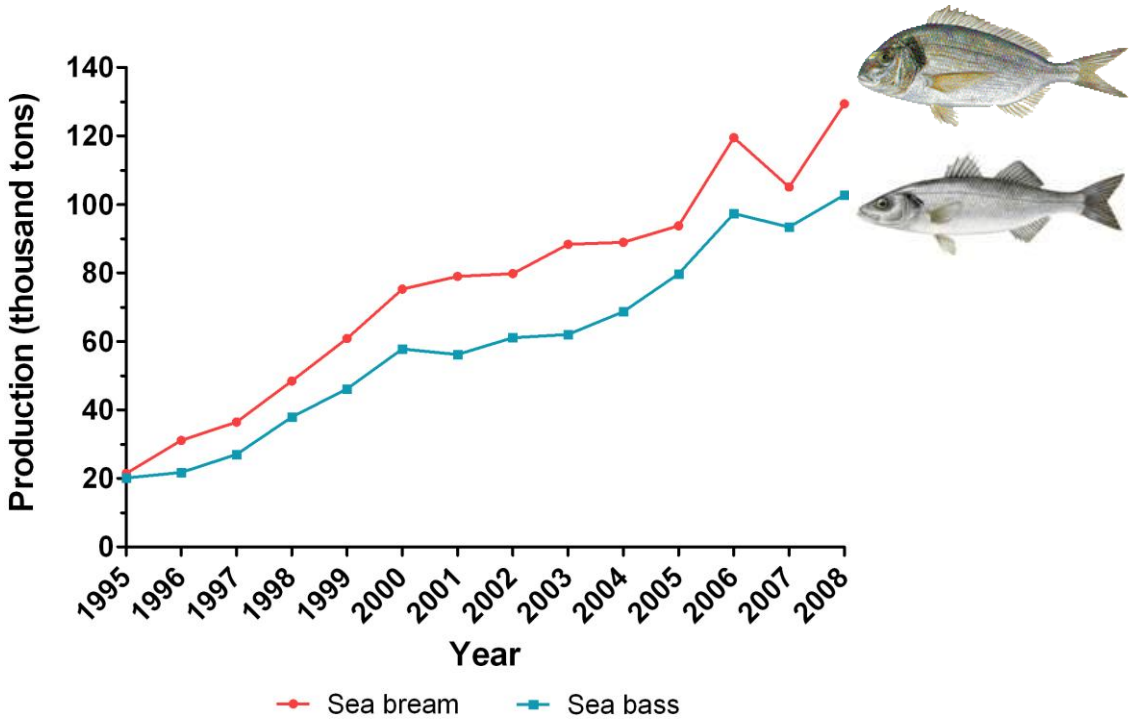

Figure 9. Production of sea bream and sea bass between 1995 and 2008 (Data from FEAP).

1.4. Need for diversification of Mediterranean marine aquaculture production

Expansion of aquaculture production is a necessity, which is raised from the continuous increase in demand for fish consumption and the stability of capture fisheries landings. In Europe, it is estimated that aquaculture production will double until 2030, exceeding 4 million tons (IUCN, 2004). However, to ensure a sustainable expansion of aquaculture production, an ecosystem approach should be applied together with correct strategies on production and marketing. One of the main strategies to ensure future expansion of Mediterranean aquaculture, besides production cost reduction and market enlargement, is the diversification of aquaculture production with new species. A trend for diversification is sustained by the diversity of environmental conditions, by the

availability of new techniques, by an increase in rearing yields, by new market trends and by the possibilities to reduce risks of disease outbreak.

At present, Mediterranean finfish marine aquaculture is dominated by the intensive and technologically sophisticated production of sea bream and sea bass. As mentioned in paragraph 1.3.3, the production of these two species accounts for 92.4% of the total Mediterranean finfish marine aquaculture. The limited number of fish species coming from Mediterranean marine aquaculture has led to market saturation for these species and the collapse of their market price in 2000 and 2009. Although, diversification of marine aquaculture fish species has been in the focus for the last couple of decades (Avault, 1993), production is still dominated by only two species. Today, few months after recovering from the last market price crisis, where sea bass and sea bream were sold less than € 3/Kg, efforts towards introduction of new species must be stepped up.

A significant increase in the number of studies related to new species for aquaculture has been observed. At present, 25 marine fish species are being investigated in the Mediterranean region (Quemener *et al.*, 2002). The selection of new candidate species must take into consideration both biological and market criteria. Ideally, new candidate species for farming should combine low production cost and high market price. An interesting method for selection of new fish species for aquaculture was proposed by Quemener *et al.* (2002) and involved the assessment of around 8000 fish species using nine criteria groups, each with a scoring index. The criteria included aquaculture potential, adaptation potential to the environment, growing out potential, rearing potential, transformation potential, practical use potential, image, consumption and flesh quality. Therefore, the selection of the correct candidate species is a crucial initial step, which will determine a lot in the results of the investigation towards establishment and commercial farming of new species. The research and development, which follows selection of a candidate new species, aims at closing the rearing cycle, constituting the foundation of commercial production and determining the market direction.

Nowadays, there is an increased interest among aquaculturists around the world for fast growing species, such as cobia (*Rachycentrom canadum*), greater amberjack (*Seriola dumerili*), meagre (*Argyrosomus regius*), common dolphin fish (*Coryphaena hippurus*) and different species of groupers (*Epinephelus* sp.) and tuna (*Thunnus* sp.). In north Europe, there is a great effort in developing farming techniques for cod (*Gadus morhua*) and halibut (*Hippoglossus hippoglossus*), whose natural stocks are considered over-exploited by capture fisheries. In general, fast growing species are carnivorous fish and their culture combines high production cost and high market price. The high production cost is mainly due to the high protein and high energy feeds that are required for optimum growth of carnivorous fish. The implications involved in this matter are further discussed in Paragraph 1.6.1. The alternative view in diversification of aquaculture production is to direct one's efforts towards building up farming of low-cost

species, which involves rearing of species situated low at the trophic pyramid. Promoting herbivorous fish to diversify aquaculture production includes important advantages, such as better energy efficiency, reduced utilization of natural resources and limited environmental impact. A comparison of a herbivorous, a first level carnivorous and a highly carnivorous fish in relation to energy efficiency is illustrated in Figure 10. Herbivorous fish has 1.5 and 2 times better energy efficiency than first level carnivorous and highly carnivorous fish, respectively (Duxbury & Duxbury, 1997). The low protein and fat content in the diet of herbivorous fish ensures a reduced utilization of natural resources, such as fish meal and fish oil used in fish feeds. The limitation in the use of fish meal and fish oil in feed production will consequently reduce the fishing pressure on small pelagic species (see Paragraph 1.6.1). Moreover, the requirement of herbivorous fish for low energy feeds reduces the release of nutrient in the water. An example of a herbivorous fish, which is considered as candidate species for Mediterranean marine aquaculture and is also considered in this thesis work, is marbled rabbitfish (*Siganus rivulatus*) (Paragraph 1.5.1). This species combines low production cost and high market price.

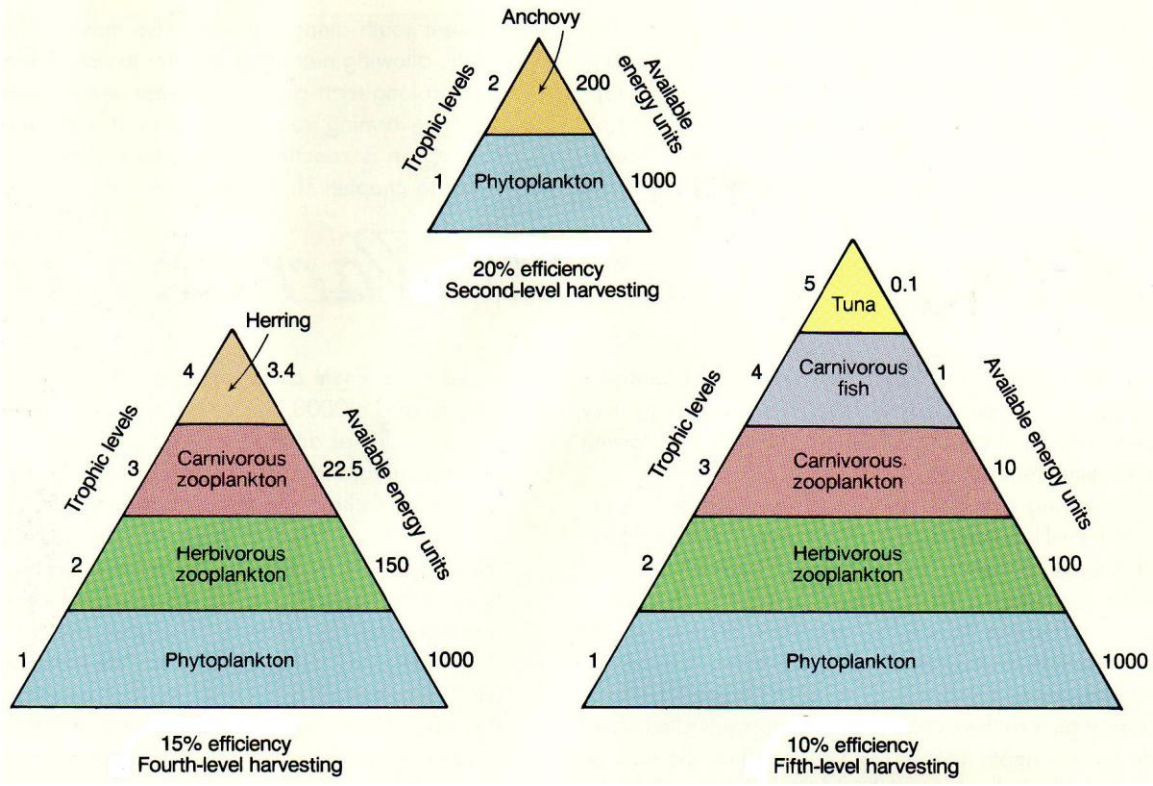

Figure 10. Energy efficiency varies among different trophic levels. For example, in order to add 1 Kg of weight, a person ate 10 Kg of tuna, to attain that weight the tuna had to consume 100 Kg of small fish, and the fish needed to consume 1000 Kg of carnivorous zooplankton, which in turn required 10,000 Kg of herbivorous zooplankton, needing

100,000 Kg of phytoplankton to supply the eventual 1 Kg gain at the top of the pyramid (Duxbury & Duxbury, 1997).

1.5. New species in Mediterranean aquaculture

As already mentioned before, research on new marine species for the diversification of Mediterranean aquaculture is limited to 25 species. Here, they are presented five of these species, which are under investigation at Meneou Marine Aquaculture Research Station (MeMARS). These species are:

- Marbled spinefoot rabbitfish (*Siganus rivulatus*),
- common pandora (*Pagellus erythrinus*),
- common dentex (*Dentex dentex*),
- greater amberjack (*Seriola dumerili*), and
- meagre (*Argyrosomus regius*).

Mollusk and crustacean species are not presented in this thesis work because their culture requires different techniques and practices.

1.5.1. Marbled Spinefoot Rabbitfish (*Siganus rivulatus*)

Taxonomy

Class:	Actinopterygii
Order:	Perciformes
Family:	Siganidae
Genus:	*Siganus*
Species:	*Siganus rivulatus*

Figure 11. *Siganus rivulatus*.

General information

The marbled spinefoot rabbitfish, *S. rivulatus* (Fig. 11), is a tropical and subtropical fish, indigenous to the Indo-West Pacific region. Since the opening of the Suez canal in 1869, rabbitfish has established itself along the eastern Mediterranean coast (until the Aegean Sea), where it grows and reproduces naturally. The body morphology of rabbitfish is ovoid in profile and remarkably compressed laterally. The head is relatively small with rounded snout and a ventral mouth which contains numerous small pointed teeth. Rabbitfish posses cycloid scales, which are buried beneath the skin. A distinguish feature of this fish is the venom glands associated with their dorsal and ventral spines. It is a euryhaline fish and it is found in depths up to 30m. Although

omnivorous, feeds mainly on benthic algae and sea grasses. In rabbitfish sexes are separate (gonochoric) and reproduction period lasts between May and August. Spawning is synchronised with the lunar cycle and occurs near the time of the full moon.

Research and development

In general, rabbitfish have been used widely for mariculture studies not only because of their whitish, firm and tasty meat but also because these fish are relatively easy to breed and can be grown at low cost (Lam, 1974). Several studies on a number of biological and physiological subjects have been done on different species of the *Siganus* genus, such as *S. guttatus* (Komatsu et al., 2006; Rahman et al., 2000a), *S. canaliculatus* (Hoque et al., 1998; Hoque et al., 1999), *S. Spinus* (Harahap et al., 2001; Harahap et al., 2002) and *S. rivulatus* (Saoud *et al.*, 2007; Saoud *et al.*, 2008a, b).

A topic, which attracted the interest of many researchers, is the annual reproductive cycle and more specifically the lunar synchronization of gonad development and spawning in rabbitfish. Hoque et al. (1999) reported that *S. canaliculatus* is a multiple spawner with an ovary belonging to the group-synchronous type of oocyte development. In Japan, spawning of this species is taking place between April and June, usually 4-7 days after the new moon (Hoque *et al.*, 1998) (Fig. 12a). Similar observations have been done for *S. spinus* by Harahap *et al.* (2001), who stated that this species undergo active vitellogenesis and spawning around the days of new moon from May to July (Fig. 12b). In *S. guttatus*, the studies of Rahman *et al.* (2000a, 2000b) showed that the gonadosomatic index (GSI) in male fish attained two peaks corresponding with the new moons in June and July, whereas in female fish the peaks in GSI were observed during the first lunar quarters in June and July, where spawning also took place (Fig. 12c). As it is the case for the above mentioned species, *S. rivulatus*, which is one of the species under study in this thesis work, has a definite spawning season, which extends from June to August according to Zaki *et al.* (1994) and Yeldan &Avsar (2000).

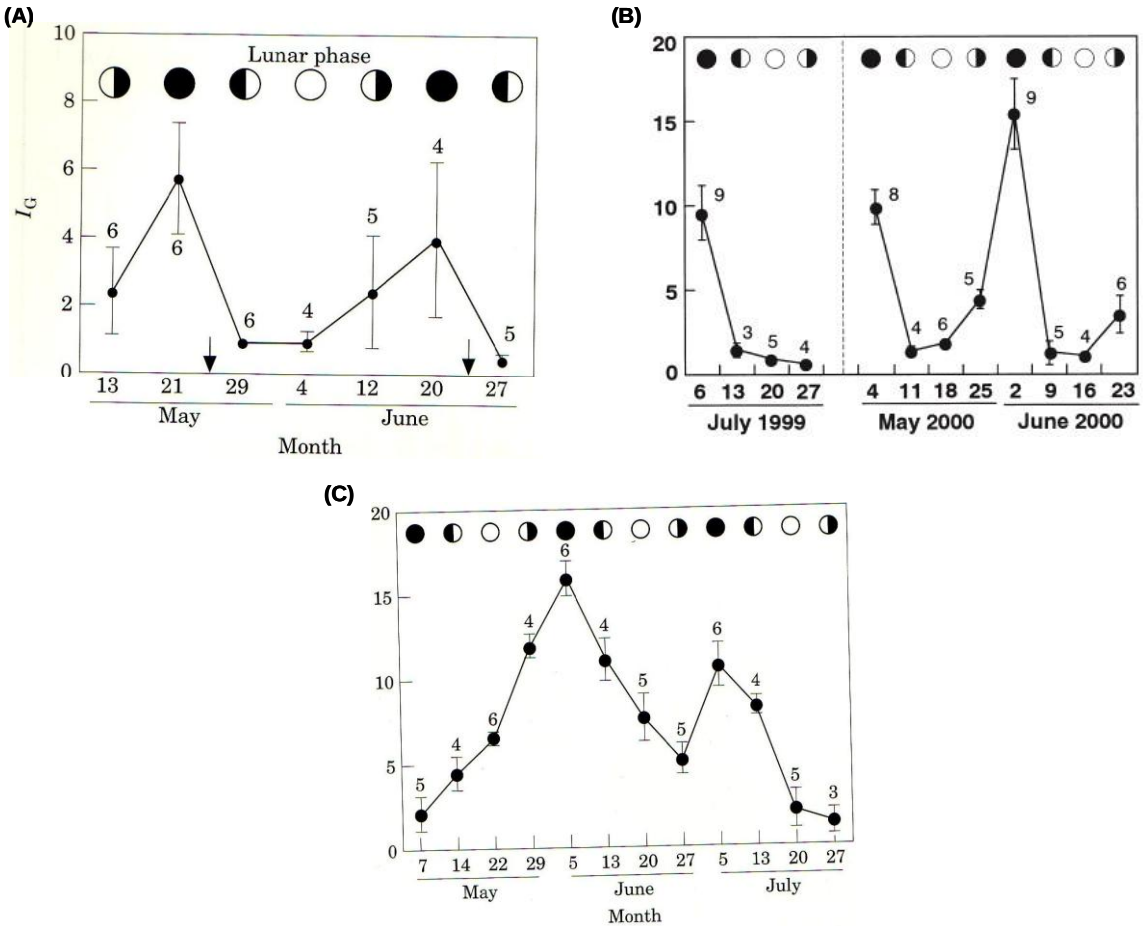

Figure 12. Changes in the gonadosomatic index (GSI or I_G) value of female (A) *S. canaliculatus* (Hoque *et al.*, 1998), (B) *S. spinus* (Harahap *et al.*, 2001) and (C) *S. guttatus* (Rahman *et al.* (2000a), during the lunar reproductive cycle. Each value represents the mean±SE. Numbers indicate fish sampled. Lunar phases are indicated as (●) new moon; (◖) first lunar quarter; (○) full moon; and (◗) last lunar quarter.

For *S. rivulatus*, it is not yet clear if the same female spawns more than one time during the spawning season, but Hoque *et al.* (1999) has reported that *S. canaliculatus* spawns at least twice during the spawning season and the total fecundity is at least 1.4-5.2 million eggs for mature fish with a fork length of 23.5-30.8 cm. From observations that have been done at the MeMARS, females of *S. rivulatus* produce small benthic adhesive eggs with a mean diameter of 630 μm and up to 12 oil globules (Stefanou & Georgiou, 1999). The adhesive property of the eggs renders their collection with egg collector and transfer impossible. For this reason, at MeMARS three alternative ways are applied in order to overcome this problem:

- Collection of eggs from the broodstock tank using aquamats® (Fig. 13),
- collection of the larvae at hatching day by overflow from the broodstock tank, and
- removal of broodstock from the tank after spawning.

Figure 13. Use of aquamats for the collection of rabbitfish eggs at MeMARS

As already mentioned above, rabbitfish species are mainly fed on benthic algae and sea grasses; a feeding habit that places them at the low levels of the trophic pyramid (Fig. 10). This is an important advantage when they are compared with candidate carnivorous fish for aquaculture, as it can be assumed that the need of this species in protein (especially coming from fish meal) will be less and their culture will be relatively environmentally friendly. However, not many studies have been performed to determine the optimum protein content and all the other nutritional elements (e.g. fatty acids, amino acids, etc.) in rabbitfish diet. Parazo (1990) tested six semipurified diets comprising three levels of protein (25, 35, 45% of dry matter) each at two levels of estimated energy (3161, 3832 Kcal/Kg) on fry of S. guttatus for 8 weeks. Based on responses for growth rate and efficiency of protein utilisation, the results suggested that a diet with 35% protein and 3832 Kcal/Kg energy was found to be best for rabbitfish fry. Kissil *et al.* (2002) performed a growth experiment with *S. rivulatus* juveniles, testing three iso-energetic feed, which contained three levels of protein (25, 35, 45% of dry matter). Growth rate for the three groups was 0.35, 0.43 and 0.46 $g.d^{-1}$ respectively, pointing out that the feed containing 35% of protein was the most cost-effective one. In the same study, it is stated that the grow-out period needed for *S. rivulatus* to arrive at the marketable size (200 g) is about 8 months, when temperature is above 20 °C.

As far as the physiological capacity of rabbitfish under captive conditions is concerned, a review of the published research work reveals that rabbitfish adapts well to culture conditions and can tolerate a large range of environmental parameters. Saoud *et*

al. (2008a) investigated the temperature requirements of *S. rivulatus* for optimal growth and survival. The results showed that fish stopped feeding at 14 and 36 °C and that the optimum temperature for growth was 27 °C, where standard growth rate was 2.61±0.05 %BW. d^{-1}. In another experiment, Saoud *et al.* (2007) tested the influence of salinity on survival, growth, plasma osmolality and gill Na^{+}-K^{+}-ATPase activity in *S. rivulatus*. Results showed that *S. rivulatus* can survive at salinities ranging from 10 to 50 ppt and that they can maintain a relatively stable blood osmolality (between 398 and 435 $mmol.K^{-1}$). Also, in four treatments at salinities of 25, 30, 35 and 40 ppt, no differences were observed in survival and growth between treatments. However, gill NKA was lowest at 35 ppt and increased at salinities above and below 35 ppt. *S. rivulatus* can also tolerate high stocking densities with no apparent differences in growth and survival. In an experiment, which was performed in 52-l aquaria, fingerlings of average weight of 6.5 g were stocked into the aquaria at four stocking densities (10, 20, 30 and 40 fish/aquarium). Survival was greater than 95% in all treatments and specific growth rate varied between 2.12 and 2.27, showing no significant difference between the treatments. The maximum density at the end of the experiment was 15 Kg/m^3 (Saoud *et al.*, 2008b). No difference in growth and survival has been also reported for *S. canaliculatus* grown in floating cage nets in two different densities (8 and 12 fish/ m^3) (Yousif *et al.*, 2005).

Another important aspect in the completion of the life cycle of rabbitfish under captivity, which requires special attention, it is the larval culture. Stefanou & Georgiou (1999) reported that larvae of *S. rivulatus* require a super small (SS) strain of rotifers (90-150μm) at first feeding, which is just 2 DPH. Research work on larvae culture at MeMARS showed that larvae of *S. rivulatus* perform better in mesocosm conditions than in intensive conditions with or without green water. However, further work should be done on defining all the environmental and nutritional requirements of the rabbitfish larvae.

Other research works that has been done on rabbitfish include sex differentiation at early stage, disease identification, genetic studies on wild fish, etc. Sex differentiation in young fish of *S. guttatus* was reported to take place after 73 DPH in male fish and after 129 DPH in female fish. During these days, it was observed the detachment of gonadal tissue, including both somatic and germ cells, into the ovarian cavity in the ovary and into the seminiferous lobules and main seminal duct in the testis (Komatsu *et al.*, 2006). The diseases that have been identified and reported on specimens from adult rabbitfish include two bacterial diseases caused by *Pseudomonas putrefaciens* (Saeed *et al.*, 1987) and *Mycobacterium marinum* (Diamant *et al.*, 1987), and one parasitic disease caused by Zschokkella helmii n. sp. (Abdel-Ghaffar *et al.*, 2008). Finally a genetic study by Azzurro *et al.* (2006) on the evaluation of the demographics and dynamic aspects of S. luridus in the Mediterranean revealed that (for the first time in a Lessepsian migrant) there was a lowering of the genetic diversity of the invading population (Mediterranean) (haplotype

diversity 0.879, nucleotide diversity 0.592) compared to the parental one (Red Sea) (haplotype diversity 0.978, nucleotide diversity 0.958).

On commercial level, production of farmed rabbitfish in the Mediterranean only exists in Cyprus, where fingerlings are supplied by MeMARS and fattening is performed in off-shore sea cages from private companies. Rabbitfish is very popular among consumers in Cyprus, but also in other east Mediterranean countries such as Lebanon, Israel and Saudi Arabia…United Arab Emirates. Rabbitfish is among the most expensive fish in Cyprus, reaching a retail price of €25/Kg in 2010. However, the development of rabbitfish culture in the Mediterranean requires further research work, which should be mainly focusing on (i) the determination of the nutritional requirements of larvae and adult fish and the development of a species specific feed, and (ii) the controlled mass production of rabbitfish fingerlings. Furthermore, much work should be done on the marketing of this species, so it gains a place in the fish markets of developed countries.

1.5.2. Common Pandora (*Pagellus erythrinus*)

Taxonomy

Class:	Actinopterygii
Order:	Perciformes
Family:	Sparidae
Genus:	*Pagellus*
Species:	*Pagellus erythrinus*

Figure 14. *Pagellus erythrinus*.

General information

P. erythrinus (Fig. 14) has an oval compressed body and its colour is pink with tiny blue dots. It is a bethopelagic species that can be found in a range of bottom substrate and in depths down to 300 m, but it is most common at 20-100 m depth. It is distributed along the Eastern Atlantic coasts and from Mediterranean Sea to Guinea-Bissau. Omnivorous in its feeding habits, feeds mainly on benthic invertebrates and small fishes. This species is protogynic hermaphrodite, and females become males first in their third year with sizes of about 17 cm. In the Mediterranean spawning takes place between April and middle July, although the ovotestes may retain some activity until the end of September (Valdes *et al.*, 2004). It is a multiple spawning fish and releases several small batches of eggs throughout the spawning period. It can reach a maximum size of 60 cm and maximum weight of 1.56 kg (Miller & Loates, 1997).

Research and development

Among the five fish that are presented in this section of the thesis, common pandora is one of the two most popular fish between consumers in Mediterranean (the other one is common dentex). The experience on the culture of common pandora at MeMARS and a literature review on this species reveals that its culture does not present any particular bottleneck. Its domestication and the closure of its life cycle have been well documented and all the different production stages are described. This is maybe due to the fact that *P. erythrinus* is coming from the same family (*Sparidae*) with the most popular fish in Mediterranean marine aquaculture (European sea bass and gilthead sea bream) and their production characteristics are very similar.

During reproduction period, common pandora does not require any hormone treatment to reach ovulation, but it is able to spawn naturally. Klaoudatos *et al.* (2004) reported that natural spawning resulted in the production of 150,000 viable eggs/Kg with an average hatching rate of 85%, whereas spawning induced with injection of HCG hormone (5000 and 250 IU/Kg) produced 16,140 and 29,940 viable eggs/Kg for the high and low dosage, respectively, with an average hatching rate of 75%. In another study, Guner *et al.* (2004) reported that 30 mature 4-7 years old females placed in a tank at a male:female ratio of 1:2, produced 33.2 million eggs out of which 97% were buoyant healthy eggs with diameters ranging from 753-801 μm.

Many studies on common pandora have been focused on larval development and culture. Klimogianni *et al.* (2004) studied the effect of temperature on yolk-sac larval development and reported that growth rate of the total length increased as temperature rose from 16° to 18°C, while in the range of 18-21°C it stabilized and was independent of water temperature. Higher survival rates occurred at 18-21°C. Suzer *et al.* (2006a) studied the effect of three different illumination levels (10, 30 and 100 lx) on the development of larvae until 30 DAH. Specific growth rates (SGR) were calculated as 8.14% d^{-1} for 10 lx, 9.08% d^{-1} for 30 lx and 8.75% d^{-1} for 100 lx, whereas survival rates were determined as 24.2%, 38.3% and 34.6%, respectively for the three illumination levels. Also, specific enzyme activities of trypsin, chymotrypsin, pepsin, amylase and lipase were higher in larvae under 30 lx illumination level. The pattern of development of the main digestive enzymes found in *P. erythrinus* larvae is similar to that described in other Sparid species (Suzer *et al.*, 2006b). Micale *et al.* (2006) described the ontogeny of the alimentary tract and its associated structures (liver, pancreas, gall bladder), indicating that at first feeding (3-4 DAH) digestive tract evolves from an undifferentiated straight tube into a four portions differentiated organ: buccopharynx, oesophagus, incipient stomach and intestine. The pancreas, liver and gall bladder were also differentiated at this stage. The osteological ontogeny in larvae and the role of temperature in developmental plasticity was studied by Sfakianakis *et al.* (2004), who determined the time of ontogeny of vertebral column, and caudal, dorsal, anal and pectoral fins. It was also stated that the

role of temperature in developmental plasticity is enhanced by the induction of abnormalities mainly in the area of the caudal fin, which reached (in total) 75% occurrence in the higher temperature regime (23°C) tested.

During on-growing of common pandora, Mihelakakis *et al.* (2001) reported that feeding frequency influences survival, growth and feed efficiency with fish fed more times daily to perform better. Highest survival rate during on-growing of young fish (mean weight of 5.08 g) was 93.3%, SGR was 1.99% d^{-1}, and feed efficiency (FE) was 97.2%. In another study, Klaoudatos *et al.* (2004) investigated the effect of pelleted and extruded diet and fish concentration during on-growing. Fish fed on extruded pellets showed higher SGR (1.08% d^{-1}) and lower FCR (1.55) than fish fed pelleted diets (SGR= 1.03% d^{-1}, FCR= 1.82). Also, low fish concentration (0.03-3.01 Kg/m3) showed higher SGR (0.69% d^{-1}) and lower FCR (1.94) than high fish concentration (SGR= 0.64% d^{-1}, FCR= 2.06). The effect of crude lipid level (16.45% and 9.4%) and satiation level (100% and 75%) on production performance of common pandora was investigated by Kousoulaki *et al.* (2007), who reported that fish growth did not differ between crude lipid level when fish were fed to satiation, while at restricted feeding fish fed high crude lipid level diet grew faster that those fed the low level diet. Finally, in the context of a study at MeMARS, which was looking at the effect of astaxanthin and light intensity on the development of normal skin colour, it has been found that common pandora can develop the normal pink colour under intensive culture conditions when it is fed on a diet with a concentration of astaxanthin between 10-30 μg/Kg of feed, and it is exposed to limited light intensity (< 50 lx).

Although, mass production practices for *P. erythrinus* do not present any particular obstacle, the production of this species is limited to minor quantities coming from Greece, Turkey and Cyprus. This is mainly due to the fact that *P. erythrinus* has to challenge sea bass and sea bream for the same market share. Therefore, the chances of common pandora to gain as much market share as possible will increase only if further research work will optimize some production parameters, such as the determination of nutritional requirements and formulation of species-specific feed.

1.5.3. Common Dentex (*Dentex dentex*)

Taxonomy

Class:	Actinopterygii
Order:	Perciformes
Family:	Sparidae
Genus:	*Dentex*
Species:	*Dentex dentex*

Figure 15. *Dentex dentex*.

General information

D. dentex (Fig. 15) has an oval compressed body and its colour is greyish or pinkish with many small black spots more or less evident. Its jaws have rows of canine teeth, with 4 to 6 anterior teeth very developed in each jaw. It is common in Mediterranean Sea, but it is also found in the Black Sea and the Eastern Atlantic Ocean from the British Isles to Mauretania, sometimes up to Senegal and Canary Islands. It usually lives in shallow water less than 50 m deep, but it can be found over rocky grounds down to 200 m depth. It is an active predator and feeds on fish, molluscs and cephalopods. Adult fish are usually solitary but young fish form schools. *D. dentex* is usually gonochoristic fish (some protandric hermaphrodite), it is a multiple spawner, exhibiting asynchronous oocyte development and breeds between April and June. Adult fish can reach a maximum length of one meter and a weight up to 14.3 Kg.

Research and development

Although studies on domestication and culture of common dentex have been carried out since 1980's, it is still considered as a new candidate species for diversification of aquaculture production, because it never achieved to arrive to a stable commercial production. In literature, there is a large quantity of studies on a variety of subjects, such as embryonic and larval development (Jug-Dujakovic *et al.*, 1995; Santamaria *et al.*, 2004; Gisbert *et al.*, 2009), larval culture (Glamuzina *et al.*, 1989; Crespo *et al.*, 2001; Gimenez & Estevez, 2008), reproduction (Chatzifotis *et al.*, 2004; Pavlidis *et al.*, 2004; Gimenez *et al.*, 2006), on-growing (Koumoundouros *et al.*, 2004; Perez-Jimenez, 2009) and nutrition (Cardenete *et al.*, 1997; Skalli *et al.*, 2004; Chatzifotis *et al.*, 2008).

Jug-Dujakovic *et al.* (1995) described the embryonic and yolk-sac larval development and reported that the mean diameter of fertilised egg was 958 μm, incubation period was 81 h at 17°C and total length of newly hatched yolk-sac larvae was

2170 ± 200 μm. Santamaria *et al.* (2004) described in detail the larval organogenesis in larvae up to 36 DAH. It is stated that up to 22 DAH, dentex larvae undergo intense organogenesis and later there is only an increase in size and complexity of the pre-existing organs. Mouth opening and eye pigmentation are observed at 4 DAH, opercular spines and inflated swim bladder are visible at 10 DAH and flexion of the notochord is observed at 23 DAH. Gisbert *et al.* (2009) concentrated on the development of digestive enzymes and observed that enzymes involved in the digestion of protein, lipid and carbohydrate were present at hatching and before the onset of exogenous feeding, with trypsin and chymotrypsin to have high specific activity at hatching and lipase to increase specific activity after hatching.

The most important bottleneck in *D.* dentex culture is the low survival rate during larval phase, mainly due to inappropriateness of the nutritional quality of food, high sensitivity to handling stress, epidemic diseases, aggressive behaviour and cannibalism. More than 20 years ago, Glamuzina *et al.* (1989) studied larval culture and stated that survival was best at a temperature of 19°C and salinity of 38 ppt. Gimenez and Estevez (2008) reported best survival when initial larval density was between 10 and 40 larvae L^{-1}, rotifer concentration at 10 rotifers mL^{-1}, photoperiod of 24 L:0 D and light intensity of at least 3.4 μmol m^{-2} s^{-1}. Koumoundouros *et al.* (2004) described the successful rearing of larvae in a semi-intensive version of mesocosm technique, where two hundred thousand eggs were introduced in two outdoor tanks of $60m^3$ volume. The growth rate was high, resulting in a production cycle of 57 days from hatching to juveniles of 50 ± 4.5 mm TL and 1.6 ± 0.4 g mean weight. Survival during the larval phase was 43-50% and during the weaning to pre-growing phase was 70% (21.7% in total).

As already mentioned above, *D. dentex* is usually gonochoristic fish, it is a multiple spawner, exhibiting asynchronous oocyte development and breeds between April and June. After histological analysis of gonads out of 448 individuals, Loir *et al.* (2001) reported that sexual differentiation occurred between 5 and 12 months of age and neither bisexual gonads nor any other indication of sexual inversion was found in fish up to 4 years of age. At two years old, all males produced milt and 67% of females observed to undergo maturation. Maximum GSI value was 6% and batch relative fecundity ranged from 32,000 and 393,000 eggs/Kg BW. Pavlidis *et al.* (2004) studied the role of sex ratio on spawning performance and reported that the highest number of eggs released, percentage of viable eggs, number of spawnings, spawning index and relatively fecundity were observed in the broodstock with sex ratio of 1:1. Gimenez *et al.* (2006) stated that low quality eggs of *D. dentex* had significant differences regarding carbohydrate composition and higher activity of enzymes such as alkaline phosphatase and pyruvate kinase.

During on-growing phase, *D. dentex* is well adapted in cages under off-shore conditions and has a higher growth rate when compared with sea bass (*Dicentrarchus*

labrax) and sea bream (*Sparus aurata*). Over a 12 month rearing period, mean weight of *D. dentex* reach 415 g, *D. labarx* 150 g and *S. aurata* 207 g (Koumoundouros *et al.*, 2004). Skalli *et al.* (2004) has determined the optimum protein-to-energy (P/E) ratio for fingerling growth during on-growing to be 23.7 g MJ^{-1} (50% CP and 22 KJ g^{-1}). However, larger fish required a P/E ratio of 19 g MJ^{-1} (43.7% CP and 22 KJ g^{-1}). In a similar study, Espinos *et al.* (2003) has proved that optimum growth and nutrient utilization of denetx fingerlings can be obtained when they are fed a diet containing around 50% CP with a lipid level ranging from 12% to 17% and having a P/E ratio from 22 to 25 g MJ^{-1}. Under these nutritional parameters, SGR ranged between 4.0 and 4.3% and FCR between 1.5 and 1.8. Cardenete *et al.* (1997) reported a daily growth rate of 0.72 g d^{-1} and an FCR of 1.8 when fish were fed on dry pellets containing 58% protein DM, 24.7 fat DM and 24.4 KJ g^{-1} of gross energy. Finally, Chatzifotis *et al.* (2008) investigated the partial substitution of fish meal (FM) by soy protein concentrate (SM) and concluded that only 25% of FM can be substituted by SM. An improvement in growth was also observed by a dietary taurine supplementation of 2 g Kg^{-1} diet.

1.5.4. Greater Amberjack (*Seriola dumerili*)

Taxonomy

Class:	Actinopterygii
Order:	Perciformes
Family:	Carangidae
Genus:	*Seriola*
Species:	*Seriola dumerili*

Figure 16. *Seriola dumerili*.

General information

The main morphological characteristics of *S. dumerili* (Fig. 16) are the elongated, fusiform and slightly laterally compressed body, covered with small scale (cycloids). Their colour is yellow-green in juveniles; in adults it is blue or olivaceous dorsally and silvery to white on the sides and belly. *S. dumerili* is a multiple spawning fish, and it may release several batches of eggs during the same spawning season. The ovary type is synchronous: at least two size groups of oocytes are present at the same time. This species is gonochoric without sexual dimorphism, and both sexes are separated. Maturity occurs at three years of age but functional breeders are 4 and 5 years old for males and females respectively. The spawning season lasts from late spring to early summer (from May to July) in the Mediterranean. It feeds primarily on fish but also invertebrates. It is

found in warm subtropical waters and it is widely distributed around the globe. It can reach maximum size of 190 cm and maximum weigh of 80.6 kg.

Research and development

Greater amberjack could not escape from the interest of aquaculturists, as it is a really fast growing species when compared to sea bass or sea bream. Reported growth rates for *S. dumerili* were up to 9.2 $g.d^{-1}$, when the fish were grown in off-shore sea cages and fed on raw fish (Mazzola *et al.*, 2000), and 3.1 $g.d^{-1}$, when the fish were grown in tanks and fed on raw fish (Garcia-Gomez, 1993). Growth rates for sea bass and sea bream do not exceed 1 $g.d^{-1}$. *S. dumerili* can also be grown on dry pellets and can still perform good, having a growth rate of 4.4 $g.d^{-1}$ (Mazzola *et al.*, 2000). Fish of more than 4 Kg each that have been kept in 15-tonne tanks at the facilities of MeMARS and fed on raw fish and squid reached growth rates of up to 20 $g.d^{-1}$. Having in mind the above information, anybody could have a reason to wonder why this species is not still cultivated in large quantities. The reason for the delayed in the development of mass commercial production of greater amberjack is the availability of fingerlings. Currently, the very small quantities of farmed *S. dumerili* are mainly coming from the collection of wild young fish.

At the moment, the problem on the production of fingerlings is focus actually on the inability to have systematical production of good quality fertilized eggs due to the existence of important reproductive dysfunctions of greater amberjack in captivity. Reproduction of *S. dumerili* has been reported in several occasions but still a predictable production of eggs can not be guaranteed by anybody. A spawning incidence was reported by Mylonas *et al.* (2004) after hormonal treatment of the broodstock (Fig. 17) using GnRHa implants. Specifically, out of the five mature female fish used in this study, only one contained oocytes at early final maturation and treated with an ethylene-vinyl acetate (EVAc) GnRHa implant at a dose of 500 μg GnRHa. Spawning was observed after 36 h, 4 and 5 days, with fertilized eggs only at 36 h and 5 days. Kozul *et al.* (2001) reported a spawning of just 200 g of fertilized eggs after hormonal treatment of broodstock with a single injection of human chorionic gonadotropin (HCG) at a dose of 1000 I.U./Kg. Natural spawning was reported by Jerez *et al.* (2006) from a broodstock consisted of 11 wild captured fish. The fish were captured at an average weight of 8 Kg and spawned naturally at an average weight of 25 Kg, after they were reared for 6 years in a 500 m^3 raceway tank. Spawning took place between April and October, coinciding with the increase in temperature from 19 to 24 °C. The total egg production was 14 million eggs, which were collected in 38 spawns, with an average of 61.75% fertilization and 16.49% hatching. The problem of reproduction does not exist in other *Seriola* species, which are cultured mainly in Japan and Australia. *S. lalandi*, *S. quinqueradiata* and *S. mazatlana* are reported to spawn systematically either naturally or with the

injection of hormone treatments (HCG or LHRHa) (Benetti, 1997; Chuda *et al.*, 2005; Moran *et al.*, 2007; Kolkovski & Sakakura, 2004).

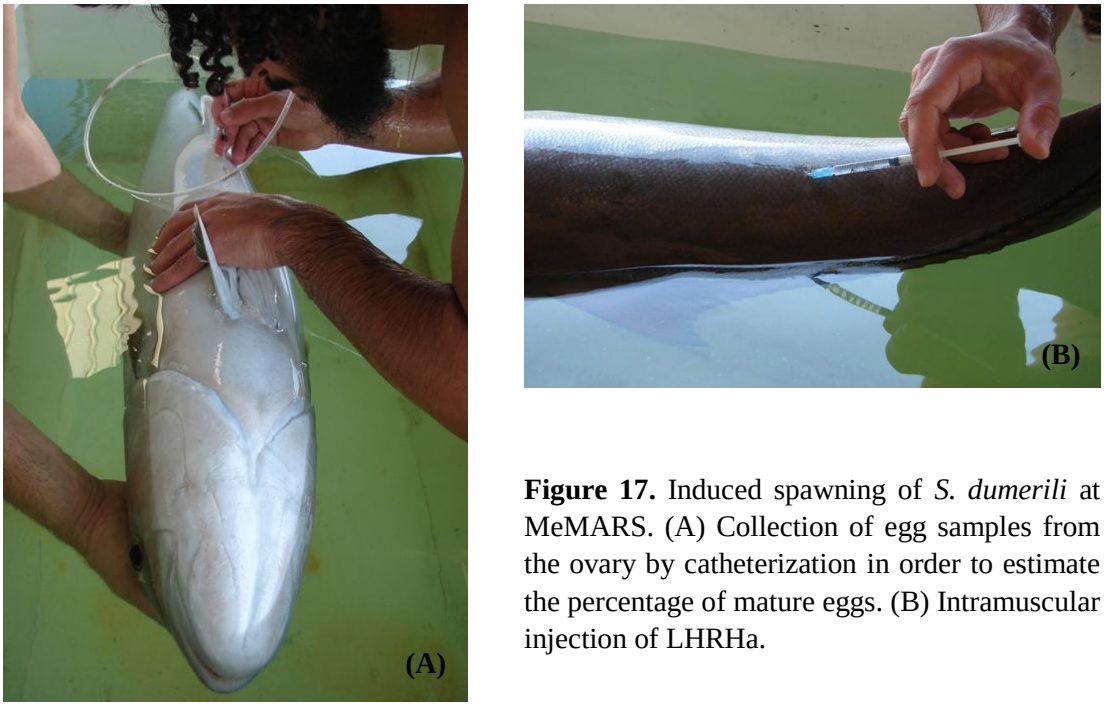

Figure 17. Induced spawning of *S. dumerili* at MeMARS. (A) Collection of egg samples from the ovary by catheterization in order to estimate the percentage of mature eggs. (B) Intramuscular injection of LHRHa.

The limitation in the production of viable eggs under captivity is the reason why larval culture in *S. dumerili* is not studied in large extend. Although, few studies exist on this life stage of *S. dumerili*, further research should be done in defining the nutritional and environmental requirements for the successful larval culture. Papandroulakis *et al.* (2005) studied the larval rearing in *S. dumerili* with mesocosm technique, which involves endogenous productivity and daily exogenous food addition. Feeding of larvae involved (with time order) phytoplankton (*Chlorella minutissima*, which was ingested unintentionally), enriched rotifers (*Brachionus rivulatus*), enriched *Artemia* sp., artificial diet and frozen sea bream eggs. After 40 DPH, survival was 3.5% and TL of larvae was 35.63 ± 6.52 mm. Hamasaki *et al.* (2009) observed that hatchery-reared larvae of *S. dumerili* appeared to selectively prey on egg-bearing rotifers, whose body sizes are relatively large among the rotifer populations in the tanks, indicating an ontogenic change in the feeding habit of the larvae.

Greater amberjack is among the most promising new candidates for diversification of aquaculture production in Mediterranean due to its high growth rate and market perspective. However, other than overcoming the obstacles of reproduction and larval culture, so the closure of its life cycle is achieved, the highly carnivorous feeding habits of this species constitute an important limitation. Therefore, research on alternative sources of protein in fish feed other than fish meal is considered vital for the realization of the culture of this species.

1.5.5. Meagre (*Argyrosomus regius*)

Taxonomy

Class:	Actinopterygii
Order:	Perciformes
Family:	Sciaenidae
Genus:	*Argyrosomus*
Species:	*Argyrosomus regius*

Figure 18. *Argyrosomus regius*.

General information

Meagre (Fig. 18) has an elongated body with a relatively big head and a large mouth. The lateral line is evident and extends onto the caudal fin. The body colour is silver-grey with bronze traits dorsally. *A. regius* is found in the Mediterranean and Black Sea and along the Atlantic coasts of Europe and the west coast of Africa. It lives in inshore and shelf waters, close to the bottom or near the surface, at depths ranging from 15 to 200 m. It is also encountered in estuaries and coastal lagoons. It feeds on fish and nektonic crustaceans and it can reach a body weight of over 50 Kg. The largest size recorded was 182 cm total length and 103 Kg of body weight. *A. regius* is a gonochoristic species and, in Mediterranean, spawns between April and July.

Research and development

Meagre is a medium grower species with growth rates higher than those of most common Mediterranean cultured species, such as sea bass and sea bream, and, consequently, a high interest for the intensive culture of this species has been gradually generated among aquaculturists the last 15 years. Cittolin *et al.* (2008) reported that juvenile meagre reached an average weight of 23.5±3.8 g and length of 121.9±5.9 mm in 73 DAH at a temperature range of 22-29°C. At MeMARS, growth trials starting with newborn larvae during a whole year, where water temperature ranged between 16 and 29°C, resulted in the production of fish with average weight of 900 g and growth rate of about 2.5 $g.d^{-1}$. Growth rates for sea bass and sea bream do not exceed 1 $g.d^{-1}$. A study on the effect of dietary lipid level in juvenile meagre revealed that the best growth performance was observed in fish fed the 17% lipid level, whereas the increase of dietary lipid level from 17% to 21% had a negative effect on growth (Chatzifotis *et al.*, 2010). Inclusion of different levels of plant proteins and fish protein hydrolysates in four experimental diets during ongrowing of meagre resulted in a reduction of growth by the inclusion of plant proteins and an increase by the addition of fish protein hydrolysates (Estevez *et al.*, 2010). In a comparison between meagre and sea bass at the same average

body weight (667.52 g) on final product quality traits, meagre showed markedly lower viscera (5.22 vs 10.84%), mesenteric fat (0.88 vs 7.85%) and muscular lipid (2.24 vs 12.78%). In general, meagre was characterized by 1.04 condition factor, 44% fillets and 94.5% dressing yield (Poli *et al.*, 2003).

Production of fry has been reported only in 1996 from a single hatchery in France, where production was very limited. Much of the research effort nowadays is focus on reproduction and optimization of larval rearing protocol. Meagre remains sexually undifferentiated until near the end of 9 months of age and attains puberty at 2 and 3 years of age for males and females, respectively (Schiavone *et al.*, 2008). Natural spawning of meagre broodstock under captivity has not yet reported. Production of viable eggs has only been achieved through artificial reproduction with administration of LHRHa hormone. Cittolin *et al.* (2008) stated that administration of 4 μg/Kg LHRHa at a broodstock comprised by male fish over 3.6 Kg and female fish over 6.1 Kg resulted in the spawning of viable eggs after 16 h. The main developmental stages in larvae have been defined as: mouth opening at 76 °C/day, first feeding at 113 °C/day, swim-bladder inflammation at 190 °C/day and metamorphosis between 266 and 502 °C/day (Cittolin *et al.*, 2008). In an experiment where two larval stocking densities have been tested on growth performance, 50 larvae.L^{-1} promoted better growth in terms of standard length, body weight and dry weight than 100 larvae.L^{-1}. Survival was better in high larval density, whereas biochemical composition of larvae was not affected by stocking density (Roo *et al.*, 2010).

1.6. Fish Nutrition

1.6.1. Protein

Proteins are organic compounds comprised of conjugated amino acids (AA) in proportions that are characteristic of each protein. This nutrient always contains carbon (50%), hydrogen (6.5%), oxygen (21.5%) and nitrogen (16%), however some proteins also contain sulphur, phosphorus and iron. Protein is necessary in fish diet for good growth, development and health. However, fish requirement is for certain amino acids that make up the proteins, rather than for protein itself.

In fish tissue, there are 20 principle AA, which are categorized as essential (indispensable) AA (EAA), semi-essential AA, and non-essential AA (NEAA) (Table 3). The EAA must be supplied by the diet, because fish cannot synthesize them, whereas the NEAA can be synthesized from EAA (sometimes are grouped with EAA). NEAA can be synthesized by transfer of an amino group to α-keto acids which can be derived from non-protein sources, such as glucose. When a particular EAA is deficient in a diet, it is referred to as a limiting amino acid because it limits the synthesis of protein. All of the

AA needed to synthesize a protein must be available or no synthesis can occur. This is why protein quality is important in fish nutrition.

Table 3. Essential, semi-essential and non-essential nature of amino acids

Essential	Non-essential and semi-essential
Arginine	Alanine
Histidine	Asparagine
Isoleucine	Aspartic acid
Leucine	Glutamic acid
Lysine	Glutamine
Threonine	Glycine
Tryptophan	Proline
Valine	Serine
Methionine	Cysteine[1]
Phenylalanine	Tyrosine[1]

[1] Semi-essential amino acids

Requirements of farmed fish species for protein and EAA is a well studied area, since protein constitutes the most expensive part of fish feed. Protein and EAA requirements vary greatly between species and size of cultured fish. In sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*), protein requirement for optimum growth is found to be 40-45%. However, during the larval or juvenile stage of these two species, protein requirement is between 50-60%, which is higher than on-growing stage. Atlantic salmon (*Salmo salar*), which is the most produced marine finfish in the world, requires a protein content of 40-45% and during juvenile stage requires 50-55%. On the other hand, a herbivorous species, such as grass carp (freshwater species) requires 30-35% crude protein in the diet. However, as already mentioned above, the most important in fish nutrition is the requirements in EAA. Table 4 summarizes the requirements in EAA of some cultured fish species.

Table 4. EAA requirements (expressed as % of dietary protein) in cultured fish species

EAA	Sea bream (*S. aurata*)	Rainbow trout (*O. mykiss*)	Common carp (*C. carpio*)
Arginine	< 2.6	5.0	4.4
Histidine	5.0	1.8	1.5
Isoleucine	2.6	2.0	2.6
Leucine	4.5	3.5	4.8
Lysine	5.0	4.5	6.0
Methionine + cysteine	4.0	3.5	2.7
Phenylalanine + tyrosine	2.9	4.5	5.7
Threonine	2.8	2.0	3.8
Tryptophan	0.6	0.5	0.8
Valine	3.0	3.2	3.4

The dietary protein requirement of fish is influenced by several factors, which can be divided into biotic and abiotic factors. Biotic factors include the metabolic specificities of each species as well as other physiological characteristics, such as activity and energetic metabolism. These can be relevant to the fish's natural feeding regime, with carnivorous species having higher protein requirements than herbivorous species. Age and size of fish have an effect on protein requirement, with small fish (larval and juvenile stage) to have higher requirements than larger fish (on-growing stage). Another biotic factor is the genetic inheritance, where selection programmes in respect to growth rate can alter absolute protein requirements. Other abiotic factors include the feeding rate and the dietary energy level. Concerning abiotic factors, temperature is the most important one. Water temperature has a very marked effect on absolute requirements, but not on relative requirements. Salinity has an indirect effect on protein requirement through stimulation or slowing down of growth or appetite, or even a slight influence on digestive utilization.

Replacement of fish meal with alternative protein sources

Fish meal is a major ingredient in formulated feeds for aquaculture, especially for carnivorous species. The current success of intensive fish farming is dependent upon the use of fish meal as a major source of dietary protein. Fish meals contain a high percentage of protein (usually 60-75%), an appreciable amount of mineral ash (10-20%) and a proportion of lipid (5-10%). Most fish meal is produced from small pelagic fish species such as sardines, anchovies, capelin, herring and menhaden, but meals are also produced from the smaller members of the cod family, and from the wastes arising from the processing of the larger gadoids, the so-called 'white fish'.

The depletion of many wild fish stocks by fishing, including some of the species mentioned above and the further expansion in aquaculture production raise the question

whether fish meal production can support aquaculture production in the future. In the 1980s, there were already discussions about the possibility of aquaculture development being slowed by a shortage of fish meal. However, 30 years later, it is clear that such a shortage has not been an absolute block for fish and shrimp farming. Today, research work on replacement of fish meal with plant-derived protein sources is aiming to (a) the decrease of the proportion of captured wild fish used as feed component, which in turn might contribute to the management of fishing pressure on wild stocks, and (b) the decrease in feed production cost as the price of fish meal is increasing.

According to FAO, production of fish meal in 2006 was around 5.46 million tons. The quantity of fish used as a raw material for fishmeal was about 20.2 million tons, representing a 14% decrease compared with 2005, and still well below the peak level of more than 30 million tons recorded in 1994. Aquaculture used 3.06 million tons of fish meal, which corresponded to 56% of total production (Fig. 19).

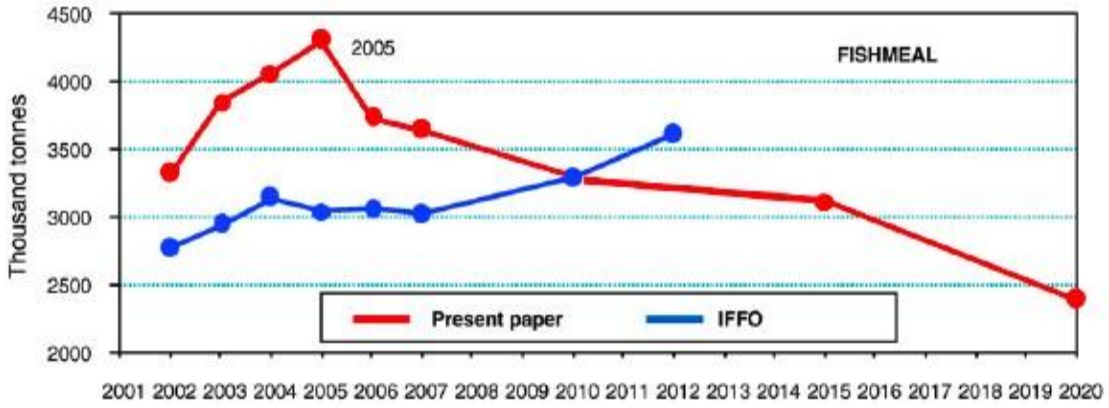

Figure 19. Current and projected use of fish meal within compound aquafeeds from 2002 to 2020 (Tacon & Metian, 2008).

The amount of wild fish it takes to produce farmed fish (fish in - fish out ratios, FIFO) constitutes a long debate in aquaculture with contradictious opinions. Tacon & Metian (2008) estimated that FIFO ratio for salmon in 2006 was 4.9:1, meaning it takes 4.9 tons of wild fish to produce 1 ton of salmon. However, a more recent study by IFFO, which presents a more realistic picture of this matter as it takes into account the aquaculture industry as a whole, the improved protein recovery percentage from whole fish and the use of by-product in fish meal production, estimated that FIFO ratio for all aquaculture was 0.52 and for salmon was 1.68:1. As far as price is concerned, fish meal remained between US$500 and US$700 per ton in the period 2000-2005. In 2006, it reached US$1,400 and since then it remained above US$1,000 per ton (Fig. 20).

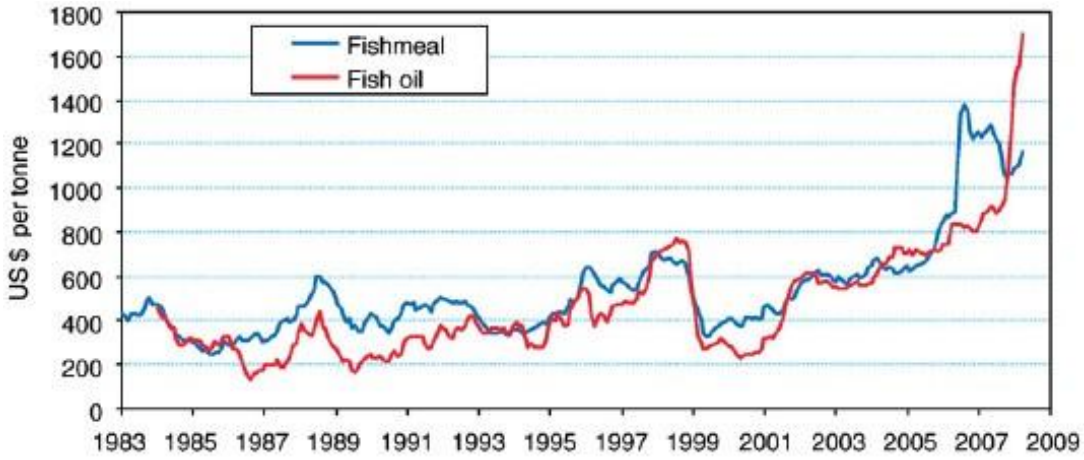

Figure 20. International market price for fish meal and fish oil (monthly average, 64/65% crude protein), any origin, wholesale (Tacon & Metian, 2008)

The increase in fish meal price and the unstable production of fish meal have induced more intensive efforts in replacing fish meal in fish feeds the last 6 years. One of the main research projects worked on this issue was the AQUAMAX projected, which was funded by EU in the context of Sixth Framework Program (FP6). The partial or complete replacement of fish meal with plant-derived protein sources is not an easy task. Many parameters are involved, such as (a) the essential amino acids profile of each source, its compatibility with the nutritional requirements of the fish and the availability level of EAAs, (b) the biological value of the protein depending on the quality of the protein as well as on protein incorporation or protein intake levels, and (c) the anti-nutritive factors and the possible means of destroying or limiting the negative effects of such substances. Partial (50% and 75%) and complete replacement of fish meal by a mixture of plant protein sources (corn gluten, wheat gluten, extruded peas, rapeseed meal and sweet white lupin) in sea bream diet resulted in progressive decrease in final body weight with plant protein inclusion. However, in partial replacement with plant protein, feed efficiency (FE) was significantly improved and specific growth rates remained unchanged or slightly reduced in comparison to fish fed the FM diet (Gomez-Requeni *et al.*, 2004; Sitja-Bobadilla *et al.*, 2005). In a similar experiment, 75% replacement of fish meal with a mixture of plant protein sources in juvenile sea bream resulted to similar weight gain and specific growth rate, higher feed intake in sea bream fed the fish meal diet and higher feed efficiency and protein efficiency in group fed the plant protein diet. Also, sea bream fed the plant protein diet had higher hepatosomatic index and lower fillet yield. Their fillet had lower moisture and higher lipid levels with lower levels of n-3 polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA), while the level of PUFA n-6 was higher (De Francesco *et al.*, 2007). In sea bass, up to 90% replacement of fish meal with plant protein sources did not cause any significant differences in growth rate, feed efficiency or in daily nitrogen gains. There was, however,

a slight increase in fat deposition in fish fed diets with plant protein sources (Kaushik *et al.*, 2004).

1.6.2. Lipids

The lipids are a heterogeneous class of water-insoluble compounds. Lipids include fats, oils and waxes. Fats are esters of glycerol and fatty acids that are solid to room temperature. Oils are glycerol esters that are liquid at room temperature, whereas waxes are esters of fatty acids with alcohols other than glycerol. Lipids are essential nutrients in fish diets and contain the three elements carbon, hydrogen and oxygen. However, unlike carbohydrates, a larger proportion of carbon and hydrogen comprise lipid molecules. Because of the larger proportion of carbon and hydrogen atoms, lipids liberate approximately 9.4 kcal of GE g^{-1} and are the best sources of energy in terms of kcal g^{-1} compared with carbohydrates (4.1 kcal of GE g^{-1}) and proteins (5.6 kcal of GE g^{-1}) (Webster & Lim, 2002).

The most important classes of lipids in animal nutrition are the triacylglycerols (TAG) or triglycerides and phospholipids (PL). TAGs are triesters of glycerol and three fatty acids (FA) and PLs are esters of two fatty acids and one phosphoric acid. Fatty acids are the key components of all lipids. The amount of hydrogen on each carbon (degree of saturation) and the length of the carbon chain determine the physical and nutritional characteristics of lipids. In fish, FA synthesis occurs mainly in the liver, by means of the FA synthetase complex. The main newly-synthesized FAs are palmitate (16:0), stearate (18:0) and myristate (14:0), in different proportions depending on species. The neosynthesized FAs or those supplied by the food can be transformed (bioconverted) into FAs with longer or more unsaturated chains, at least to a certain extent.

Lipid supply in the diet of fish is important firstly to satisfy essential fatty acids (EFA) requirements, which are not synthesized by the organism. EFAs are necessary for cellular metabolism, as well as for the maintenance of membrane structure integrity. Lipids also serve as a vector during intestinal absorption of liposoluble vitamins and carotenoid pigments. Finally, as already mentioned before, lipids provide energy, an important role due to the fact that fish poorly digest complex carbohydrates.

EPA and DHA in fish diet

The aquatic environment is characterized by a great richness in polyunsaturated fatty acids (PUFA) and in particular, long chain PUFAs (20 carbon atoms or more) or HUFA. HUFAs of the n-3 series (n-3 PUFA) are those for which fish have the highest requirements, in contrast to higher terrestrial vertebrates. As mentioned before, HUFAs can be produced by bioconversion of PUFAs, such as linoleic acid (n-6, 18:2) and

linolenic acid (n-3, C18:3), which cannot be synthesized by fish and thus should be supplied in the food. There are very great differences between species in their capacity to convert C18 FAs into HUFAs, which is high in freshwater fish but low in marine fish. Therefore, in marine fish the truly important EFAs and consequently the ones that should be supplied in food are the HUFAs, such as eicosapentanoic acid (n-6 20:5 or EPA), docosahexanoic (n-3 22:6 or DHA) and arachidonic (n-6 20:4 or AA) (Corraze *et al.*, 2001). Furthermore, the competition between DHA and EPA, and EPA and AA for the same metabolic and enzymatic pathways requires that not only the individual amounts but also the optimum ratios of these EFAs are important.

The majority of research work, which has been done on the determination of nutritional requirements of marine fish in EPA, DHA and EPA/DHA ratio, concerns the most popular farmed species. Ibeas *et al.* (1997) concluded that an EPA/DHA ratio of 2/1 (6.44 and 3.19 % in the diet, respectively) in the diet improved the condition of juvenile gilthead sea bream. In sea bass, Skalli & Robin (2004) suggested that EFA requirement was about 0.7% n-3 HUFA of the dry diet. EFAs are usually added in the feeds through the addition of marine fish oil, which is produced from small pelagic fish such as menhaden, herring and anchovies, or wastes arising from the processing of the large fish such as cod-liver. However, as it is the case for fish meal (Paragraph 1.6.1), it is questionable whether fish oil production can support further expansion in aquaculture production. For fish oil, the role of aquaculture is even greater than for fish meal, with close to 87% of production consumed by the sector (salmonids are responsible for more than 55% of the sector's share). According to FAO, total fish oil production in 2006 was about 0.9 million tons, out of which 0.78 million tons were used in the production of aquafeeds (Fig. 21).

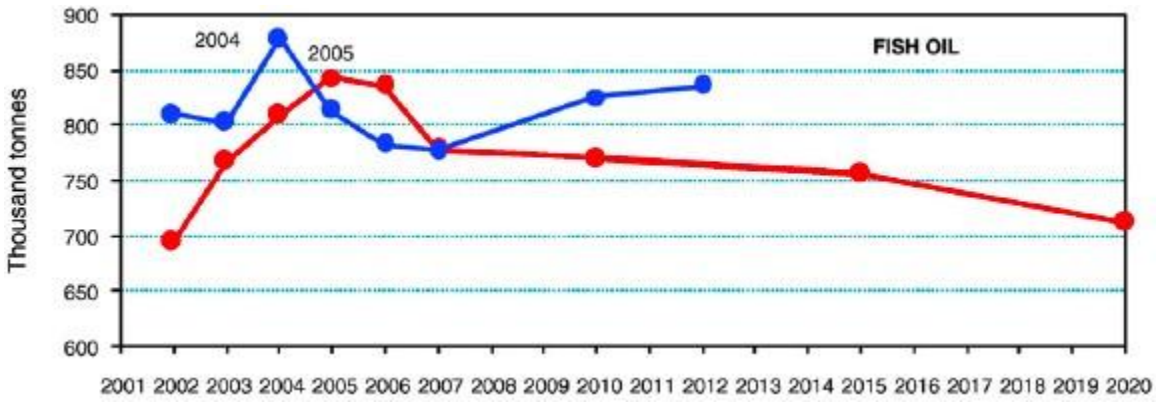

Figure 21. Current and projected use of fish meal within compound aquafeeds from 2002 to 2020 (Tacon & Metian, 2008).

In early 2008, fish oil prices soared to an all-time record of US$ 1,700/ton, compared with US$ 915/ton one year earlier (Fig. 20). The extremely high price of fish oil and the depletion of some fish stocks used in the production of fish oil and fish meal (Fishmeal

Information Network, 2008) render the replacement of fish oil with vegetable oil as a prerequisite for further expansion of aquaculture production. Two of the main projects of the last 10 years that investigated the replacement of fish oil with vegetable oil, were the RAFOA and AQUAMAX projects funded by FP5 and FP6 of the EU. Different vegetables, such as rapeseed, linseed and soybean, were used in these projects in order to generate suitable blends to replace fish oil. Oils differ markedly in their fatty acid compositions, with fish oils to be unique in being rich in n-3 HUFAs, specifically EPA and DHA (Table 5).

Table 5. Values of the fatty acid compositions of different oils (data from AQUAMAX project)

	Saturates	**Monoenes**	**18:2 ω-6**	**18:3 ω-3**	**EPA**	**DHA**
Rapeseed	7	60	21	10	0	0
Linseed	12	18	17	54	0	0
Soybean	15	22	54	8	0	0
Camelina	10	29	18	39	0	0
Palm	66	26	7	0	0	0
Capelin	19	59	2	0.7	5.5	3.5
Anchovy	26.6	23.8	1.2	0.6	20.2	13.5

In sea bass and sea bream replacement of up to 60% of fish meal with vegetable meal had no detrimental effects on growth or feed conversion (Montero *et al.*, 2005; Mourente & Bell, 2006; Benedito-Palos *et al.*, 2007). However, feed intake and growth rates were reduced by about 10% when fish were fed with 100% vegetable oil diet. In both species, flesh DHA & EPA concentration were reduced by ~50% when fish were fed 60% vegetable oil, while in sea bream fed 100% vegetable oil the reduction was ~65%. Trials on recovery of fatty acid profiles by a fish oil finishing diet presented an increase in DHA & EPA concentration but not a complete restoration (Montero *et al.*, 2005; Diaz-Lopez *et al.*, 2009; Fountoulaki *et al.*, 2009).

Importance of EPA and DHA for human health

The long chain omega-3 fatty acids (ω-3 FAs), EPA and DHA, are essential in the human diet for proper growth, development and good health. The best source of these ω -3 FAs is seafood, especially finfish (the fatty tissue of finfish) but also crustaceans and shellfish. Both EPA and DHA are vital for human biological functions, as they are in every cell of human body. For the example, around 25% of the fat in the brain of humans is DHA. DHA is also the preferred FA for the correct construction and functioning of all membranes in the body, particularly those in very active tissue such as nerves and active muscle. Also, both EPA and DHA are important in the cardiovascular system. EPA in particular contributes to the anti-inflammatory response. It is the building block of a

group of cell messengers called eicosanoids. These affects blood pressure, blood clotting, immune function, allergic response, reproduction and gastric secretion (IFFO, www.iffo.net). Table 6 summarizes the specific health benefits of EPA and DHA.

Table 6. Some of the conditions for which there is evidence of specific health benefits of EPA and DHA (IFFO, www.iffo.net)

Already endorsed by health authorities
Prevention of reoccurrence of cardiac infarction
Reduction in cardiovascular disease
Conditions where there is significant scientific evidence of preventative or therapeutic benefits
Brain and nervous system development
Rheumatoid arthritis
Psychiatric disorders, including depression and schizophrenia
Dementia and Alzheimer's Disease
Psoriasis
Conditions for which there is some evidence of benefits
Asthma in children
Vision
Averting progression towards Type 2 diabetes
Behaviour and concentration, including ADHD (attention-deficit hyperactivity disorder) and dyslexia
Obesity
Cystic fibrosis

According to World Health Organization, the recommended daily intake of ω-3 FAs is 500 mg for healthy adults and more for those with known heart conditions or an excess of fatty triglycerides in the blood. It has been demonstrated that one to four serves (about 100 g per serve) of finfish a week can give the recommended daily intake (Fisheries Research and Development Corporation, 2004).

1.6.3. Vitamins

Vitamins are complex organic substances that are required by animals in relatively small amounts for the maintenance of optimal growth, health and reproduction. Since fish cannot synthesize vitamins at all or can only synthesize in insufficient quantity for normal development, growth and maintenance, they must be supplied in the diet. Each vitamin performs a specific function in the body and one vitamin cannot substitute for or

replace another vitamin. Vitamins have been classified into two groups based on their solubility characteristics: fat-soluble and water-soluble (Table 7). These characteristics influence the way in which the vitamins are absorbed by the gastrointestinal tract, transported and stored in the body tissues. Fat-soluble vitamins are absorbed from the intestinal tract along with lipids and any condition that increases lipid absorption will increase the absorption of fat-soluble vitamins. Absorption of water-soluble vitamins is simpler because water is constantly absorbed from the intestine into the bloodstream.

Table 7. Classification of vitamins and examples of their biological functions

Fat-soluble vitamins	
Retinol (vitamin A)	Normal growth, vision, reproduction
Cholicalciferol (vitamin D)	Calcium and phosphate metabolism/regulation
Trocopherols (vitamin E)	Antioxidant, muscle and RBC function
Menadione (vitamin K)	Brood clotting
Water-soluble vitamins	
Thiamin (vitamin B1)	Energy metabolism, nerve function
Riboflavin (vitamin B2)	Cellular energy metabolism
Niacin (nicotinic acid)	Energy metabolism, nerve function
Pantothenic acid (vitamin B5)	Energy metabolism, nerve function
Pyridoxine (vitamin B6)	Protein metabolism and utilization
Cyanocobalamin (vitamin B12)	Nerve function, RBC formation and function
Biotin (vitamin H)	Fatty acid synthesis, glucose metabolism
Folacin (folate)	Embryonic development, gut function
Ascorbic acid (vitamin C)	Antioxidant, collagen synthesis, immune responses

Water-soluble vitamins are generally not stored in the body and excess vitamins are excreted; however, excess fat-soluble vitamins can be stored in the body. This bears a danger, since excesses of fat-soluble vitamins in fish diets may cause physiological or health problems. Moreover, not all the vitamins present in the diet ingredients are in available forms. For instance, niacin in many cereal grains is bound to protein and cannot be absorbed unless the ingredient is treated with an alkali. Further, vitamins are destroyed during diet processing and storage, due to heating, oxidation, sunlight or mould growth. Thus, some vitamins may need to be added to the diet in excess of requirements due to the anticipated losses during diet processing, production and storage. Deficiency symptoms will occur if a single vitamin is omitted from the diet of a species that requires it. Determination of vitamin requirements of fish is difficult and time-consuming, and quantitative requirements are known for very few species.

Vitamin C in broodstock diet

Vitamin C, also known as ascorbic acid, is synthesized from glucose and other simple sugars by plants and many animal species. However, fish cannot convert glucose to ascorbic acid because they lack the enzyme L-gulonolactone oxidase. Thus, a dietary source of vitamin C must be provided to meet their nutritional requirement. In fish feed production, crystalline ascorbic acid is unstable, especially during extrusion, even with coated vitamin C preparations. Therefore, more stable forms of vitamin C, such as ascorbyl-2-sulphate, ascorbyl-2-monophosphate or polyphosphate, are currently used in fish feeds (Storebakken, 2002).

Vitamin C is important (i) in the formation and maintenance of collagen, (ii) in the metabolism of the amino acids proline, lysine, tyrosine and tryptophan, (iii) in the absorption and transport of iron, (iv) in the metabolism of lipids and cholesterol, (v) as an antioxidant in the protection of vitamin A and E and highly unsaturated fatty acids, (vi) in the development of strong bones and (vii) in the metabolism of folic acid. However, because of the extremely labile character of this vitamin, deficiencies have frequently been observed in fish fed artificial diets, a fact that has attracted the interest and have been studied by many researches. Alexis *et al.* (1997) studied the effect of several concentrations of ascorbic acid in the diet of sea bream (*Sparus aurata*) juveniles and observed that vitamin C deficiency caused pathological signs such as extensive tubular damage, glomerulonephritis, and inflammatory response of the haemopoetic tissue producing granuloma. Also, gross deficiency signs were observed like anorexia, internal and external haemorrhages, scale loss and depigmentation. Finally, the wound healing response showed a direct correlation to ascorbate level in the diet. In rainbow trout (*Oncorhynchus mykiss*), deficiency in vitamin C caused anorexia and lethargy, symptoms that in turn affected negatively growth and survival rates (Matusiewicz *et al.*, 1994).

Apart from all the above mentioned functions and its involvement in many reactions, the fact that vitamin C is found in high concentrations in gonadal tissue points out its important role in reproduction (Sandnes & Braekkan, 1981; Dabrowski *et al.*, 1994). Broodstocks of sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus auarata*) fed AA supplemented diet (2000 mg AA/Kg feed) produced fertilized eggs, which contained concentrations of total ascorbate of 218.5 ± 17.7 and 122.4 ± 5.1 μg/g wet weight respectively. This was significantly higher than the groups of broodstock fed a sufficient AA for normal growth, which contained only 155.9 ± 6.9 and 103.9 ± 3.5 μg/g wet weight respectively (Terova *et al.*, 1998a). Using the same groups of broodstock, it has been also observed increased collagen synthesis in embryos and fasting larvae coming from the broodstocks fed an additional dose of AA (Terova *et al.*, 1998b). In milkfish (*Chanos chanos*) broodstock, supplementation of 0.1% of vitamin C in the feed resulted in higher percentage of spawns with higher percentage egg viability, hatching and cumulative survival rate of larvae (Emata *et al.*, 2000). Finally, in a different kind of

experiment where eggs of rainbow trout (*Oncorhynchus mykiss*) were enriched by immersion in different forms and concentrations of ascorbic acid, results suggest that when broodstock rainbow trout don not have enough vitamin C in their ovaries, immersion of eggs in 1000 mg L^{-1} of neutralized AA may be useful (Falahatkar *et al.*, 2006).

1.6.4. Carotenoids

Carotenoids are a family of over 600 natural fat-soluble pigments, the colour range of which extends from yellow to deep red. They are produced within microalgae, phytoplankton and higher plants. They are synthesized through the isoprenoid pathway, which also produces such diverse compounds, such as fatty acids, steroids, sterols, and vitamins A, D, E and K. Carotenoids are also found in animals, but animals cannot synthesize them, thus must be supplied through their diet. Most studies on carotenoids in aquatic animals involve the salmonids and penaeid shrimps because of the economic importance of pigmentation (Choubert, 2001). The pink coloration of salmon flesh or shrimp carapaces is considered as a criterion of quality.

Carotenoids are polyenic compounds characterized by a system of conjugated double bonds. The most widespread carotenoids used in aquaculture industry are: (i) β-carotene, (ii) canthaxanthin and (iii) astaxanthin. The colour in carotenoid substances is caused by the chromophore, which is made up of at least seven conjugated double bonds. β-carotene is orange in colour, whereas canthaxanthin and astaxanthin are pink.

Carotenoids are liposoluble compounds and their absorption is linked to that of lipids and their digestibility is influenced by the lipid content of the diet. However, digestibility of carotenoids depends mainly on their form and nature. In the case of astaxanthin, which exists in three forms (diester, monoester and free form), studies have been shown that it is more digestible in its esterified form than its free form (Choubert, 2001).

The role of carotenoids in fish is not limited in the pigmentation of skin and flesh in several species (Kalinowski et al., 2005; Booth et al., 2004; Tejera et al., 2007; Choubert et al., 2006), but it involves the function of carotenoid molecules as precursors of vitamin A, the regulation of immune responses and possible involvement in physiological phenomena such as growth and reproduction (Nakano et al., 1995; Rehulka, 2000).

Astaxanthin in broodstock diet

Astaxanthin is a red pigment, which has two hydroxyl groups in positions 3 and 3' and two ketone groups in positions 4 and 4' (Fig. 22). It can be found in both free and esterified forms (mono- or diester), but the latter form has been found to be more digestible. It is the main natural pigment in salmonids and many crustaceans, as it is documented to have better deposition and accumulation to the flesh and carapace than other carotenoids (Torrissen, 1989; Chien & Jeng, 2003).

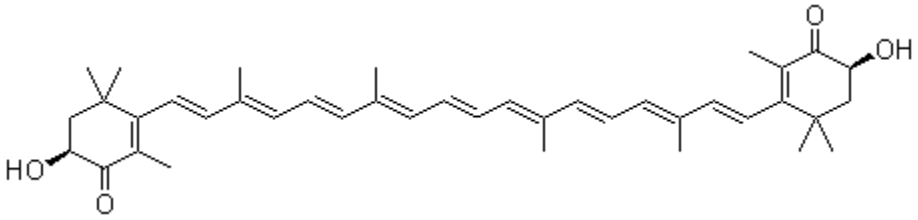

Figure 22. Formulae of astaxanthin (3,3'-dihydroxy-β, β-carotene 4, 4'-dione)

Apart from the benefits mentioned above about astaxanthin and carotenoids in general, astaxanthin is also reported to show a potent antioxidative activity in fish (Bell *et al.*, 2000; Nakano et al., 1995) and an improved resistance to low dissolved oxygen stress in crustaceans (Chien & Shiau, 2005). Another function of astaxanthin supplementation in fish feed, which is related to the experimental section of this thesis work, is its effect on reproductive performance and on the quality of produced eggs and larvae. Vassallo-Agius *et al.* (2001a) reported that broodstock of striped jack (*Pseudocaranx dentex*) fed steam dry pellets containing 10 ppm astaxanthin and squid meal had an improved overall spawning performance (total larval production was 678.1×10^3 larvae/Kg-female), which was similar to the fish fed on raw fish mix (total larval production was 679.9×10^3 larvae/Kg-female). Also, Vassallo-Agius *et al.* (2001b) stated that striped jack broodstock produced three times more eggs when it was fed a diet with a supplementation of staxanthin. In another experiment with broodstock of yellowtail (*Seriola quinqueradiata*), astaxanthin supplementation (30 mg/Kg) in soft-dry pellets (a-SDP) improved egg production, fertilization rates, hatching rates and larval survival at 3 DAH, when compared with a broodstock group fed basal soft-dry pellets (SDP) (Table 8) (Vassallo-Agius *et al.*, 2001c). However, Verakunpiriya *et al.* (1997) suggested that astaxanthin supplementation in the diet of yellowtail broodstock beyond 30 mg/Kg diminished the quality of produced eggs.

Table 8. Spawning results of the two trials for yellowtail fed the soft-dry pellet (SDP) and the astaxanthin-SDP (a-SDP) diets (Vassallo-Agius *et al.*, 2001b)

	Trial 1		**Trial 2**	
	SDP	**a-SDP**	**SDP**	**a-SDP**
Egg production ($\times 10^3$ Kg^{-1} female)	51.0	90.9	90.0	93.6
Fertilization rate (%)	65.7	79.8	94.2	96.2
Hatching rate (%)	63.0	73.2	40.3	65.3
Larval survival (%)	61.8	59.3	32.4	60.0

In rainbow trout (*Oncorhynchus mykiss*) broodstock fed diets containing 5 different concentrations of astaxanthin, which varied between 0.07 to 92.91 mg/Kg, produced eggs had an astaxanthin concentration ranging from 2.03 to 29.79 mg/Kg. The astaxanthin content in the eggs and fertilization rate, eyed-egg percentage and percentage hatch were significantly correlated, suggesting that supplements of astaxanthin are required for optimum reproduction in rainbow trout (Ahmadi *et al.*, 2006). Finally, in Atlantic salmon (*Salmo salar*) fed diets either with or without 100 mg/Kg astaxanthin supplementation, the astaxanthin concentration of the eggs was not related to the fertilization rate and the survival of eggs from fertilization to hatching. Also, the free embryos that hatched from eggs with high astaxanthin concentration did not perform better than those hatched from eggs with a low astaxanthin concentration. These findings indicated that astaxanthin is of little value as a measure of egg quality in Atlantic salmon (Christiansen & Torrissen, 1997).

1.6.5. Carbohydrates

Carbohydrates are organic compounds composed of carbon, hydrogen and oxygen in proportions which almost always correspond to the crude formula $(CH_2O)_n$. They are one of the major classes of nutrients besides proteins and lipids. Carbohydrates are abundant in plants because they are the storage form of energy in plants, in contrast to animals, which store energy as lipid. Carbohydrates typically fall into three main groups: monosaccharides, oligosaccharides and polysaccharides. Monosaccharides are simple sugars and are rarely found in the nature. Rather, they occur as components of complex carbohydrate molecules. Oligosaccharides contain between two and ten monosaccharides that are chemically bonded together. Most oligosaccharides are the result of catabolism of polysaccharides. Polysaccharides are large sugar complexes that contain repeating chains of monosaccharides.

Although carbohydrates are not indispensable in fish feed, they constitute an inexpensive source of energy. In the absence of carbohydrates, there is increased

utilization of proteins and lipids as an energy source. In many species, a dietary carbohydrate supply appears to be necessary as it improves growth and especially protein utilization (Kaushik, 2001). In the wild, fish food is low or sometimes even completely lacking in carbohydrates, with the exception of chitin which is not very digestible. The poor ability of fish to make use of dietary carbohydrates is a reflection of the scarcity of carbohydrates in the aquatic environment.

1.7. Energy metabolism and respiration

Fish, like all animals, need energy to live. They can utilize energy only from the oxidation of organic compounds (carbohydrates, lipids and proteins), which are provided by the digestion of food and the turnover of cells and tissues. The energy of ingested food is partitioned into various fractions in terms of animal utilization (Fig. 23). Gross energy (GE) is the energy released as heat when an organic substance is completely oxidized to carbon dioxide (CO_2) and water. It can be determined by combustion of the food in a calorimeter. Digestible energy (DE) corresponds to gross energy (GE) ingested, less the GE of faeces, which generally varies between 10 and 30% of the GE of the food. Metabolisable energy (ME) is the DE less the energy lost by the fish as gill and urinary excreta. Finally the net energy (NE) of food is its ME less energy expenditure linked to food consumption and utilization. NE of food is available for maintenance, muscle activity and productive purposes, such as growth and reproduction.

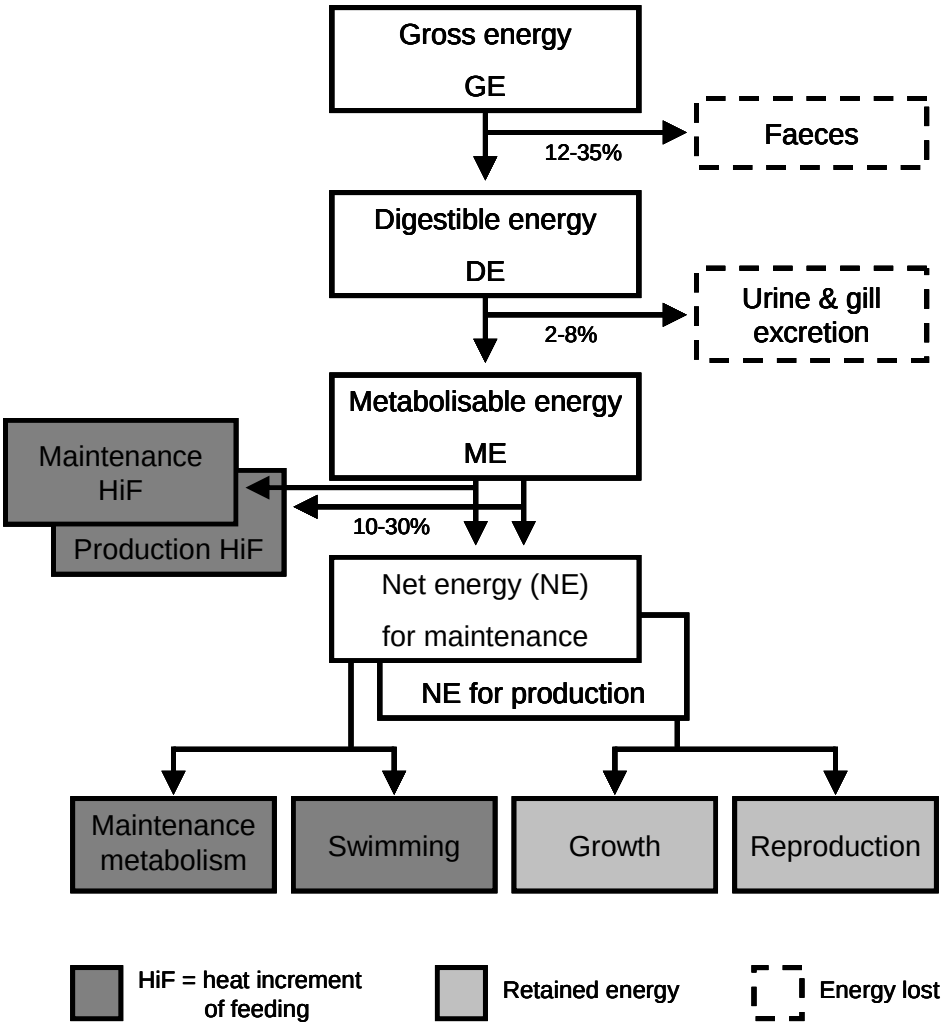

Figure 23. Partition of dietary energy in fish.

According to the life history of each fish, consumed energy is partitioned into its major functional processes of metabolism, growth and reproduction. For example, largemouth bass, which employs an "ambush" type of feeding behaviour, partitions ingested energy differently than actively swimming skipjack tuna, which forages continuously. Both species allocate similar proportions of consumed energy to standard metabolism, specific dynamic action (food assimilation) and waste losses, but tunas devote a much larger fraction of their energy intake to activity than do basses (Fig. 24) (Adams & Breck, 1990).

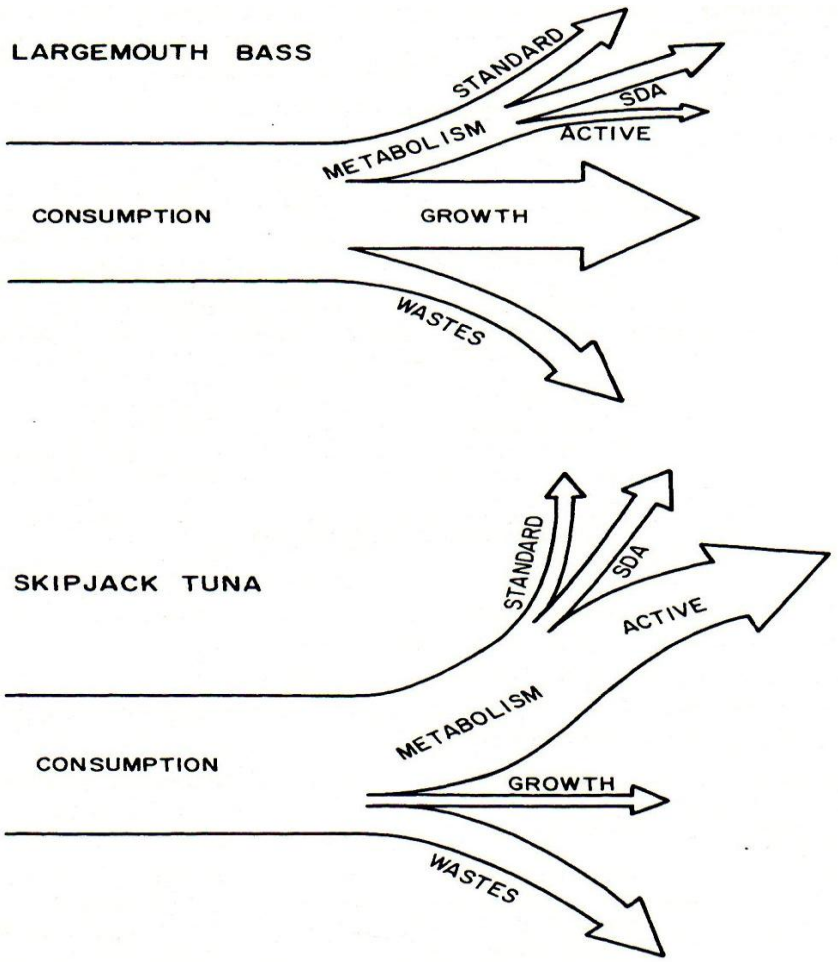

Figure 24. Allocation of consumed energy into the major components of the bioenergetics budget at maximum rations in the relatively sedentary largemouth bass and the actively foraging skipjack tuna. Widths of arrows are scaled to represent actual proportions of consumed energy allocated to each process. SDA is specific dynamic action (Adams & Breck, 1990).

The energy requirements of fish depend on the species and physiological stage of the animal itself. They also vary in relation to environmental factors, such as oxygen and temperature. In aerobic metabolism, oxygen is used to convert the energy contained in food to high-energy chemical bonds, such as those formed during the citric acid cycle, which is the primary producer of adenosine triphosphate (ATP). In turn, hydrolysis of ATP is the source of energy at the cellular level. Oxygen uptake by fish through respiration is therefore a vital function in energy metabolism and oxygen consumption rate is a conventional metabolic measure for fish because dissolved oxygen can be easily determined. Aerobic metabolism in fish can be categorized as standard, resting routine, routine, swimming and active, depending on the locomotive status of the fish. Water temperature influences most biochemical and physiological processes in fish, due to the fact that fish are ectotherms, and their body temperature varies with water temperature.

Hence, the control of metabolic activity by temperature conditions is generally considered central for fish to optimize the use of their ecosystem in time and space.

1.8. Quality of livestock

Aquaculture being the most rapidly growing animal food-producing sector of the last few decades attracts many investments on new and large projects. However, the fast development and growth of the sector bears some dangers, which can create serious problems on individual business or the aquaculture sector as a whole. One of the main dangers, apart from rough planning and implementation of new business projects, unsustainable development and bad management practices, is the bad quality of livestock and products. Quality of livestock and products is an important chapter for aquaculture sector, where lots of emphasis must be given on to ensure best possible results. Within the objectives of the experimental part of this thesis work is the assessment of the quality of livestock at different production stages.

The assessment of the quality of livestock in aquaculture is all down to performance in terms of growth, survival, reproduction, physiology, behaviour, etc. Giving the high intensity and magnitude of some aquaculture practices, quality of livestock should be no less than optimum in order for the fish to be able to perform as expected. For example, a hatchery that is producing more than 50 million fry of sea bass and sea bream using stocking densities of over 100 larvae/l, exercises such a pressure on the fish (broodstock and larvae) that if their quality is not good enough, the production targets will never be achieved.

The good performance of broodstock and the production of an adequate volume of good quality eggs and larvae to support hatchery operations are necessary to offset the huge investments done in Mediterranean marine aquaculture. The quality of broodstock, although an under-estimated parameter in fish farming, it constitutes an important prerequisite for the production of strong and healthy fish. Only the last few years feed companies produce broodstock specific feeds that can meet the nutritional needs of the parent fish and ensure the production of high quality eggs and larvae. Variations in the quality of eggs and larvae are observed in all the hatcheries and possible improvement on this aspect will be of great economic value. In most research studies and hatcheries, egg quality is a combination of several parameters, such as fertilization and hatching rate, morphometric measurements, and egg content in proteins, collagen, lipids and carbohydrates. Larval quality is also based on a number of parameters, such as survival, morphometric measurements, viability against hunger and stress, and larvae content in proteins, lipids, and enzymes. This combination of parameters both in egg and larval quality, give rather an objective picture of the quality, once some of the above-mentioned parameters are also affected by environmental, technological and handling factors and do

not cover all the possible quality indicators. Therefore, the application of more sophisticated techniques and analysis that will decipher the strengths and weaknesses of an organism is necessary so as to give the appropriate tools and information to the scientists to assess better the quality of livestock.

1.8.1. Genomics

Gemonic studies on animals give the opportunity to meet and understand the different functions of an organism in great detail and assess their quality. With respect to biodiversity, genomics can be used to study and evaluate specific nutritional strategies, reproduction techniques, animal welfare and response to endocrine disruptor pollutants. However, today genomic data concerning fish are limited. Only two species, zebrafish (*Danio rerio*) and pufferfish (*Fugu rubripes*) have their genome detailed annotated and mapped (Kelly *et al.*, 2000; Aparicio *et al.*, 2002). Information on species important for fisheries and aquaculture are scarce, rendering difficult the study of functional genomics, transcriptomics and proteomics on species important for human nutrition. However, the last five years several genomic studies have produced expressed sequence tag (EST) collections for species involved in Mediterranean aquaculture. Chini *et al.* (2006 and 2008) have identified several genes of known function in other organisms and many new genes in sea bass (*Dicentrarchus labrax*) and bluefin tuna (*Thunnus thynnus*). These studies can lead to further genomic studies on specific subjects of great interest, such as nutrition, metabolism and reproduction under captivity and conservation of wild stocks. Recent studies on different breeding protocols in rainbow trout (*Oncorhynchus mykiss*) have used genomic tools to assess egg quality (Bonnet *et al.*, 2007a, Bonnet *et al.*, 2007b). The results revealed that control of ovulation using either hormonal induction or a manipulated photoperiod can induce differences in the egg mRNA abundance of specific genes. An example of such genes is prohibitin 2 (PHB2), which has been found to be negatively correlated with the developmental potential of the egg.

Two genomic studies, which are related to this work, involve the molecular cloning and characterization of hypoxia-inducible factor-1 (HIF-1) and L-ascorbic acid transporter SVCT1. HIF-1 is a heterodimer composed of α and β subunits. HIF-1β is generally found to be constitutively expresses in the nucleus and to be insensitive to changes in O_2 availability, whereas stabilization of HIF-1α and its nuclear accumulation are acutely regulated by hypoxia. In sea bass, the number of HIF-1α mRNA copies was found to be significantly increased in liver tissue in response to both acute and chronic hypoxia, whereas it remained unchanged in fish exposed to hyperoxic conditions (Terova *et al.*, 2008). SVCT1 mediate concentrative, high-affinity L-ascorbic acid transport that is stereospecific and is driven by the Na^+ electrochemical gradient. SVCT1 is mainly confined to epithelial systems, such as to intestine, kidney and liver. Although initial identification of this gene came from rat complementary DNA libraries (Tsukaguchi et

al., 1999), identification of this gene also in fish will be a major step in understanding the importance of L-ascorbic acid transport in regulating the supply and metabolism of vitamin C.

2. Materials and Methods

2.1. A preliminary study on physiology and reproduction of marbled rabbitfish (*S. rivilatus*)

This work was carried out between May and August 2009, when a total of 55 wild rabbitfish were collected during eight different dates. Fish samples were collected using rod and line at the south (N=18) and north (N=37) part of Cyprus island (Fig. 25).

Figure 25. Sampling locations (in circles), two at the north and one at the south part of the island.

The specimens were stored in a fridge at 4°C for one day and then they were measured for total weight (TW), total length (TL), dress weight (DW) and weight of gonad and liver. For length measurements, a millimetrically calibrated measuring board was used and measurements were done to the nearest 0.5 cm. For weight measurements, TW and DW were measured to the nearest 0.5 g, and weight of gonad and liver at the nearest 0.0001 g. Then, the measurements were used to calculate the gonadosomatic index (GSI), hepatosomatic index (HSI), dress out percentage (DOP) and condition factor (K), by the following equations:

$GSI = (\text{Weight of gonad} / TW) \times 100$

$HSI = (\text{Weight of liver} / TW) \times 100$

$DOP = (DW/TW) \times 100$

$K = (TW / TL^3) \times 100$

The same measurements were performed on a number of farmed rabbitfish, which were cultured at the facilities of MeMARS (see Paragraph 2.3).

2.2. Effect of temperature on energy metabolism in marbled rabbitfish (*S. rivulatus*)

The experimental set-up used involved a static respirometer, which contained a non-circulating, fixed volume of water of 7 liters. The respirometry chamber was built from a plastic container to which an oxygen probe (Catvis® Oxyguard) and a sampling tube were fitted (Fig. 26). The respirometer was placed on a magnetic machine to facilitate a light stirring of the water. The temperature, oxygen concentration and time were recorded via a computer camera during the whole duration of the measurements. Before starting the respirometry measurements and in order to confirm that the experimental set-up was working properly, the experiment ran without any fish in the respirometry chamber for one hour. Oxygen concentration remained stable at 100% saturation indicating that no external parameters were interfering with the respirometry chamber.

All the fish used in this experiment were two years old and were cultured at the facilities of MeMARS. Respirometry measurements were performed at four different water temperatures, 17, 20, 23 and 26 °C. For each water temperature, five measurements were done with five different individual fish. The mean weight of the fish (for all treatments) was 161±32 g. Each group of five-fish was first placed in a 30-liter fiberglass tank in order to be acclimatized for 72 hours in the corresponding water temperature. During acclimatization period, the system was flow-through and the water was first passing from a boiler in order to be heated to the desirable water temperature. One hour before the start of respirometry measurements each fish was transferred in the respirometry chamber, where water was flowing through, in order to adapt and arrive to a resting state. The measurement was initiating with the interruption of water flow and the sealed fitting of oxygen probe. The data collected were passed on a tubular and a graphing form in order to facilitate result analysis. Oxygen consumption rate was calculated for all respirometry measurements done, using the following equation:

$$M_{O_2}\ (mgO_2\ /g\ BW/min) = (C_{O_2(A)} - C_{O_2(B)}) \times V\ /\ W\ /\ T$$

where,
M_{O_2} = O_2 consumption rate (mg O_2/h),
$C_{O_2(A)}$ = O_2 concentration in water (mg O_2/L) at the start of the measurement period,
$C_{O_2(B)}$ = O_2 concentration in water (mg O_2/L) at the end of the measurement period,
V = volume of respirometer (L),
W = total fish biomass (g), and
T = time elapsed during measurement period (min).

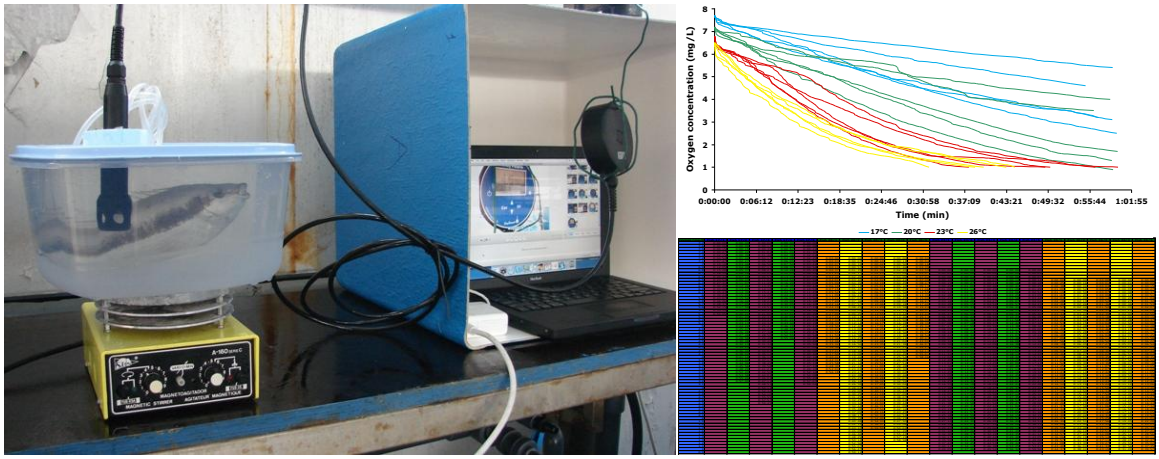

Figure 26. Experimental set-up for respirometry. Oxygen concentration, temperature, and time were recorded via a computer camera and then the data were transferred to a tubular and graphic form.

Identification of HIF-1a gene

Preparation of total RNA and first-strand (cDNA) synthesis.

Total RNA was extracted from *Siganus rivulatus* (marbled spinefoot) liver using the PureYield RNA Midiprep System (Promega, Italy), following the manufacturer's protocol. The quantity of the RNA was calculated at an absorbance of 260 nm. The integrity and relative quantity of RNA was assessed by electrophoresis. After extraction, 3 µg of total RNA was reverse transcribed into cDNA in a volume of 12 µl, containing 1 µl of oligo dT16 primer (50 pmol) and 1 µl of 10 mM dNTPs. This mix was heated at 65 °C for 15 min and chilled on ice, and then 4 µl of 5X reverse transcription buffer, 2 µl of 0.1 M DTT, 1 µl RNaseOUT, and 200 units of Moloney murine leukemia virus reverse transcriptase were added to a final volume of 20 µl, as described in the M-MLV Reverse Transcriptase kit (Invitrogen). After incubation at 37 °C for 50 min, the reaction was stopped by heating to 75 °C for 15 min.

Cloning and sequencing

To perform PCR, a 4-µl aliquot of the resulting cDNA was amplified with 1 µl GoTaq Polymerase (Promega) in 50 µl of the final volume, which contained 5 µl buffer, dNTPs 10 mM, and 50 pmol of each of the designed HIF-1α RT-PCR primer sets (Fig. 34). A total of 30 PCR amplification cycles (10 touchdown) were performed for all primer sets, using an automated Thermal Cycler (Mycycler, Biorad). The annealing temperatures depended on the melting temperatures of the primer set used. An aliquot of each sample was then electrophoresed on 2 % agarose gel in 1X TAE buffer (Eppendorf) and bands were detected by ethidium bromide staining. The positive control consisted in

a master mix of cDNA and cytoplasmatic β–actin primers (accession n° AY148350) (Terova *et al.*, 2007a, 2007b, 2008), while the negative control consisted of total RNA added to the RT reaction mixture without reverse transcriptase and subsequently amplified using the same set of primers and the same conditions. The negative control confirmed the absence of genomic contamination. The PCR products from HIF-1α primer amplifications were cloned using the pGEM®-T Easy cloning vector system (Promega, Italy) and subsequently sequenced in both directions (T7 and SP6).

Sequence and phylogenetic analysis

The amino acid sequences of *Siganus rivulatus* HIF-1α were analyzed using a translator program which is available at the open reading frame finder (NCBI, http:// www.ncbi.nlm.nih.gov). Sequence identities were verified using the BLAST program and NCBI database. Sequences were aligned using the ClustalW program and adjusted using Multiple Sequence Alignments Editor & Shading Utility, GeneDoc, version 2.6.002 Copyright © 2000 by Karl Nicholas (www.psc.edu/biomed/genedoc).

2.3. Effect of nutritional protein level on growth performance of marbled rabbitfish (*S. rivulatus*)

The experimental set-up involved six circular 500-liter fiberglass tanks connected to a water flow-through system (Fig. 27). Each tank was stocked with 50 hatchery reared rabbitfish, with an initial mean weight of 32±1.9 g/fish. The initial mean stocking density was 3.2±0.2 Kg/m^3. Seawater was pumped from the nearby coast, and filtered by means of a sand filter. Each tank had its own water inlet and aeration (by means of air blower) in each tank was provided by an air diffuser. Water flow was set at 3.5 l/min and air flow at 150 ml/min, both were stable throughout the experiment period. Outlet water was leaving from each tank through an overflow stand-pipe and then it was passing from a settling tank and a UV sterilizer before it returns to the sea. Physical water parameters, including oxygen concentration, temperature and salinity, were recorded daily. Water salinity was constant at 39 ppt and temperature varied according to the natural temperature of the sea. Oxygen concentration was at 100% saturation throughout the experiment and photoperiod was natural.

Figure 27. Tanks used for the feeding experiment in rabbitfish.

The tanks were divided in two treatments (three tanks in each treatment) in order to test the two experimental diets with different protein content, 35% (Treatment A) and 44% (Treatment B). The composition of the feeds can be seen at Table 9. The fish were fed by hand twice a day (8:00 and 12:00) with dry pellets (3 mm Ø) until satiation (ad libitum). Satiation was determined when the fish showed no more interest in the food. The fish were not fed the day before each sampling. The experiment lasted for one year and survival was above 94% in all tanks.

During each sampling day, all the fish in each tank were anesthetized using 2-phenoxyethanol at a dose of 0.2 ml/l and the TW of the whole population was recorded. Then, three fish from each tank were sacrificed by an overdose (0.35 ml/l) of anesthetic and data on TW, DW and weight of gonad and liver were recorded. By using these data, GSI, HSI, DOP and K were calculated by the equations mentioned in paragraph 2.1. Growth performance was expressed as specific growth rate (SGR), calculated by the following equation:

$$SGR\ (\%\ BW.d^{-1}) = [(\ln W_t - \ln W_0) / t] \times 100$$

where,
W_t = body weight at time t, and
W_0 = body weight at time = 0 (start of the experiment).

Table 9. Composition of the feeds used in the experiment

	Treatment A	Treatment B
Protein (%)	35	44
Lipid (%)	5	18
Ash (%)	15	11
Fibers (%)	4	2
Calcium (%)	2.3	1.7
Phosphorous (%)	0.8	1.6
Gross Energy (KJ/g DM)	17.9	21.6

2.4. Effect of vitamin C and astaxanthin supplementation in broodstock diet on reproductive performance, and egg and larval quality in common pandora (*P. erythrinus*)

The experiment took place at the facilities of MeMARS and the fish used were descended from cultured specimens (F_1 generation). The fish were three years old and they were cultured under the same conditions until the start of this experiment. Out of the 200 mature fish kept in a 15-ton tank, twelve male and twelve female fish were selected after examination of their gonads by catheterization on 4th of March, 2008. Before catheterization, all the fish were semi-anesthetized using 2-phenoxyethanol at a dose of 0.2 ml/l of water. Full anesthetization was performed in a 50-liter tank at a dose of 0.25 ml/l just before treating each fish. Catheterization was performed by means of a hematocrit (Heinz Herenz®) of 1.5 mm diameter, which was inserted in the gonad duct of the fish. Then, with a gentle suction sperm or egg cells were collected and observed under a microscope (Leica® DMLS) at 10× magnification. Twenty four fish (12 males and 12 females) were selected to participate in this experiment.

The selected fish were distributed in three round fiberglass 3-ton tanks with conical bottom (Fig. 28), so that each tank had eight fish at a sex ratio of 1:1. The average body weight of each group of broodstock is shown at Table 10. Seawater was supplied to the tanks at a flow rate of 0.2 l/s and oxygen was supplied by means of atmospheric air through an air compressor at a flow rate of 150 ml/min . A net with large mesh openings was placed on top of the tank in order to prevent fish from jumping out when a noise or light disturbed them. Throughout the experimental period (4th March – 21st June), salinity was constant at 39 ppt and temperature ranged from 17 – 24 °C.

Table 10. Average body weight of the three groups of broodstock.

Number of fish	Average body weight (g)		
	Group A	**Group B**	**Group C**
4 Males	569±165	622±173	656±185
4 Females	523±275	518±182	506±173

The three broodstock groups were fed different experimental diets that were produced at MeMARS. The first feed (Diet A), which was taken as the control diet, was a conventional dry feed that is used to the fattening of seabream, the second feed (Diet B) was the conventional feed with a supplementation of vitamin C (2000 mg/Kg) and the third feed (Diet C) was the conventional feed with a supplementation of vitamin C (2000 mg/Kg) and astaxanthin (30 mg/Kg). The vitamin C was added by means of a spray-dried powder of stabilized (phosphorylated) vitamin C product, which is called Rovimix® Stay-C® 35, and the astaxanthin by means of an unesterified astaxanthin product, which is called Carophyll® Pink. The feeds were produced by grinding first the conventional dry feed, which was Aquasol® pellets of 5 mm diameter, using an Omega® T12 Grinder. Then the powdered conventional feed was divided in three parts and the appropriate amounts of vitamin C and astaxanthin were added and mixed in Diets B and C. Water (200 ml/kg) and gelatin (23 g/Kg) were added in each diet and then were mixed until a soft homogeneous paste was created. Finally the paste was cut in 5 mm pellets and the pellets were let to dry in room temperature for 12 hours. The produced pellets had a dry matter of 68.4%. The production of the three diets was done once a week until the end of the experiment. Feed was supplied to the fish daily by an automatic clockwork feeder between 8 am and 5 pm. Feeding ratio ranged between 1.8 and 3.2% of body weight.

Figure 28. One of the 3-ton broodstock tanks.

Spawning started on 4th of April 2008 and lasted until 21st of June. All groups spawned naturally and the eggs were collected daily using an egg-collector at the overflow pipe of each tank. The egg-collector was done by a PVC tube and a mesh net of 500 μm (Fig. 29). During the spawning season, salinity was constant at 39 ppt and temperature ranged from 19 to 24ºC.

Figure 29. Egg collector.

Egg morphological measurements

The eggs were collected every morning and separated between fertilized and non-fertilized eggs. The separation was done by letting the eggs to rest for 10 minutes in a bucket with 10 l of seawater; the fertilized eggs were floating whereas the non-fertilized eggs were sinking. A sample of 30 fertilized eggs was then taken from each group in order to measure the diameter of the eggs and oil droplets. Measurements were done to the nearest 0.5 μm by using an ocular micrometer (Leica® DMLS) at 10× magnification.

Hatching rate and starvation test

Fertilized eggs were stocked into 96-well microtitre plates, one egg per well containing 200 μl of filtered, sterilized seawater. Overall, 192 eggs from each group were placed in 2 microtitre plates. The plates were held in an incubator at 19 ºC until all the larvae die. This experiment was repeated three times. Hatching rate was taken as the number of eggs hatched into larvae expressed as percentage of fertilized eggs. For the starvation test, the survival of larvae which hatched in the microtitre plates was recorded daily. Survival was taken as the percentage of larvae survived out of the initial number of larvae. From the number of surviving larvae and survival duration, the survival activity index (SAI) was calculated by the following equation:

$$SAI = 1/N \, {}_k\Sigma_{i=1} \, (N\text{-}h_i) \times i$$

where
N = total number of larvae,
h_i = cumulative mortality by the ith day, and
k = number of days elapsed until all larvae died due to starvation.

Egg incubation and larvi-culture

Fertilized eggs from each group were incubated in 300-l round tanks with conical bottom (Fig. 30). Egg hatching was observed after 36 h at 20 ºC and yolk-sac absorption was completed on 2nd DAH. Feeding started on 3rd DAH, where rotifers were supplied at a concentration of 5 rotifers/ml until 14th DAH. *Artemia* nauplii were introduced in the feeding regime on 12th DAH and continued to be supplied until the end of the larvi-culture that lasted up to 17th DAH. Rotifers were cultured with yeast and INVE® Protein Selco and were enriched again with INVE® Protein Selco. *Artemia* was enriched with INVE® DHA Protein Selco.

Figure 30. Larval culture of *P. erythrinus*. The buckets above the tanks were used for the supply of phytoplankton (*Nannochloropsis* sp.) and rotifers (*Brachionus plicatilis*).

Samples of eggs have been taken from all groups on spawning and of larvae on 0, 2, 5, 7, 9, 13 and 17 DAH. During each sampling day, two samples from each group were collected. The first sample was about 300 g of eggs or larvae that were stored in eppendorfs, then dipped in liquid nitrogen and finally stored in -80ºC freezer. The second sample was 10-20 eggs or larvae that were fixed in PFA 4% reagent. These samples were then analyzed using genomic tools to identify the L-ascorbic acid transporter, SVCT gene.

Identification of SVCT gene

Preparation of total RNA and first-strand (cDNA) synthesis

Total RNA was extracted from *Pagellus erythrinus* embryos [stages 9 days post hatching (dph) and 13 dph] using the PureYield RNA Midiprep System (Promega, Italy), following the manufacturer's protocol. The quantity of the RNA was calculated at an

absorbance of 260 nm. The integrity and relative quantity of RNA was assessed by electrophoresis. After extraction, 3 µg of total RNA was reverse transcribed into cDNA in a volume of 12 µl, containing 1 µl of oligo dT16 primer (50 pmol) and 1 µl of 10 mM dNTPs. This mix was heated at 65 °C for 15 min and chilled on ice, and then 4 µl of 5X reverse transcription buffer, 2 µl of 0.1 M DTT, 1 µl RNaseOUT, and 200 units of Moloney murine leukemia virus reverse transcriptase were added to a final volume of 20 µl, as described in the M-MLV Reverse Transcriptase kit (Invitrogen). After incubation at 37 °C for 50 min, the reaction was stopped by heating to 75 °C for 15 min.

Cloning and sequencing

To perform PCR, a 4-µl aliquot of the resulting cDNA was amplified with 1 µl GoTaq Polymerase (Promega) in 50 µl of the final volume, which contained 5 µl buffer, dNTPs 10 mM, and 50 pmol of each of the designed SVTC RT-PCR primer sets (Fig. 46). A total of 30 PCR amplification cycles (10 touchdown) were performed for all primer sets, using an automated Thermal Cycler (Mycycler, Biorad). The annealing temperatures depended on the melting temperatures of the primer set used. An aliquot of each sample was then electrophoresed on 2 % agarose gel in 1X TAE buffer (Eppendorf) and bands were detected by ethidium bromide staining. The positive control consisted in a master mix of cDNA and cytoplasmatic β–actin primers (accession n° AY148350) (Terova *et al.*, 2007a, 2007b, 2008), while the negative control consisted of total RNA added to the RT reaction mixture without reverse transcriptase and subsequently amplified using the same set of primers and the same conditions. The negative control confirmed the absence of genomic contamination. The PCR products from SVTC primer amplifications were cloned using the pGEM®-T Easy cloning vector system (Promega, Italy) and subsequently sequenced in both directions (T7 and SP6).

Sequence and phylogenetic analysis

The amino acid sequences of *Pagellus erythrinus* SVTC were analyzed using a translator program which is available at the open reading frame finder (NCBI, http:// www.ncbi.nlm.nih.gov). Sequence identities were verified using the BLAST program and NCBI database. Sequences were aligned using the ClustalW program and adjusted using Multiple Sequence Alignments Editor & Shading Utility, GeneDoc, version 2.6.002 Copyright © 2000 by Karl Nicholas (www.psc.edu/biomed/genedoc).

2.5. Statistical analysis

The statistical analysis of the results was performed using the GraphPad® Prism 5 software. Data on respiration measurements, growth experiment, egg and larvae quality and starvation test were statistically treated using one-way ANOVA. If the variances

were normally distributed, the Tukey-Kramer multiple comparison test was applied for comparison of treatment means. When variances were not normally distributed, the Kruskal-Wallis nonparametric test and Dunn's multiple comparison test were applied. When two groups were compared, data were analyzed using Student's *t*-test.

3. Results

3.1. A preliminary study on physiology and reproduction of marbled rabbitfish (*S. rivilatus*)

The morphometric measurements of wild rabbitfish (N=55) captured between May and August 2009, revealed that TW and TL have a linear relationship, which is described by the equation y=0.068x+11.04 (Fig. 31). The condition factor was then relatively stable and the average value was 1.65±0.17. HSI was also relatively stable among different sizes of fish, having an average value of 1.4±0.4. Average DOP was found to be 73.7±3.7%. GSI of female fish was found to be high (>5) only during sampling few days before the full moon. However, in male fish, high GSI values were observed throughout the sampling period. The highest GSI values were 14 for male and 12.3 for female fish.

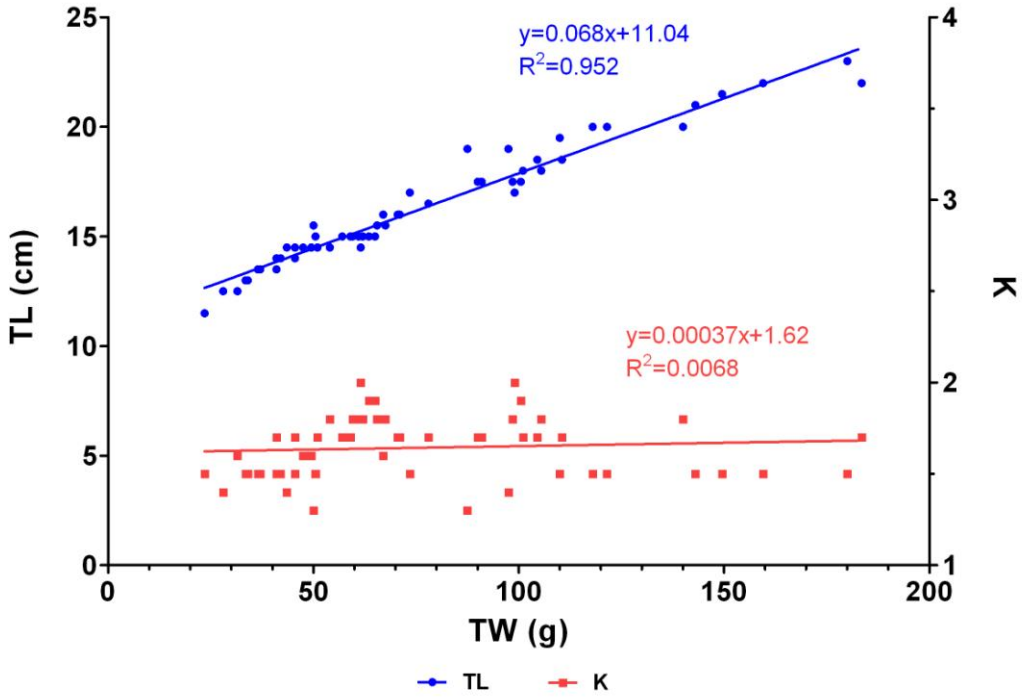

Figure 31. TW plotted against TL, producing a linear relationship. Condition factor remains relatively stable no matter the size of fish.

3.2. Effect of temperature on energy metabolism in marbled rabbitfish (*S. rivulatus*)

The oxygen consumption rate (M_{O_2}) measured at four different temperatures was plotted against oxygen concentration (Fig. 32). M_{O_2} was taken as the average value

out of the five measurements done at each water temperature. At all temperatures, M_{O_2} is decreasing as dissolved oxygen concentration in the water decreases. The relationship between the two variables is linear and it is described by the equations shown on the graph. M_{O_2} is higher at the higher temperature for any given oxygen concentration above 2 mg/l. Below this concentration fish were starting to lose their stability and probably the control of their senses.

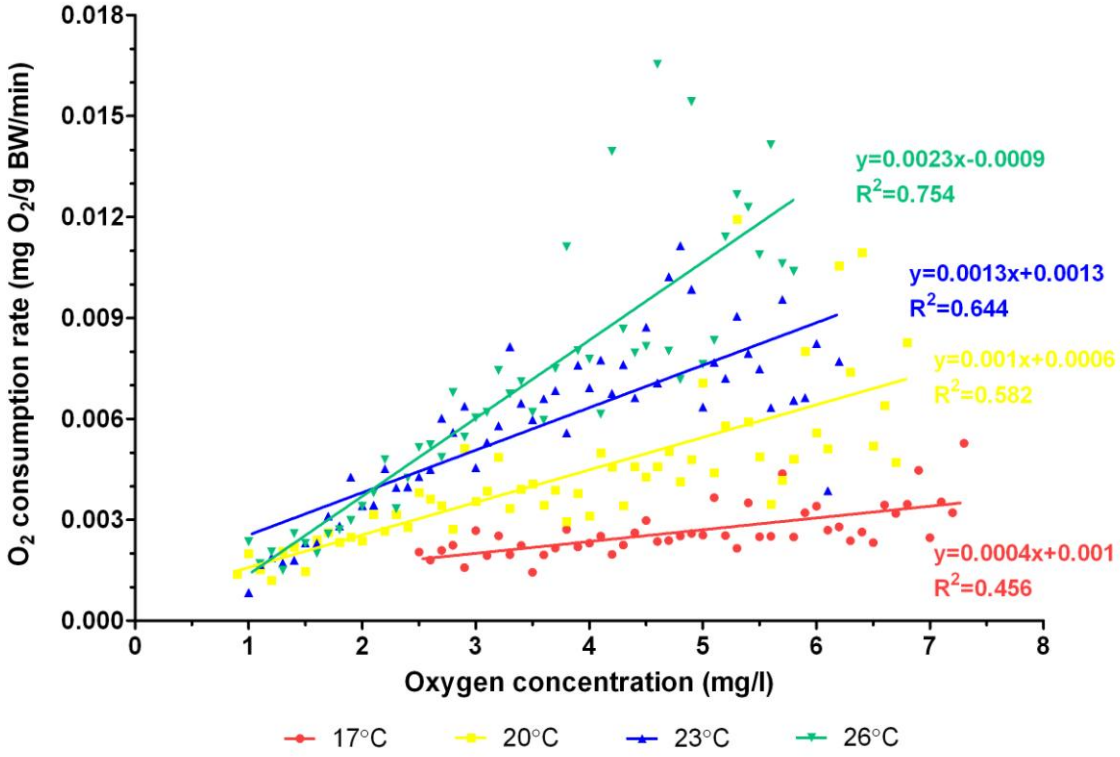

Figure 32. Oxygen consumption rate (MO_2) against oxygen concentration at four different temperatures. The equations describe the linear relationship between the two variables at the given temperatures.

After the observation that M_{O_2} changes with oxygen concentration, to compare the M_{O_2} at the four different temperatures, the same range of oxygen concentration was taken into account (4.6 – 6.5 mg/l). The mean values of M_{O_2} for the different temperatures (17, 20, 23 and 26 °C) were 2.4×10^{-3}, 4.0×10^{-3}, 6.2×10^{-3} and 1.1×10^{-2} mg O_2/g BW/min, respectively. M_{O_2} has an exponential relationship with temperature, which is described by the equation $y = 0.0001e^{0.167x}$ (Fig. 33). However, this relationship is valid for a range of temperatures, where fish can maintain a metabolic function. The low and high critical temperatures for rabbitfish must be below 17 °C and above 26 °C respectively.

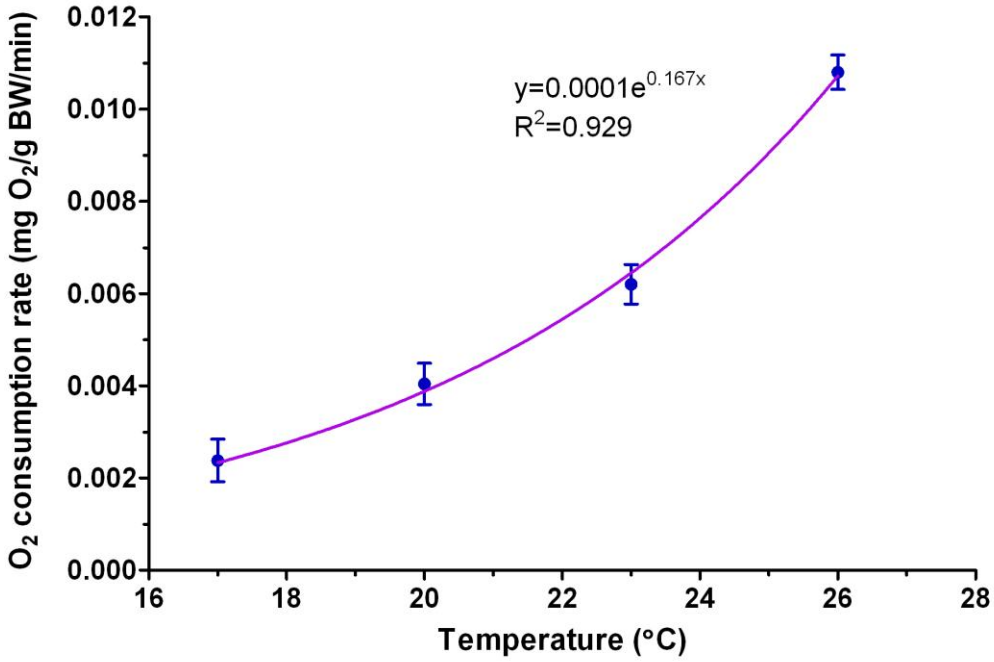

Figure 33. Exponential relationship between oxygen consumption rate (MO_2) and temperature. Temperature range is between 17 °C and 26 °C.

Identification of HIF-1a gene

Cloning and characterization of Siganus rivulatus HIF-1α cDNA

A BlastN search was performed (http://www.ncbi.nlm.nih.gov/BLAST/) on the complete, nonredundant Genbank nucleotide database for orthologues of HIF-1 α in other fish species. A multiple sequence nucleotide alignment was then carried out on coding sequences for HIF-1 α and a strategy based on regions of strong nucleotide conservation was used to design the primers (Fig. 34).

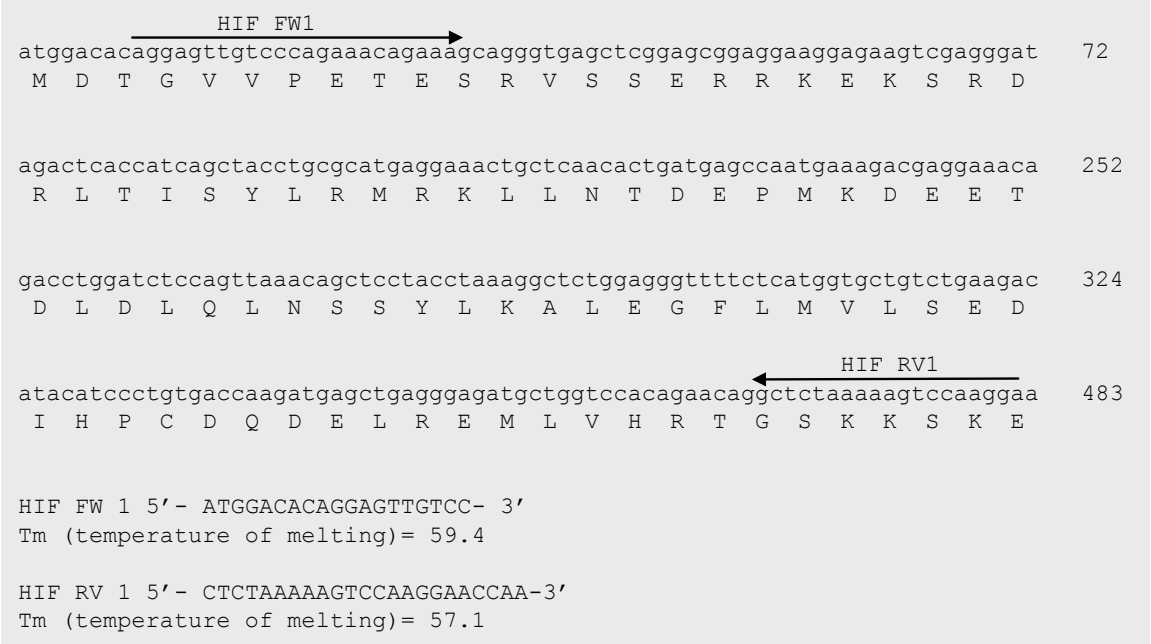

Figure 34. The nucleotide sequence of *Siganus rivulatus* HIF-1α (accession n° GU249151.1), with the deduced amino acids shown below the sequence in single-letter code. Nucleotides are numbered to the left. The locations of the primers used in PCR of the transcript are indicated by solid horizontal arrows. The 5'-3' primer sequences are indicated, too.

The cDNA fragment obtained using the aforementioned primers was subsequently cloned into the pGEM-T plasmid and characterized by nucleotide sequence analysis on both strands. Then, a partial coding sequence (~ 484 bp) for *Siganus rivulatus* HIF-1 α was determined that shared a high sequence identity with the HIF-1 α proteins of various vertebrate species.

Conceptual translation of the cDNA predicts a protein of 161 amino acids (aa) homologous to the HIF-1α s of other species as indicated by sequence analysis on the NCBI database. The sequence was deposited to the GenBank with accession number GU249151 and is presented in Figure 35.

•Siganus rivulatus hypoxia-inducible factor (HIF-1a) mRNA, partial cds

```
LOCUS       GU249151                484 bp    mRNA    linear   VRT 27-DEC-2009
DEFINITION  Siganus rivulatus hypoxia-inducible factor (HIF-1a) mRNA, partial
            cds.
ACCESSION   GU249151
VERSION     GU249151.1  GI:281484994
KEYWORDS    .
SOURCE      Siganus rivulatus (marbled spinefoot)
  ORGANISM  Siganus rivulatus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Actinopterygii; Neopterygii; Teleostei; Euteleostei; Neoteleostei;
            Acanthomorpha; Acanthopterygii; Percomorpha; Perciformes;
            Acanthuroidei; Siganidae; Siganus.
REFERENCE   1  (bases 1 to 484)
  AUTHORS   Rimoldi,S., Terova,G., Anastasiades,G. and Saroglia,M.
  TITLE     Molecular cloning of HIF-1a in rabbitfish (Siganus rivulatus)
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 484)
  AUTHORS   Rimoldi,S., Terova,G., Anastasiades,G. and Saroglia,M.
  TITLE     Direct Submission
  JOURNAL   Submitted (30-NOV-2009) Department of Biotechnology and Molecular
            Sciences, University of Insubria, Via Dunant, 3, Varese, VA 21100,
            Italy
FEATURES             Location/Qualifiers
     source          1..484
                     /organism="Siganus rivulatus"
                     /mol_type="mRNA"
                     /db_xref="taxon:216359"
     gene            1..>484
                     /gene="HIF-1a"
     CDS             1..>484
                     /gene="HIF-1a"
                     /codon_start=1
                     /product="hypoxia-inducible factor"
                     /protein_id="ADA70345.1"
                     /db_xref="GI:281484995"
                     /translation="MDTGVVPEKKRVSSERRKEKSRDAARCRRGKESEVFYQLAQELP
                     LPHSVSSSLDKASIMRLTISYLRMRKLLTPNEPMAKEQTDLDVQLNSSYLKALEGFLM
                     VLSEDGDMIYISENINKCLGLAQFDLTGHSVFDFIHPCDQEELREMLIHRTGSKKSKE
                     P"
ORIGIN
        1 atggatacag gagttgtccc agaaaagaaa agggtgagct cggagcgcag gaaggagaag
       61 tcaagagatg cagcgcgatg ccgccgtggg aaggagtcgg aggtcttcta ccagctggcc
      121 caggagctgc ccctgcccca cagcgtcagc tccagcctgg acaaagcctc catcatgagg
      181 ctcaccatca gctacctgcg catgaggaag ctgcttactc cgaatgaacc aatggcaaag
      241 gagcaaacag accttgacgt gcagcttaac agctcctacc tgaaggccct tgagggcttt
      301 cttatggtgc tgtctgaaga tggagatatg atctacatct ctgagaacat caacaagtgc
      361 ctgggcctgg cacaatttga tctgacggga cacagcgtgt ttgacttcat tcatccatgt
      421 gaccaggagg agctgaggga gatgttgatc cacagaacag gctctaaaaa gtccaaggaa
      481 ccaa
```

Figure 35. *Siganus rivulatus* hypoxia-inducible factor (HIF-1a) mRNA, partial cds and protein sequence deposited in GenBank database with the following accession number: GU249151).

ClustalW analysis

The % homologies for alignments of HIF-1α for different species, including the sizes of DNA and protein, are presented in Table 11. The alignment of the N termini of the amino acid sequence of *S. rivulatus* HIF-1α with that of sea bass (*Dicentrarchus labrax*) and other vertebrate species is shown in Fig. 36 and 37, respectively. *Siganus rivulatus* HIF-1α shares a remarkably high sequence similarity with the human HIF-1α protein in the bHLH (basic helix-loop-helix domain) (Fig. 37), in particular with the residues 17-74 (numbering according to human HIF-1α).

Table 11. Shared homologies (%) between HIF-1α coding sequences in different fish, avian and mammalian species

	Species	Coding sequence size (bp)	Protein size (aa)	Homology with *S. rivulatus* (%)
HIF-1α	*Siganus rivulatus*	483	161	-
	Dicentrarchus labrax	2265	755	87
	Micropogonias undulatus	2259	752	87
	Gymnocephalus cernuus	2247	748	82
	Perca fluviatilis	2250	749	82
	Stizostedion lucioperca	2250	749	82
	Pachycara brachycephalum	2243	747	80
	Zoarces viviparous	2244	747	80
	Platichthys flesus	2337	778	78
	Gasterosteus aculeatus	2271	756	76
	Oryzias melastigma	2247	748	72
	Onchorhynchus mykiss	2301	766	71
	Esox lucius	2292	763	70
	Thymallus thymallus	2238	745	70
	Danio rerio	2334	777	61
	Aspius aspius	2322	773	60
	Ctenopharyngodon idellus	2325	774	60
	Ictalurus punctatus	2331	776	59
	Carassius carassius	2331	776	59
	Acipenser gueldenstaedtii	2355	784	58
	Gymnocypris przewalskii	2322	773	58
	Gallus gallus	2436	811	58
	Xenopus laevis	2403	800	56
	Homo sapiens	2481	826	56
	Spalax judaei	2475	824	54
	Oryctolagus cuniculus	2460	819	55
	Mus musculus	2433	810	54
	Ratus norvegicus	2472	823	54
	Sus scrofa	2475	824	55
	Bos taurus	2472	823	55

```
S.rivulatus    ATGGATACAGGAGTTGTCCCAGAAA-AGAAA--AGGGTGAGCTCGGAGCGCAGGAAGGAG  57
               ||||| |||||||||||||||||| |||||  |||||||||||||||| |||||||||
Seabass        ATGGACACAGGAGTTGTCCCAGAAACAGAAAGCAGGGTGAGCTCGGAGCGGAGGAAGGAG  60

S.rivulatus    AAGTCAAGAGATGCAGCGCGATGCCGCCGTGGGAAGGAGTCGGAGGTCTTCTACCAGCTG  117
               ||||| || ||||| |||||||||||| || |||||||||||| ||||| || ||| |||||
Seabass        AAGTCGAGGGATGCGGCGCGATGCCGGCGCGGGAAGGAGTCAGAGGTGTTTTACGAGCTG  120

S.rivulatus    GCCCAGGAGCTGCCCCTGCCCCACAGCGTCAGCTCCAGCCTGGACAAAGCCTCCATCATG  177
               ||||||||||||||| |||||||||||| |||||||||||||||||||||| ||||| || |||
Seabass        GCCCAGGAGCTGCCACTGCCCCACAGTGTCAGCTCCAGCCTGGACAAGGCCTCAATAATG  180

S.rivulatus    AGGCTCACCATCAGCTACCTGCGCATGAGGAAGCTGCT-TACTCCGAATGAACCAATGGC  236
               || |||||||||||||||||||||||||||||| |||||  || | | |||| ||||| |
Seabass        AGACTCACCATCAGCTACCTGCGCATGAGGAAACTGCTCAACACTG-ATGAGCCAAT-G-  237

S.rivulatus    AAAG--GAGCAAACAGACCTTGA-CGTGCAGCTT-AACAGCTCCTACCTGAAGGCCCTTG  292
               ||||  ||| |||||||||| || | | ||| || |||||||||||||| ||||| || |
Seabass        AAAGACGAGGAAACAGACCTGGATC-TCCAG-TTAAACAGCTCCTACCTAAAGGCTCTGG  295

S.rivulatus    AGGGCTTTCTTATGGTGCTGTCTGAAGATGGAGATATGATCTA-CATCTCTGAGAACATC  351
               |||| ||||| |||||||||||||||| |||||||||||||| | |||| ||||| |||
Seabass        AGGGTTTTCTCATGGTGCTGTCTGAAGACGGAGATATGATCTATC-TCTCGGAGAATATC  354

S.rivulatus    AACAAGTGCC-TGGGCCTGGCACAATTTGATCTGACGGGACACAGCGTGTTTGACTTCAT  410
               |||||||||| |||| |||||||| ||||| ||||| |||||||| ||||||||||||||
Seabass        AACAAGTGCCTTGGG-CTGGCACAGTTTGACCTGACTGGACACAGTGTGTTTGACTTCAT  413

S.rivulatus    TCATCCATGTGACCAGGAGGAGCTGAGGGAGATGTTGATCCACAGAACAGGCTCTAAAAA  470
               ||||| |||||||| || |||||||||||||| || ||||||||||||||||||||||||
Seabass        ACATCCCTGTGACCAAGATGAGCTGAGGGAGATGCTGGTCCACAGAACAGGCTCTAAAAA  473

S.rivulatus    GTCCAAGGAACCAA  484
               ||||||||||||||
Seabass        GTCCAAGGAACCAA  487
```

Figure 36. Alignment of the partial cDNA sequence of *Siganus rivulatus* HIF-1α (GenBank accession n° GU249151) with that of another fish species, sea bass (D*icentrarchus labrax*) (GenBank accession n° DQ171936).

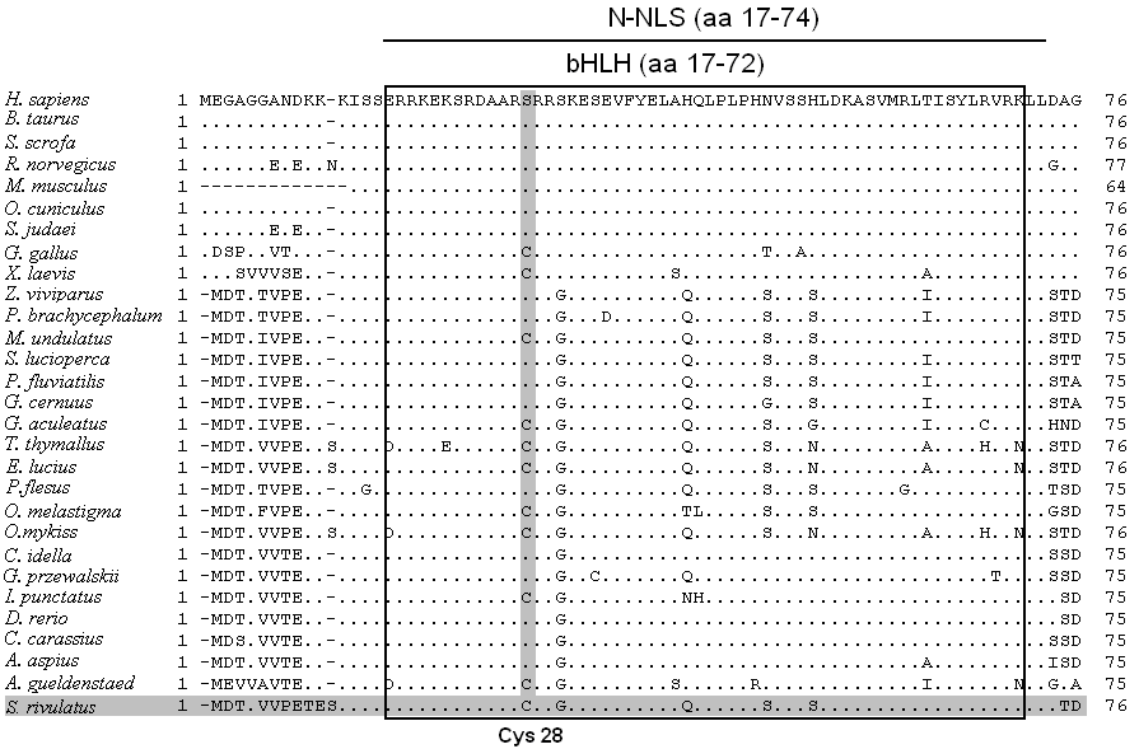

Figure 37. Alignment of the N-terminal part of HIF-1α amino acid sequence of *S. rivulatus* with all thus far available HIF-1α sequences of fish and other vertebrates species. Amino acids are designated by single-letter codes. The N-terminal nuclear localization signal (N-NLS) residues 17-74 (numbering according to human HIF-1α) is indicated with a solid line, and the basic helix-loop-helix (bHLH) domain is indicated inside a box. The aminoacids that aligns with human position 28 (Ser) are shadowed like the *S. rivulatus* sequence. The sequences shown are available from the NCBI GenBank database.

3.3. Effect of nutritional protein level on growth performance of marbled rabbitfish (*S. rivulatus*)

During the growth experiment using two different diets, temperature fluctuated as shown in Figure 38.

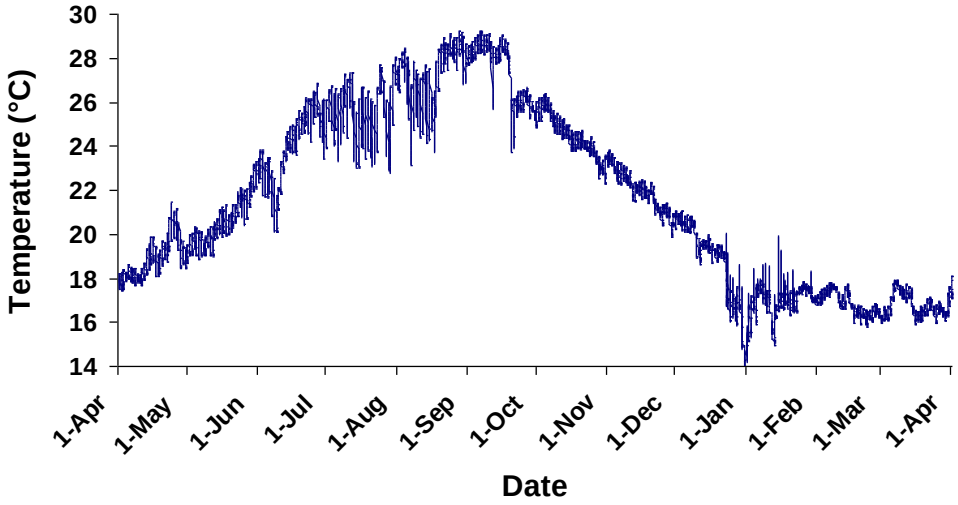

Figure 38. Temperature fluctuation during experimental period.

In both treatments, the initial mean weight of the fish in each tank was 32±1.9 g/fish. After one year of feeding, the final mean weights of the fish were 118.6±3.8 g/fish for Treatment A and 123.3±15 g/fish for Treatment B (TWs were calculated out of the whole population) (Fig. 39). It was observed that fish stopped getting any weight when temperature dropped below 18 °C. During that period SGRs were -0.01% g BW.d^{-1} for both treatments. For the rest of the period (when temperature was above 18 °C), SGRs were 0.63 and 0.66 % gBW.d^{-1} respectively. Both treatments had similar growth pattern and there was not significance difference between them.

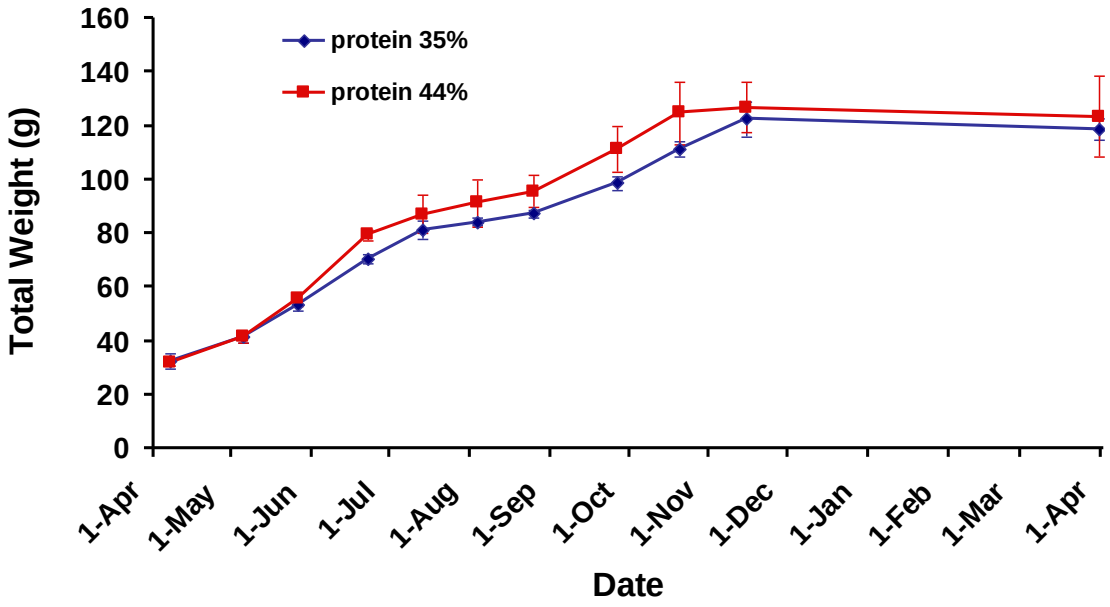

Figure 39. Annual growth curves for *S. rivulatus* using two different diets.

HSI was significantly different in the two treatments, with Treatment A to have a mean HSI of 2.5±0.8 and Treatment B of 3.0±0.9. Both treatments were also significant different in HSI with the wild fish (paragraph 3.1), which had a mean value of 1.4±0.4 (Fig. 40).

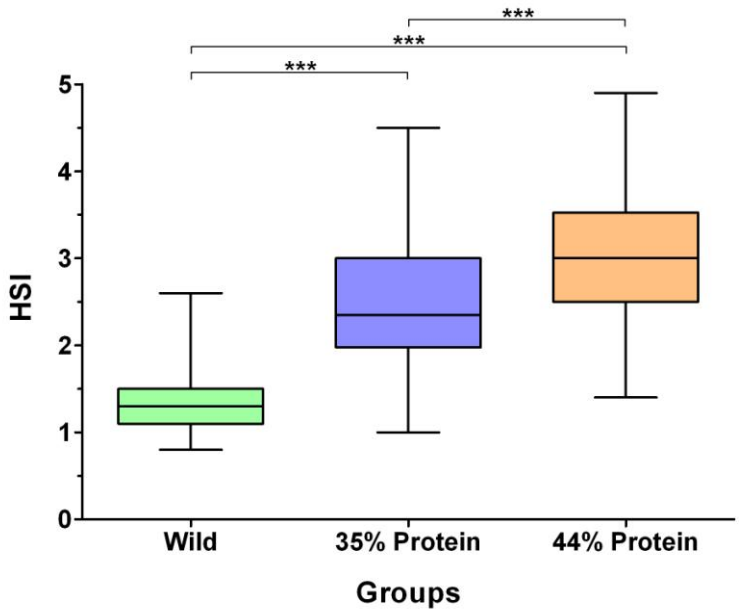

Figure 40. HSI in the wild fish (paragraph 3.1) and in group A (35% protein) and B (44% protein). Box shows the 25 and 75 percentile and the line in the middle of each box shows the median value.

Mean DOPs in groups A and B were 79.6% and 78.0% respectively. Between groups A and B there was not significance difference, but both groups were significant different with wild fish, which had a mean DOP of 73.7% (Fig. 41).

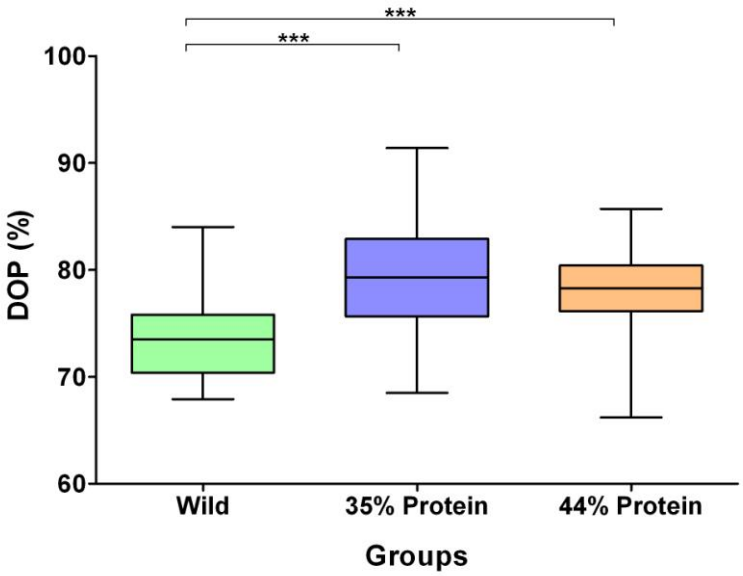

Figure 41. DOP in the wild fish (paragraph 3.1) and in group A (35% protein) and B (44% protein). Box shows the 25 and 75 percentile and the line in the middle of each box shows the median value.

The GSI in groups A and B showed the same pattern like the wild fish (paragraph 3.1), with the female fish to attain high GSI before the full moon from May until August and the male fish to sustain a high GSI throughout this period. The highest GSI values for groups A and B were 20.2 and 15.8 respectively for males, and 14.7 and 12.2 respectively for females.

3.4. Effect of vitamin C and astaxanthin supplementation in broodstock diet on reproductive performance, and egg and larval quality in common pandora (*P. erythrinus*)

During the experimental period temperature fluctuated as shown in Figure 42. All groups of broodstock spawned naturally between 4th of April and 21st of June 2008.

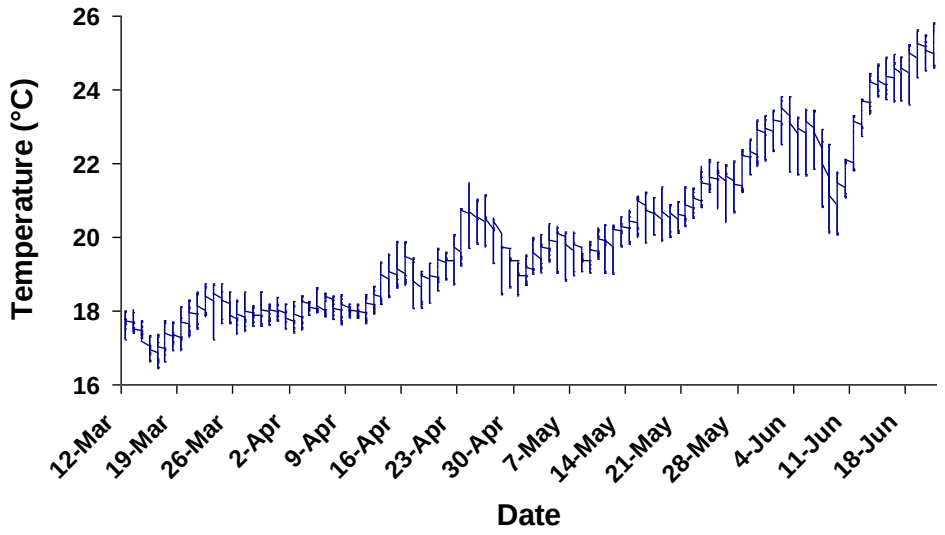

Figure 42. Temperature fluctuation during experimental period.

They spawned isolated pelagic eggs, which had spherical shape and a single oil droplet. The average diameter of eggs and oil droplets and the hatching rate in each group are shown in Table 12.

Table 12. Average values (±SD) for egg diameter, oil droplet and hatching rate in the three groups

	Group A	**Group B**	**Group C**
Egg diameter (μm)	734±25[a]	840±18[b]	784±16[c]
Oil droplet diameter (μm)	186±3[a]	205±4[b]	188±3[a]
Hatching rate (%)	50±8[a]	95±4[b]	85±5[b]

Group B produced the larger eggs in respect to egg and oil droplet diameter, having a significance difference with groups A and C (Fig. 43 & 44). Hatching rate was significantly higher in groups B and C compared to group A.

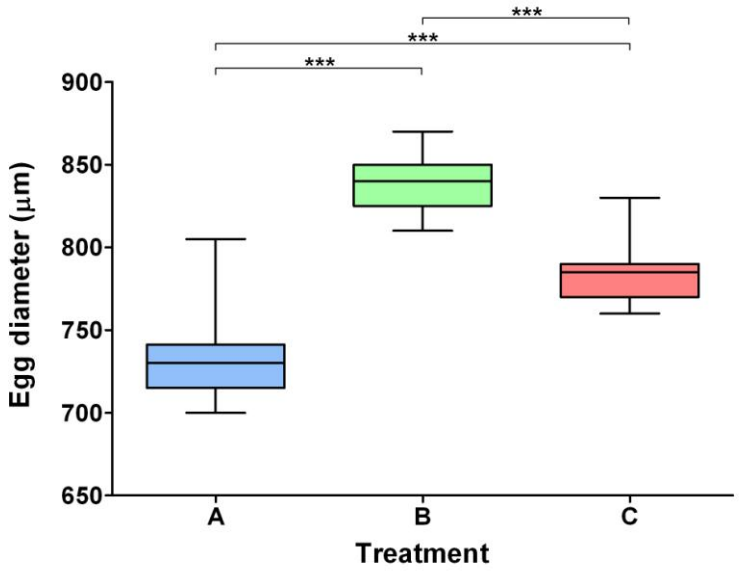

Figure 43. Egg diameter in the three treatments. Box shows the 25 and 75 percentile and the line in the middle of each box shows the median value.

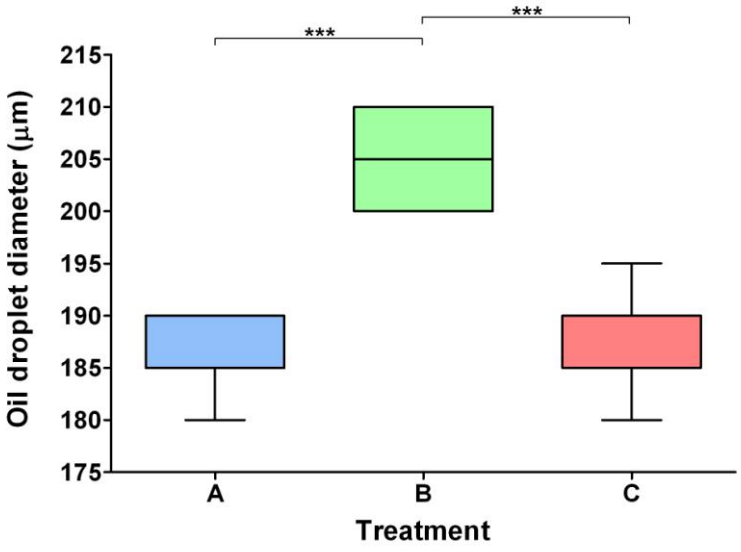

Figure 44. Oil droplet diameter in the three treatments. Box shows the 25 and 75 percentile and the line in the middle of each box shows the median value.

During the starvation test, survival arrived to 0% at 6 DAH for all groups. However, the SAI of group B was significantly higher than that of groups A and C. Mean survival throughout the starvation tests is shown in Figure 45.

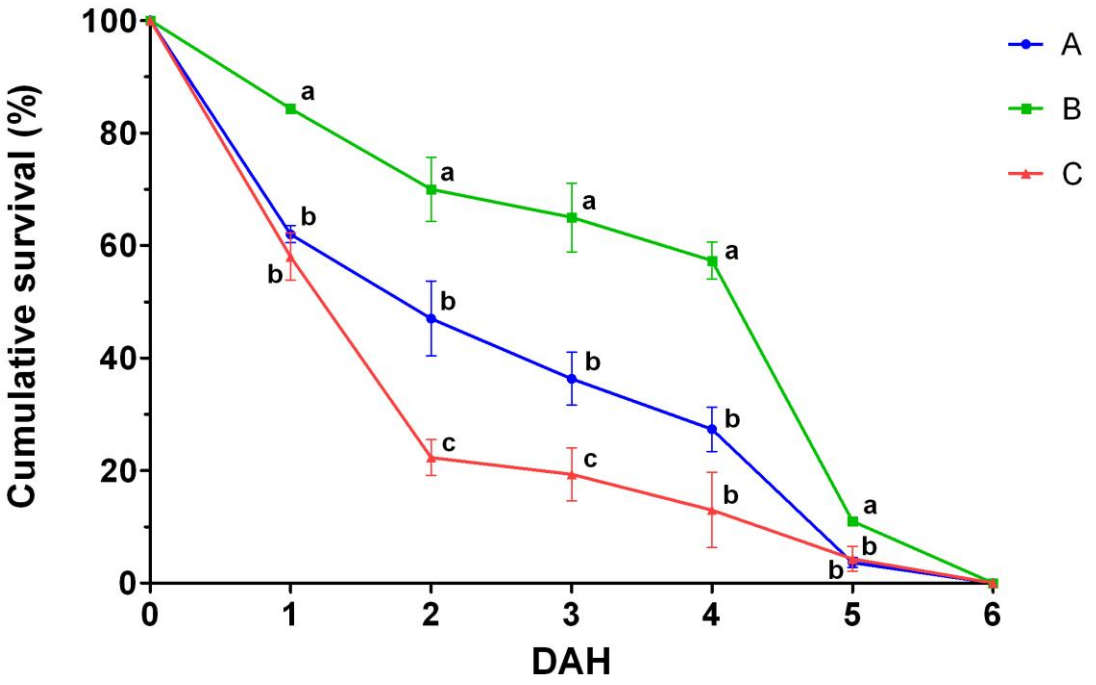

Figure 45. Cumulative survival of larvae during starvation test.

Identification of SVCT gene

Cloning and characterization of Pagellus erythrinus SVTC cDNA.

A BlastN search was performed (http://www.ncbi.nlm.nih.gov/BLAST/) on the complete, nonredundant Genbank nucleotide database for orthologues of SVTC in other fish species. A multiple sequence nucleotide alignment was then carried out on coding sequences for SVTC and a strategy based on regions of strong nucleotide conservation was used to design the primers (Fig. 46, Table 13).

```
Homo              ATGAGGGCCCAAGAGGACCTCGAGGGCCGGGCACAGCATGAAA----------------- 43
Rattus            ATGAAAGCTCAGGAGGACCCCGGGAGCTCAAAGCAGCATGAATGCCCAGATTCAGCAGGG 60
Dicentrarchus     ------------------------------------------------------------


Homo              ----CCACCAGGGACCCCTCGACCCCGCTACCCACAGAGCCTAAGTTTGACATGTTGTAC 99
Rattus            ACTTCCACCAGGGACCAGCAGGCACCTTTGCCCGCGGAGCCCAAGTTTGACATGTTGTAC 120
Dicentrarchus     ------------------------------------------------------------


Homo              AAGATCGAGGACGTGCCACCTTGGTACCTGTGCATCCTGCTGGGCTTCCAGCACT-ACCT 158
Rattus            AAGATTGAGGACGTGCCACCATGGTACCTGTGTATCCTGCTGGGCTTCCAGCATT-ACCT 179
Dicentrarchus     ------------------------------------------GGGCTGCAGCATTTACCT 18
                                                             **  * ***** * ****


Homo              GACATGCTTCAGTGGTACCATCGCCGTGCCCTTCCTGCTGGCTGAGGCGCTGTGTGTGGG 218
Rattus            GACATGCTTCAGTGGTACCATTGCTGTGCCCTTCCTCCTGGCTGAGGCGCTGTGTGTGGG 239
Dicentrarchus     GACTTGTTTCAGCGGCACTGTAGCAGTGCCATTTCTTCTGGCTGAGGCCATGTGTATCGG 78
                  *** ** ***** ** **  * ** ***** ** ** ***********  ***** * **


Homo              CCACGACCAGCACATGGTTAGTCAGCTCATCGGCACCATCTTCACGTGCGTGGGCATCAC 278
Rattus            CCGCGACCAGCACATGATCAGTCAGCTCATTGGTACCATCTTCACCTGCGTGGGTATCAC 299
Dicentrarchus     TCGAGACCAGAACACCATCAGCCAGCTGATTGGAACCATTTTCACCACCGTCGGACTTAC 138
                   *  ****** ***   * ** ***** ** ** ***** *****   *** **  * **


Homo              CACTCTCATCCAGACCACCGTGGGCATCCGGCTGCCGCTGTTCCAGGCCAGTGCCTTTGC 338
Rattus            CACTCTCATTCAGACTACAGTGGGCATCCGGCTGCCGCTGTTCCAGGCCAGTGCCTTTGC 359
Dicentrarchus     AACCCTGATCCAGACCACCGTGGGAATCAGACTTCCATTGTTTCAGGCCAGTGCATTTGC 198
                   ** ** ** ***** ** ***** *** * ** **  **** *********** *****


Homo              ATTTCTGGTTCCAGCCAAAGCCATACTGGCTCTGGAGAGATGGAAATGCCCCCCGGAAGA 398
Rattus            GTTTCTGGTTCCAGCCAAGGCTATCCTGGCCTTGGAGAGGTGGAAGTGTCCTCCAGAAGA 419
Dicentrarchus     TTTTCTGATTCCTGCACAAGCCATCCTCAGTCTGGACCGCTGGAGGTGTCCCAGTGAAGA 258
                   ****** **** **  * ** ** **     ****  * ****  ** **    *****


Homo              GGAGATCTACGGTAACTGGAGTCTGCCCCTGAACACCTCTCATATTTGGCACCCACGGAT 458
Rattus            GGAGATCTACGGTAACTGGAGTATGCCCCTGAACACCTCTCATATCTGGCATCCTCGGAT 479
Dicentrarchus     GGAGATTTATGGGAACTGGAGTCTTCCACTCAACACCTCACACATCTGGCAGCCACGCAT 318
                  ****** ** ** ********* * ** ** ******** ** ** ***** ** ** **


Homo              ACGGGAGGTCCAGGGTGCAATCATGGTGTCCAGCGTGGTGGAGGTGGTGATTGGCCTGCT 518
Rattus            TCGGGAGGTCCAGGGTGCAATCATGGTGTCCAGCGTGGTAGAGGTGGTGATTGGGCTGTT 539
Dicentrarchus     CAGAGAGATCCAGGGTGCCATCATCATGTCCAGCATTGTGGAGGTTGTGATCGGTCTGTG 378
                    * *** ********** *****  ******** * ** ***** ***** ** ***


Homo              GGGGCTGCCTGGGGCCCTGCTCAACTACATTGGGCCTCTCACAGTCACCCCCACTGTCTC 578
Rattus            GGGGCTGCCTGGGGCCCTGCTCAGCTACATTGGACCTCTCACAGTCACCCCCACTGTCTC 599
Dicentrarchus     TGGGTTGCCAGGTCTGCTGCTGGACTACATCGGTCCACTCACTGTCACTCCAACTGTCTC 438
                   *** **** **    *****   ****** ** ** ***** ***** ** ********


Homo              CCTCATTGGCCTTTCTGTCTTCCAAGCTGCTGGCGACCGAGCTGGCTCCCACTGGGGCAT 638
Rattus            CCTTATCGGTCTCTCTGTTTTCCAAGCTGCTGGCGACCGAGCTGGCTCCCATTGGGGCAT 659
Dicentrarchus     ACTGATTGGCCTGTCGGTCTTCACCACAGCTGGAGACAGAGCTGGGTCTCACTGGGGCCT 498
                   ** ** ** ** ** ** ***    * ***** *** ******* ** ** ****** *


Homo              CTCAGCTTGCTCCATTCTCCTGATCATCCTCTTCTCCCAGTACCTGCG--CAACCTCACC 696
Rattus            TTCGGCTTGCTCCATTCTACTGATCGTCCTGTTCTCCCAGTATCTACG--CAACCTCACC 717
Dicentrarchus     GTCAACATTGTGTATTCTGCTGATCGCGCTGTTTGCTCAGTACCTAAGAGCGACATCAC- 557
                   **  * *  *  ***** ******   ** **  * ***** **  *  * ** ****


Homo              TTCCTGCTGCCTGTCTACCGCTGGGGCAAGGGCCTCACTCTCCTCCGCATCCAGATCTTC 756
Rattus            TTCCTGTTGCCTGTTTACCGATGGGGCAAGGGTCTTACTCTCTTCCGCATCCAGATCTTT 777
Dicentrarchus     TTCCTGTT-CCTGTTTACAGCAGGAAGAAAGGCCTGACCTCCACCAGAGTCCAGATCTTT 616
                  ****** * ***** *** *  **   ** ** ** **   *  * *  **********


Homo              AAAATGTTTCCTATCATGCTGGCCATCATGACCGTGTGGCTGCTCTGCTATGTCCTGACC 816
```

```
Rattus          AAGATGTTTCCGATCGTGCTGGCCATCATGACCGTGTGGCTACTCTGCTATGTGCTGACT 837
Dicentrarchus   AAAACGTTTCCTATCATTCTGGCCATCATGTTTGTTTGGCTCGTTTGTTACATTCTCACT 676
                ** * ****** *** * ************   ** *****  * ** **  * ** **

Homo            TTGACAGACGTGCTGCCCACAGACCCAAAAGCCTATGGCTTCCAGGCACGAACCGATGCC 876
Rattus          CTGACAGACGTGCTGCCCGCAGATCCCACAGTCTACGGTTTCCAGGCTCGAACTGATGCC 897
Dicentrarchus   CTGACCAACCTGTTGCCGAGCGACCCCAGTCGCTACGGACACAAGGCCCGGACCGATGCC 736
                 ****  ** ** ****    ** ** *    *** **   * **** ** ** ******

Homo            CGTGGTGACATCATGGCTATTGCACCCTGGATCCGCATCCCCTACCCCTGTCAGTGGGGC 936
Rattus          CGAGGGGACATCATGGCTATCTCTCCCTGGATCCGGATCCCCTACCCATGTCAATGGGGC 957
Dicentrarchus   CGTGGGGACATCATGGCTTCATCACCCTGGTTCAGAGTCCCCTATCCTTGCCAGTGGGGG 796
                ** ** ************    * ****** ** *  ******* ** ** ** *****

Homo            CTGCCCACGGTGACTGCGGCTGCTGTCCTGGGAATGTTCAGCGCCACTCTGGCAGGCATC 996
Rattus          CTACCCACAGTGACCGTGGCTGCAGTTCTGGGAATGTTCAGCGCCACACTGGCGGGCATC 1017
Dicentrarchus   TTGCCAGTGGTAACAGTTGCCGGGACGCTGGGGATGCTGAGTGCCACCATGGCGGGCATC 856
                 * **    ** ** *  ** *     ***** *** * ** *****  **** ******

Homo            ATTGAGTCCATCGGAGATTACTACGCCTGTGCCCGCCTGGCTGGTGCACCACCCCCTCCA 1056
Rattus          ATCGAGTCCATCGGTGATTACTATGCCTGCGCCCGGTTGGCTGGAGCACCACCCCCTCCA 1077
Dicentrarchus   GTGGAGTCAATTGGTGATTATTATGCCTGTGCTCGTCTGTCTGGAGCCACACCGCCTCCA 916
                 * ***** ** ** ***** ** ***** ** **  ** **** **  **** ******

Homo            GTACATGCTATCAACAGGGGCATCTTCACCGAAGGCATTTGCTGCATCATCGCGGGGCTA 1116
Rattus          GTCCATGCTATCAACAGGGGGATTTTCACCGAAGGCGTCTGCTGCATCATCGCTGGGCTA 1137
Dicentrarchus   GTCCATGCCATCAACAGGGGCATCTTCAC------------------------------- 945
                ** ***** *********** ** *****

Homo            TTGGGCACGGGCAACGGGTCCACCTCGTCCAGTCCCAACATTGGCGTCCTGGGAATTACC 1176
Rattus          CTGGGCACAGGCAACGGGTCCACCTCTTCCAGCCCCAACATCGGGGTCCTAGGGATTACC 1197
Dicentrarchus   ------------------------------------------------------------

Homo            AAGGTGGGCAGCCGGCGCGTGGTGCAGTATGGTGCGGCTATCATGCTGGTCCTGGGCACC 1236
Rattus          AAGGTGGGCAGCCGAAGAGTCGTGCAGTATGGTGCAGGTATCATGCTAATCCTGGGGGCC 1257
Dicentrarchus   ------------------------------------------------------------

Homo            ATCGGCAAGTTCACGGCCCTCTTCGCCTCGCTCCCTGACCCCATCCTGGGGGGCATGTTC 1296
Rattus          ATTGGCAAATTCACAGCTCTCTTCGCCTCACTGCCGGACCCCATCCTGGGAGGGATGTTC 1317
Dicentrarchus   ------------------------------------------------------------

Homo            TGCACTCTCTTTGGCATGATTACAGCTGTGGGGCTGTCCAACCTGCAATTTGTGGACATG 1356
Rattus          TGCACTCTTTTCGGTATGATCACCGCTGTGGGACTGTCCAATCTGCAGTTTGTGGACATG 1377
Dicentrarchus   ------------------------------------------------------------

Homo            AACTCCTCTCGCAACCTCTTCGTGCTGGGATTTTCCATGTTCTTCGGGCTCACGCTGCCC 1416
Rattus          AACTCCTCCCGCAACCTCTTTGTATTGGGATTCTCCATGTTCTTCGGCCTCACGCTACCC 1437
Dicentrarchus   ------------------------------------------------------------

Homo            AATTACCTGGAGTCCAACCCTGGCGCCATCAATACAGGCATTCTTGAAGTGGATCAGATT 1476
Rattus          AACTACCTGGATTCCAACCCAGGTGCCATCAACACAGGCGTTCCTGAAGTGGATCAGATC 1497
Dicentrarchus   ------------------------------------------------------------

Homo            CTGATTGTGCTGCTGACCACGGAGATGTTTGTGGGCGGGTGCCTTGCTTTCATACTTGAC 1536
Rattus          CTAACTGTGCTGCTGACCACAGAGATGTTTGTTGGTGGCTGTCTTGCTTTCATACTGGAC 1557
Dicentrarchus   ------------------------------------------------------------

Homo            AACACAGTGCCAGGGAGCCCAGAGGAGCGTGGTCTGATACAGTGGAAAGCTGGGGCTCAT 1596
Rattus          AACACAGTACCAGGGAGCCCAGAGGAAAGAGGTCTGATACAGTGGAAAGCCGGGGCACAC 1617
Dicentrarchus   ------------------------------------------------------------
```

```
Homo              GCCAACAGTGACATGTCTTCCAGCCTCAAGAGCTACGATTTCCCCATTGGGATGGGCATA 1656
Rattus            GCCAACAGTGAGACGCTGGCCAGTCTCAAGAGCTACGATTTCCCGTTCGGGATGGGCATG 1677
Dicentrarchus     ------------------------------------------------------------

Homo              GTAAAAAGAATTACCTTTCTGAAATACATTCCTATCTGCCCAGTCTTCAAAGGATTTTCT 1716
Rattus            GTAAAAAGGACCACCTTTTTTAGATACATCCCCATCTGCCCAGTCTTCAGAGGATTCTCT 1737
Dicentrarchus     ------------------------------------------------------------

Homo              TCAAGTTCAAAAGATCAGATTGCAATTCCAGAAGACACTCCAGAAAATACAGAAACTGCA 1776
Rattus            AAGA---CAGAAAATCAGCCTGCAGTTCTAGAAGACGCTCCAGACAACACAGAAACTGGG 1794
Dicentrarchus     ------------------------------------------------------------

Homo              TCTGTGTGCACCAAGGTCTGA 1797
Rattus            TCTGTGTGTACCAAGGTCTGA 1815
Dicentrarchus     ---------------------
```

Figure 46. Alignment of the cDNA sequences of Sodium dependent Vitamin C Transporter of *Homo sapiens* (GenBank accession n° AF170911) and *Rattus norvegicus* SVTC (GenBank accession n° AF080452) and sea bass (D*icentrarchus labrax*) (GenBank accession n° AJ965488).

Table 13. Primer sequences used for the molecular cloning of Sodium dependent Vitamin C transporter (SVTC) in *P. erythrinus*

Primers	**Sequence 5' – 3'**	**Purpose**
SVCT1rat- fw1	GAGCCCAAGTTTGACATGTTGTA	RT-PCR
SVCT1hs- fw1	ATGAGGGCCCAAGAGGAC	RT-PCR
SVCT1dl- fw1	GGGCTGCAGCATTTACCT	RT-PCR
SVCT1dl-rev2	ATCAACAGGGGCATCTTCAC	RT-PCR
SVCT1dl-rev2	TCTGTGTGCACCAAGGTCTGA	RT-PCR
b-Actin FW	GAAGATGAAATCGCCGCACT	RT-PCR
b-Actin Rv	TTAGAAGCATTTGCGGTGCA	RT-PCR

The cDNA fragment obtained using the aforementioned primers was subsequently cloned into the pGEM-T plasmid and characterized by nucleotide sequence analysis on both strands. Then, a partial coding sequence (~ 772 bp) for *Pagellus erythrinus* SVTC was determined that shared a high sequence identity with the SVTC proteins of other vertebrate species.

Conceptual translation of the cDNA predicts a protein of 257 amino acids (aa) homologous to the SVTC of other species as indicated by sequence analysis on the NCBI database. The sequence was deposited to the GenBank with accession number EU883994 and is presented in Figure 47.

•**GenBank: EU883994.1**

```
LOCUS       EU883994                772 bp    mRNA    linear   VRT 20-AUG-2008
DEFINITION  Pagellus erythrinus sodium-dependent vitamin C transporter mRNA,
            partial cds.
ACCESSION   EU883994
VERSION     EU883994.1  GI:196167574
KEYWORDS    .
SOURCE      Pagellus erythrinus (common pandora)
  ORGANISM  Pagellus erythrinus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Actinopterygii; Neopterygii; Teleostei; Euteleostei; Neoteleostei;
            Acanthomorpha; Acanthopterygii; Percomorpha; Perciformes;
            Percoidei; Sparidae; Pagellus.
REFERENCE   1  (bases 1 to 772)
  AUTHORS   Rimoldi,S., Terova,G., Cora,S., Bernardini,G., Gornati,R. and
            Saroglia,M.
  TITLE     Clonning and sequencing of sodium-dependent vitamin C transporter 1
            (SVCT1) in common pandora (Pagellus erythrinus)
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 772)
  AUTHORS   Rimoldi,S., Terova,G., Cora,S., Bernardini,G., Gornati,R. and
            Saroglia,M.
  TITLE     Direct Submission
  JOURNAL   Submitted (14-JUL-2008) Biotechnology and Molecular Sciences,
            University of Insubria, Via Dunant,3, Varese, VA 21100, Italy
FEATURES             Location/Qualifiers
     source          1..772
                     /organism="Pagellus erythrinus"
                     /mol_type="mRNA"
                     /db_xref="taxon:50593"
     CDS             <1..>772
                     /note="SVCT1"
                     /codon_start=3
                     /product="sodium-dependent vitamin C transporter"
                     /protein_id="ACG71107.1"
                     /db_xref="GI:196167575"
                     /translation="LFQASAFAFLVPAQAILSLDRWKCPSEEEIYGNWSLPLNTSHIW
                     QPRIREIQGAIIISSVVELVIGLCGLPGLLLEYIGPLTITPTVSLIGLSVFKTAGDRA
                     GSHWGLSALCIFFILLFAQYLRSTSVPVPFYSRKKGLTTTRVQIFKMFPIILAILLVW
                     LVCYIFTLTNLLPTDPNYYGHKARTDARGDIIASAPWFRVPYPCQWGLPVITVAGVLG
                     MLSAIMAGIVESIGDYYACARLSGATPPPIHAINRGIFT"
ORIGIN
        1 cattgtttca ggccagtgca tttgctttcc tggttcctgc acaagccatc ctcagtctgg
       61 accgctggaa gtgtcccagc gaggaggaga tttatgggaa ctggagtctt ccactcaaca
      121 cctcgcacat ctggcagcca cgcatcagag agatccaggg ggccatcatc atttccagtg
      181 ttgtggagct tgtgattggt ctgtgtgggt tgccaggttt gctgttggag tacattggcc
      241 cgctcaccat cactccaact gtctcactga tcggcttgtc tgtttttaaa acagctggag
      301 acagagccgg gtcccactgg ggcctgtcag cactgtgtat tttctttatc ttgctatttg
      361 ctcagtacct gagatcgaca tcagttcctg ttccttttta cagcagaaag aaaggactga
      421 ccaccaccag agtgcaaatc ttcaagatgt tccctatcat ccttgccatc ctgttggttt
      481 ggcttgtttg ctacatcttc actctgacca acctgttgcc gaccgacccc aattattacg
      541 gacacaaggc ccggaccgat gcccgcggtg acatcattgc ttcagcaccc tggttcagag
      601 tcccctaccc ctgccagtgg gggttgccgg tgataacggt tgccggggtt ctgggaatgc
      661 tgagtgcaat catggcaggt attgtggagt ccattggtga ttattacgcc tgcgctcgtt
      721 tgtctggagc cacgcccccc ccgatccatg ccatcaacag gggcatcttc ac
```

Figure 47. *Pagellus erythrinus* sodium-dependent vitamin C transporter (SVTC) mRNA, and protein sequence deposited in GenBank database with the following accession number: EU883994.

ClustalW analysis

The % homologies for alignments of SVTC for different species, including the sizes of DNA and protein, are presented in Tab.2. As shown, *Pagellus erythrinus* SVTC shares a remarkably high sequence similarity with the human, rat and sea bass SVTC protein (Fig. 46, Table 14)

Table 14. Shared homologies (%) between SVTC coding sequences in different fish and mammalian species

	Species	**Coding sequence size (bp)**	**Protein size (aa)**	**Homology with *P. erythrinus* (%)**
SVTC	*Pagellus erythrinus*	772	257	-
	Dicentrarchus labrax	945	315	88
	Homo sapiens	2170	598	72
	Ratus norvegicus	2472	604	54

4. Discussion

The observation of high GSI values from May until August and peaks in GSI for females to be just before full moon, indicates that spawning is synchronized with the lunar cycle and takes place three or four times a year. This is in contrast with the findings of Yeldan &Avsar (2000), who observed only two peaks in GSI between July and August. Also, Zaki *et al.* (1994) determined the spawning period of *S. rivulatus* from early June to July after histological examination of the gonads coming from wild specimens. These differences may be due to latitudinal and/or genetical differences, which are important in determining the duration of the spawning season. Moreover, the observation of high GSI values in fish, which are small in size, can be explained by the findings of Ghanawi *et al.* (2010), who reported the existence of slow-growing rabbitfish that never reach large body size.

The high HSI in cultured fish in relation to the wild specimens is due to the diet of the fish. Among other functions, liver in fish serves as a storage area for fats and carbohydrates. Therefore, HSI was significantly correlated with the increase in the lipid content of the feeds. As already mentioned before, wild rabbitfish are mainly fed with algae and sea grasses, which have very low lipid content. The correlation between lipid level in feed and HSI has been also documented in farmed sea bass and sea bream (Yildiz *et al.*, 2007; Bonaldo *et al.*, 2010).

The similar growth in fish fed either with 35% or 44% protein content feed gives an indication about the nutritional protein requirements of *S. rivulatus*. Although the experimental feeds were not isoenergetic, the low protein and lipid content in treatment A performed as well as the high protein and lipid content in group B. These results are supported by the findings of Kissil *et al.* (2002), who concluded that feed containing 35% protein and 7% lipid was the most cost-effective diet for fattening of *S. rivulatus*. Recent experimental studies from Saoud *et al.* (2010) showed that optimal dietary protein requirement for juvenile marbled spinefoot rabbitfish is between 35% and 40% crude protein with feeding frequency of three times per day at 4% body weight, although good growth was obtained also with a 30% crude protein diet when fish were fed ad libitum. Using a 40% protein diet, it was found that an 8% lipid diet resulted in best growth of *S. rivulatus* juveniles. A low nutritional protein requirement was reported also for other species of the *Siganidae* family. Parazo (1990) stated that best growth rate and efficiency of protein utilization in *S. guttatus* were obtained using a diet with 35% protein and 3882 Kcal/Kg energy. In *S. canaliculatus*, best growth and feed efficiency was observed when fish were fed a dry crumble containing 31% crude protein (Tacon *et al.*, 1990).

The growth rates obtained in this study were 0.43 and 0.45 $g.d^{-1}$ (SGRs were 0.63 and 0.66 $\%gBW.d^{-1}$) for treatments A and B, respectively. These growth rates were similar to the ones obtained in the study of Kissil *et al.* (2002), which were 0.43 and 0.46

$g.d^{-1}$ for the diets with 35 and 45% protein, respectively. The pause in growth that was observed when temperature dropped below 18°C constitutes a disadvantage in the grow-out phase of this species. Saoud *et al.* (2008a) reported that marbled spinefoot rabbitfish actively consumed feed until the temperature reached 16°C, but both fish activity and feeding frenzy slowed down. At 14°C, feeding stopped completely. Moreover, the optimum temperature for growth was found to be at 27°C. The temperature range for living, feeding and optimum growth in rabbitfish constitutes a restriction to the development of its farming in all the longitudinal and latitudinal area of Mediterranean Sea. In east Mediterranean, the effects of low temperatures are limited, because seawater temperatures drop below 18°C for a period of about three months. In addition, the high seawater temperatures (25-30°C) retained for fout to five months and the small marketable size of marbled spinefoot rabbitfish, compensate the pause in growth during winter months.

The two diets tested in the growth experiment with *S. rivulatus* were based on fish meal and fish oil. However, it is worth mentioning again that the natural diet of marbled spinefoot rabbitfish, which is based on algae and sea grasses, gives the potential to successful replacement of fish meal and fish oil with proteins and lipids of vegetable origin. Therefore, further studies should be done towards this direction, as successful fattening of *S. rivulatus* with vegetable sources of protein and lipids will represent a strong advantage against other candidate species for diversification of aquaculture with carnivorous feeding habits.

The results on oxygen consumption rate (M_{O_2}) under different temperatures emphasize the important role of temperature on fish metabolism. Increasing temperature both reduces oxygen solubility and increases oxygen demand. In *S. rivulatus*, M_{O_2} increased from 2.4×10^{-3} mg O_2/g BW/min at 17°C to 1.1×10^{-2} mg O_2/g BW/min at 26°C. Similar M_{O_2} were reported by Guinea & Fernandez (1997) for *S. aurata*, which varied from 2.3×10^{-3} mg O_2/g BW/min at 16°C to 4.8×10^{-3} mg O_2/g BW/min at 21°C. However, higher M_{O_2} (compared at same temperatures) were reported for Atlantic salmon and brook charr by Atkins & Benfey (2008). In Atlantic salmon, M_{O_2} varied from 1.5×10^{-3} mg O_2/g BW/min at 12°C to 6.1×10^{-3} mg O_2/g BW/min at 18°C, whereas in brook charr, M_{O_2} varied from 1.9×10^{-3} mg O_2/g BW/min at 9°C to 3.8×10^{-3} mg O_2/g BW/min at 15°C. Differences in metabolic rates at the same water temperatures are indicative of differences in thermal optima, where organisms maximize metabolic efficiency for physiological functions such as growth, feeding and digestion.

The decrease in M_{O_2} while oxygen concentration was decreasing shows an ability of the fish to adapt with low oxygen levels. The higher the temperature, the faster the decrease in M_{O_2} was. This alteration in M_{O_2} constitutes one of the physiological mechanisms that fish employ to cope with low and/or variable oxygen levels in the environment (DallaVia *et al.*, 1994). The first option for fish to accommodate a decrease

in ambient oxygen levels is to increase the ventilation of the gills and the blood perfusion through the gills. During severe hypoxia, the last option of the fish is to increase anaerobic respiration, which will lead to death if it lasts for long (Virani & Rees, 2000). Thereby, fish become "oxygen regulators", because they regulate their oxygen uptake so that oxygen consumption is maintained at a steady level over a more or less wide range of ambient oxygen concentrations (Nilsson, 2008).

Many of the adjustments done by the fish to cope with hypoxia, depend to a large extent on changes in the expression of genes that encode diverse groups of physiologically relevant proteins. In the present study, a partial cDNA clone representing the HIF-1α in *Siganus rivulatus* was obtained. The sequence was deposited in the GenBank with accession n° GU249151. HIF-1α is the subunit that determines the biological activity of the heterodimer HIF-1, its β subunit being constitutively expressed in the nucleus and not significantly affected by oxygen concentration. The predicted *S. rivulatus* HIF-1α amino acid sequence shows extensive sequence similarity to human HIF-1α in the bHLH (basic helix-loop-helix domain). The bHLH region, which is known to be responsible for DNA binding and dimerization, is critical as changes in gene expression under hypoxic conditions can only occur after HIF is bound to DNA. Amino acid substitutions in this domain are known to affect DNA binding to HIF-1 (Lando *et al.*, 2000). In *S. rivulatus*, a hypoxia-sensitive species, the amino acid that aligns with human position 28 in the bHLH domain is cysteine, like in salmonids, and not serine, like in the hypoxia-tolerant cyprinids. Rytkönen *et al.* (2007) were the first to hypothesize that the substitution cysteine/serine in this position correlates with a species-specific oxygen demand. It also coincides in our species, although the authors then concluded that such a correlation was not present in all the fish species they analyzed from a phylogenetic point of view. Another functionally important sequence motif described in all known members of the HIF-1 α protein family is also found in the *S. rivulatus* HIF-1α: the N-terminal nuclear localization signal (N-NLS) residues 17-74 (numbering according to human HIF-1α) that mediate nucleocytoplasmic trafficking of the HIF-1 α protein (Ema *et al.*, 1997; Luo and Shibuya, 2001).

Fish represent an extremely large and divergent vertebrate group. Among fish HIF-1α, great variations exist in homology. This is different from the situation observed in other vertebrate groups, such as mammals, in which homology among mammalian HIF-1α is very high. *S. rivulatus* HIF-1α is, in fact, most homologous to that of Atlantic croaker (*Micropogonias undulatus*), showing an approximately 87% aa identity. Homology with the rainbow trout (*Onchorhynchus mykiss*) and zebrafish (*Danio rerio*) is lower, being 71 and 61%, respectively, while homology with crucian carp (*Carassius carassius*) HIF-1α is even lower, with merely a 59% aa identity. The overall high degree of HIF-1α sequence conservation through evolution is not only consistent with its essential role in various responses to hypoxia, but also suggests that its biological action may be equally well conserved. In fact, the mechanism of degradation and stabilization of

HIF-1α protein is most likely the same in fish as in mammals (Soitamo *et al.*, 2001), although in fish (rainbow trout and chinook salmon) stabilization occurred at much higher oxygen levels than in mammals, suggesting a role for oxygen-regulated gene expression in the normal physiology of fish.

High maternal ascorbic acid intakes are beneficial for egg production and egg and larvae quality (Sandnes *et al.*, 1984; Blom & Dabrowski, 1996; Terova *et al.*, 1998a). This is in accordance with the results of the present study, since supplementation of vitamin C (2000 mg/Kg) in the broodstock diet of common pandora improved significantly the quality of produced eggs and larvae. All three egg quality criteria (egg diameter, oil droplet diameter and hatching rate) were better in group B than in the other two groups. Also, SAI, which indicates larval tolerance to starvation, was significantly higher in group B than in groups A and C. SAI was found to reflect the activity of larvae and to be correlated with success of mass production of larvae of Japanese flounder, yellowtail and striped jack (Furuita *et al.*, 2003).

During gonadal growth in oviparous teleosts the precursor of the egg yolk proteins, vitellogenin, is synthesized in the liver under the control of oestrogenic hormones. The serum levels of oestradiol-17β and vitellogenin are affected by the availability of ascorbic acid, which is believed to influence the endocrine functions in maturing fish (Waagbo *et al.*, 1989). Deposition of ascorbic acid in the egg yolk reserves is important for the synthesis of collagens during the development of the embryo and for praline and lysine hydroxylation. Sandnes *et al.* (1984) stated that rainbow trout broodstock fish should be fed adequate amounts of vitamin C to provide eggs with more than 20 μg ascorbic acid/g. This concentration of ascorbic acid in the eggs resulted in better hatchability and better survival of the embryos. Blom & Dabrowski (1996) demonstrated that ascorbic acid is efficiently transferred to embryos and offsprings from eggs with high concentration of vitamin C. An initial high ascorbic acid status of the egg protected hatched rainbow trout fry against mortality even when first feed had a low content of ascorbic acid (20 mg/Kg). The low dietary ascorbic acid level had an evident negative effect on survival only after 18 weeks after initiation of exogenous feeding. However, it resulted in a significant lower individual weight starting at week 7. Therefore, a high ascorbic acid diet for both broodstock fish and larvae is vital for good egg hatchability, overall survival (before and after yolk sac absorption) and growth. The quantity of ascorbic acid supplementation (2000 mg/Kg) in the broodstock feed during this study was chosen according to Terova *et al.* (1998a), who experimented on broodstock of sea bass and sea bream. In the study of Terova *et al.* (1998a), the concentration of total ascorbate in fertilized eggs was 218.5 and 122.4 μg/g wet weight respectively. In the studies mentioned above on rainbow trout, an improvement on the quality of eggs and larvae was observed already when broodstock feed was supplemented with 300 μg/Kg. In this study, feed supplementation with 2000 μg ascorbic acid/Kg did not have any negative effect on the broodstock of common pandora, but further

investigation on the nutritional requirements on this vitamin is necessary in order to maximize the advantages on reproductive performance and egg and larvae quality.

Dietary astaxanthin is thought to have an important role in the reproduction of fish in both marine and freshwater fish. However, in this study, the supplementation of astaxanthin (30 mg/Kg) in the broodstock feed did not have any additional improvement in egg and larvae quality, compared to group B, where there was only supplementation of vitamin C. On the contrary, some of the quality criteria (egg diameter, oil droplet diameter and SAI) were significantly lower than in group B. In literature, inclusion of astaxanthin into broodstock diet was found to enhance egg quality in yellowtail (*S. quinqueradiata*), striped jack (*P. dentex*) and rainbow trout (*O. mykiss*) (Verakunpiriya *et al.*, 1997; Vassallo-Agius *et al.*, 2001a; Ahmadi *et al.*, 2006). The astaxanthin content in the eggs produced from five broodstock groups of rainbow trout, which were fed diets with astaxanthin concentration between 0.07 and 92.9 mg/Kg, varied between 2.03 and 29.79 mg/Kg, respectively. The fertilization rate, eyed-egg percentage and percentage hatch were significantly correlated with the astaxanthin content in the eggs (Ahmadi *et al.*, 2006). In striped jack the inclusion of 10 mg astaxanthin/Kg of feed improved the overall spawning performance and egg quality (Vassallo-Agius *et al.*, 2001a). Verakunpiriya *et al.* (1997) tried four different levels of astaxanthin (0, 20, 30 and 40 mg/Kg) supplementation in the feed of yellowtail and concluded that the best egg quality was observed when fish were fed 30 mg/Kg astaxanthin. Egg quality criteria were significantly lower in fish that were fed 40 mg/Kg astaxanthin, indicating the existence of a maximum astaxanthin concentration, beyond which the quality of eggs diminished. These results indicate that the 30 mg astaxanthin/Kg of feed used in this study for common pandora may be beyond the critical maximum concentration at which astaxanthin has a positive effect on egg quality. However, it can also be the case that astaxanthin does not really play a vital role in reproduction performance and egg and larvae quality in common pandora. This is the case in Atlantic salmon, where astaxanthin content in eggs (0 to 14.7 mg/Kg) was not correlated with fertilization rate and survival of eggs and larvae (Christiansen & Torrissen, 1997). Although, the astaxanthin content in the eggs of common pandora was not measured, the red colour of the eggs produced from group C indicates that common pandora incorporate carotenoids into the eggs. However, further research should be done in order to make clear whether astaxanthin has a beneficial effect in reproductive performance and quality of eggs and larvae, and ultimately estimate the nutritional requirement of astaxanthin in common pandora.

As already mentioned before, adequate amount of vitamin C in fish enhances reproductive performance and quality of eggs and larvae. Vitamin C is also involved in other enzymatic reactions, which aim at the formation and maintenance of collagen, the metabolism of amino acids, the absorption and transport of iron and the protection of tissues from oxidative damage. Absorption of vitamin C is facilitated by two transporters, which are the Hexose transporters (GLUTs) and the Sodium-Ascorbate co-transporters

(SVCTs). In the present study, a partial cDNA clone representing the SVCT in *P. erythrinus* was obtained. The sequence was deposited in the GenBank with accession n° EU883994. SVCT1 and SVCT2 each mediate concentrative, high-affinity L-ascorbic acid transport that is stereospecific and is driven by the NA+ electrochemical gradient. Despite their close sequence homology and similar functions, the two isoforms of the transporter are discretely distributed: SVCT1 is mainly confined to epithelial systems (intestine, kidney, liver), whereas SVCT2 serves a host of metabolically active cells and specialised tissues in the brain, eye and other organs (Tsukaguchi *et al*., 1999).

The increase of biodiversity in eastern Mediterranean marine aquaculture through the introduction of new species is an approach, which can lead to the sustainable development of the sector, the increase in production, the stability of a healthy marketing environment, the development of new technology and the diversification of aquaculture products. The magnitude of biodiversity available for aquaculture and the potential of certain species for farming render this approach as a safe route towards the future. The fish species presented in this study are considered suitable for the diversification of aquaculture products. At present, the information gathered in relation to their biology, physiology and culture techniques can sustain the initiation of their commercial mass production, but further research is needed to gain more knowledge towards their particular biological needs and functions.

5. References

Abdel-Ghaffar F., Ali M.A., Al Quraishy S., Entzeroth R., Abdel-Baki A.S., Al Farraj S. and Bashtar A.R., 2008. *Zschokkella helmii* n. sp. (*Myxozoa*: *Myxosporea*), a new parasite of marbled spinefoot *Siganus rivulatus* (Forsskal 1775), Red Sea, Egypt: light and transmission electron microscopy. Parasitological research 102, 183-192.

Adams S.M. and Breck J.E., 1990. Bioenergetics. In: Methods for fish biology. Eds. C.B. Schreck and P.B. Moyle. American Fisheries Society, USA, pp. 389-415.

Ahmadi M.R., Bazyar A.A., Safi S., Ytrestoyl T. and Bjerkeng B., 2006. Effects of dietary astaxanthin supplementation on reproductive characteristics of rainbow trout (*Oncorhynchus mykiss*). Journal of Applied Ichthyology 22, 388-394.

Alexis M.N., Karanikolas K.K. and Richards R.H., 1997. Pathological findings owing to the lack of ascorbic acid in cultured gilthead bream (*Sparus aurata* L.). Aquaculture 151, 209-218.

Aparicio S., Chapman J., Stupka E., Putnam N., Chia J.M., Dehal P., Christoffels A., Rash S., Hoon S., Smit A., Gelpke M.D., Roach J., Oh T., Ho I.Y., Wong M., Detter C., Verhoef F., Predki P., Tay A., Lucas S., Richardson P., Smith S.F., Clark M.S., Edwards Y.J., Dogget N., Zharkikh A., Tavtigian S.V., Pruss D., Barnstead M., Evans C., Baden H., Powell J., Glusman G., Rowen L., Hood L., Tan Y.H., Elgar G., Hawkins T., Venkatesh B., Rokhsar D. and Brenner S., 2002. Whole-genome shotgun assembly and analysis of the genome of *Fugu rubripes*. Science 297, 1301-1310.

Atkins M.E. and Benfey T.J., 2008. Effect of acclimation temperature on routine metabolic rate in triploid salmonids. Comparative Biochemistry and Physiology, Part A 149, 157-161.

Avault J.W, 1993. Ten requirements for culturing a "new" species: a checklist. Aquaculture Magazine 19, 68-73.

Azzurro E., Golani D., Bucciarelli G. and Bernardi G., 2006. Genetics of the early stages of invasion of the Lessepsian rabbitfish *Siganus luridus*. Journal of Experimental Marine Biology and Ecology 333, 190-201.

Basurco B. and Lovatelli A., 2003. The aquaculture situation in the Mediterranean Sea. Predictions for the future. In: International Conference on Sustainable Development of the Mediterranean and Black Sea Environment, Thessalonica (Greece), 29-31 May 2003.

Beaumont N.J., Austen M.C., Mangi S.C. and Townsend M., 2008. Economic valuation for the conservation of marine biodiversity. Marine Pollution Bulletin 56, 386-396.

Bell J.G., McEvoy J., Tocher D.R. and Sargent J.R., 2000. Depletion of α-Tocopherol and astaxanthin in Atlantic salmon (*Salmo salar*) affects autoxidative defense and fatty acid metabolism. The Journal of Nutrition 130(7), 1800-1808.

Benedito-Palos L., Navarro J.C., Sitja-Bobadilla A., Bell J.G., Kaushik S. and Perez-Sanchez J., 2008. High levels of vegetable oils in plant protein-rich diets fed to gilthead sea bream (*Sparus aurata* L.): growth performance, muscle fatty acid profiles and histological alterations of target tissues. British Journal of Nutrition 100, 992-1003.

Benetti D.D., 1997. Spawning and larval husbandry of flounder (*Paralichthys woolmani*) and Pacific yellowtail (*Seriola mazatlana*), new candidate species for aquaculture. Aquaculture 155, 307-318.

Bianchi C.N. and Morri C., 2000. Marine biodiversity of the Mediterranean Sea: Situation, problems and prospects for future research. Marine Pollution Bulletin 40 (5), 367-376.

Blom J.H. and Dabrowski K., 1996. Ascorbic acid metabolism in fish: is there a maternal effect on the progeny? Aquaculture 147, 215-224.

Bonaldo A., Isani G., Fontanillas R., Parma L., Grilli E. and Gatta P.P., 2010. Growth and feed utilization of gilthead sea bream (*Sparus aurata* L.) fed to satiation and restrictively at increasing dietary energy levels. Aquaculture International 18(5), 909-919.

Bonnet E., Fostier A. and Bobe J., 2007a. Microarray-based analysis of fish egg quality after natural or controlled ovulation. BMC Genomics 8, 55.

Bonnet E., Montfort J., Esquerre D., Hugot K., Fostier A. and Bobe J., 2007b. Effect of photoperiod manipulation on rainbow trout (*Oncorhynchus mykiss*) egg quality: A genomic study. Aquaculture 268, 13-22.

Booth M.A., Warner-Smith R.J., Allan G.L. and Glencross B.D., 2004. Effects of dietary astaxanthin source and light manipulation on the skin colour of Australian snapper *Pagrus auratus* (Bloch & Schneider, 1801). Aquaculture Research 35, 458-464.

Bouchet P., 2006. The magnitude of marine biodiversity. In: The exploration of marine biodiversity: Scientific and technological challenges. Eds. Carlos M. Duarte. Fundacion BBVA, Bilbao, Spain 2006, 31-62.

Chatzifotis S., Muje P., Pavlidis M., Agren J., Paalavuo M. and Molsa H., 2004. Evolution of tissue composition and serum metabolites during gonadal development in the common dentex (*Dentex dentex*). Aquaculture 236, 557-573.

Chatzifotis S., Polemitou I., Divanach P. and Antonopoulou E., 2008. Effect of dietary taurine supplementation on growth performance and bile salt activated lipase activity of common dentex, *Dentex dentex*, fed a fish meal/soy protein concentrate-based diet. Aquaculture 275, 201-208.

Chatzifotis S., Panagiotidou M, Papaioannou N., Pavlidis M., Nengas I. and Mylonas C.C., 2010. Effect of dietary lipid levels on growth, feed utilization, body composition and serum metabolites of meagre (*Argyrosomus regius*) juveniles. Aquaculture 307(1-2), 65-70.

Chien YH. and Shiau WC., 2005. The effects of dietary supplementation of algae and synthetic astaxanthin on body astaxanthin, survival, growth, and low dissolved oxygen stress resistance of kuruma prawn, *Marsupenaeus japonicus* Bate. Journal of Experimental Marine Biology and Ecology 318, 201-211.

Chini V., Rimoldi S., Terova G., Saroglia M., Rossi F., Bernardini G. and Gornati R., 2006. EST-based identification of gene expressed in the liver of adult sea bass (*Dicentrarchus labrax*, L.). Gene 376, 102-106.

Chini V., Cattaneo A.G., Rossi F., Bernardini G., Terova G., Saroglia M. and Gornati R., 2008. Genes expressed in Blue Fin Tuna (*Thunnus thynnus*) liver and gonads. Gene 410, 207-213.

Choubert G. Carotenoids and pigmentation. In: Nutrition and feeding of fish and crustaceans, Guillaume J., Kaushik S., Bergot P. and Metaille R., Chichester, UK, Praxis Publishing Ltd, 2001, p. 183-196

Choubert G., Mendes-Pinto M.M. and Morais R., 2006. Pigmenting efficacy of astaxanthin fed to rainbow trout *Oncorhynchus mykiss*: Effect of dietary astaxanthin and lipid sources. Aquaculture 257, 429-436.

Christiansen R. and Torrissen O.J., 1997. Effects of dietary astaxanthin supplementation on fertilization and egg survival in Atlantic salmon (*Salmo salar* L.). Aquaculture 153, 51-62.

Chuba H., Nakao T., Arakawa T. and Matsuyama M., 2005. Effects of oocyte diameter on ovulation time, quantity and quality of eggs in yellowtail Seriola quinqueradiata in inducing ovulation with HCG. Nippon Suisan Gakkaishi 71(6), 942-946.

Cittolin G., Borgoni N., Angelini M. and Lorenzini C., 2008. L'allevamento dell'*Argyrosomus regius*. In: Acquacoltura in Toscana. Studi e analisi di settore. pp. 49-61.

Cognetti G. and Curini-Galletti M., 1993. Biodiversity conservation problems in the marine environment. Marine Pollution Bulletin 26 (4), 179-183.

Costanza R., D'Arge R., DeGroot R., Farber S., Grasso M., Hannon B., Limburg K., Naeem S., O'Neill R.V., Paruelo J., Raskin R.G., Sutton P. and Van den Belt M., 1997. The value of the world's ecosystem services and natural capital. Nature 387, 253-260.

Craig R.K., 2004. Protecting international marine biodiversity: International treaties and national systems of marine protected areas. Journal of Land Use and Environmental Law 20(2), 337-373.

Crespo S., Marin de Mateo M., Santamaria C.A., Sala R., Grau A. And Pastor E., 2001. Histopathological observations during larval rearing of common dentex *Dentex dentex* L. (Sparidae). Aquaculture 192, 121-132.

Dabrowski K., Matusiewicz M. and Blom J.H., 1994. Hydrolysis, absorption and bioavailability of ascorbic acid esters in fish. Aquaculture 124, 169-192.

DallaVia, J., Thillart, G.V.D., Cattani, O., deZwaan, A., 1994. Influence of long-term hypoxia exposure on the energy metabolism of Solea solea. II. Intermediary metabolism in blood, liver, and muscle. Marine Ecology Progress Series. 111, 17-27.

De Francesco M., Parisi G, Perez-Sanchez J., Gomez-Requeni P., Medale F., Kaushik S.J., Mecatti M. and Poli B.M., 2007. Effect of high-level fish meal replacement by plant proteins in gilthead sea bream (*Sparus aurata*) on growth and body/fillet quality traits. Aquaculture Nutrition 13, 361-372.

Diamant A., Banet A., Ucko M., Colorni A., Knibb W. and Kvitt H., 2000. Mycobacteriosis in wild rabbitfish *Siganus rivulatus* associated with cage farming in the Gulf of Eilat, Red Sea. Diseases of Aquatic Organisms 39, 211-219.

Diaz-Lopez M., Perez M.J., Acosta N.G., Tocher D.R., Jerez S., Lorenzo A. and Rodriguez C., 2009. Effect of dietary substitution of fish oil by *Echium* oil on growth, plasma parameters and body lipid composition in gilthead seabream (*Sparus aurata* L.). Aquaculture Nutrition 15, 500-512.

Duxbury A.C. and Duxbury A.B., 1997. Production and life. In: An introduction to the world's oceans. Eds. A.C. Duxbury and A.B. Duxbury. Wm. C. Brown Publishers, Fifth edition, USA, pp. 367-385.

Ema, M., Taya, S., Yokotani, N., Sogawa, K., Matsuda, Y., Fujii-Kuriyama, Y., 1997. A novel bHLH-PAS factor with close sequence similarity to hypoxia inducible factor I alpha regulates the VEGF expression and is potentially involved in lung and vascular development. Proc. Natl. Acad. Sci. USA. 94, 4273-4278.

Emata, A.C., Borlongan, I.G. and Damaso, J.P., 2000. Dietary vitamin C and E supplementation and reproduction of milkfish *Chanos chanos* Forsskal. Aquaculture Research, 31, 557-564.

Espinos F.J., Tomas A., Perez L.M., Balasch S. and Jover M., 2003. Growth of dentex fingerlings (*Dentex dentex*) fed diets containing different levels of protein and lipid. Aquaculture 218, 479-490.

Estevez A., Trevino L, Kotzamanis Y., Karacostas I., Tort L. and Gisbert E., 2010. Effects of different levels of plant proteins on the ongrowing of meagre (*Argyrosomus regius*) juveniles at low temperatures. Aquaculture Nutrition 10, 1-11.

Falahatkar B., Dabrowski K., Arslan M. and Rinchard J. (2006) Effects of ascorbic acid enrichment by immersion of rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) eggs and embryos. Aquaculture Research 37, 834-841.

FAO, 2007. The state of world fisheries and aquaculture 2006. FAO Fisheries and Aquaculture Department, Food and Agriculture Organisation of the United Nations, Rome.

FAO, 2009. The state of world fisheries and aquaculture 2008. FAO Fisheries and Aquaculture Department, Food and Agriculture Organisation of the United Nations, Rome.

FEAP, 2008. Production and price reports of member associations of the F.E.A.P. Federation of European Aquaculture Producers, FEAP Secretariat, 2008.

Fisheries Research and Development Corporation, 2004. What's so healthy about seafood? A guide for seafood marketers. Fisheries Research and Development Corporation, Australian Government, Second Edition, 2004.

Fishmeal Information Network, 2008. FIN dossier 2008. Annual review of the feed grade fish stocks used to produce fishmeal and fish oil for the UK market. Grain and Feed Trade Association, UK.

Fountoulaki E., Vasilaki A., Hurtado R., Grigorakis K., Karacostas I., Nengas I., Rigos G., Kotzamanis Y., Venou B. and Alexis M.N., 2009. Fish oil substitution by vegetable oils in commercial diets for gilthead sea bream (*Sparus aurata* L.); effects on growth performance, flesh quality and fillet fatty acid profile. Recovery of fatty acid profiles by a fish oil finishing diet under fluctuating water temperatures. Aquaculture 289, 317-326.

Furuita H., Yamamoto T., Shima T., Suzuki N. and Takeuchi T., 2003. Effect of arachidonic acid levels in broodstock diet on larval and egg quality of Japanese flounder *Paralichthys olivaceus*. Aquaculture 220, 725-735.

Garcia-Gomez A., 1993. Primeras experiencias de crecimiento de juveniles de *Seriola* mediterranea (*Seriola dumerili*, Risso 1810) alimentados con una dieta semihumeda. Bol. Inst. Esp. Oceanogr. 9(2), 347-360.

GFCM, 2008. Trend and issues of marine and brackish Mediterranean aquaculture. General Fisheries Commission for the Mediterranean, Committee on Aquaculture, Sixth Session, Tirana, Albania, 17-19 December 2008.

Ghanawi J., Saoud I.P. and Shalaby S.M., 2010. Effect of size sorting on growth performance of juvenile spinefoot rabbitfish, *Siganus rivulatus*. Journal of the World Aquaculture Society 41(4), 565-573.

Gimenez G., Estevez A., Lahnsteiner F., Zecevic B., Bell J.G., Henderson R.J., Pinera J.A. and Sanchez-Prado J.A., 2006. Egg quality criteria in common dentex (*Dentex dentex*). Aquaculture 260, 232-243.

Gimenez G. and Estevez A., 2008. Effect of larval and prey density, prey dose and light conditions on first feeding common dentex (*Dentex dentex* L.) larvae. Aquaculture Research 39, 77-84.

Gisbert E., Gimenez G., Fernandez I., Kotzamanis Y. and Estevez A., 2009. Development of digestive enzymes in common dentex *Dentex dentex* during early ontogeny. Aquaculture 287, 381-387.

Glamuzina B., Jug-Dujakovic J. and Katavic I., 1989. Preliminary studies on reproduction and larval rearing of common dentex, *Dentex dentex* (Linnaeus 1758). Aquaculture 77, 75-84.

Gomez-Requeni P., Mingarro M., Calduch-Giner J.A., Medale F., Martin S.A.M., Houlihan D.F., Kaushik S. and Perez-Sanchez J., 2004. Protein growth performance, amino acid utilization and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilthead sea bream (*Sparus aurata*). Aquaculture 232, 493-510.

Guinea J. and Fernandez F., 1997. Effect of feeding frequency, feeding level and temperature on energy metabolism in *Sparus aurata*. Aquaculture 148, 125-142.

Guner Y., Ozden O., Altunok M., Koru E. and Kizak V., 2004. Spawning and larvae production of common pandora, *Pagellus erythrinus* L. The Israeli Journal of Aquaculture 56(3), 209-217.

Hamasaki K., Tsuruoka K., Teruya K., Hashimoto H., Hamada K., Hotta T. and Mushiake K., 2009. Feeding habits of hatchery-reared larvae of greater amberjack *Seriola dumerili*. Aquaculture 288, 216-225.

Harahap A.P., Takemura A., Nakamura S., Rahman MD.S. and Takano K., 2001. Histological evidence of lunar-synchronized ovarian development and spawning in the spiny rabbitfish *Siganus spinus* (Linnaeus) around the Ryukyus. Fisheries Science 67, 888-893.

Harahap A.P., Takemura A., Rahman MD.S., Nakamura S. and Takano K., 2002. Lunar synchronization of sperm motility in the spiny rabbitfish *Siganus spinus* (Linnaeus). Fisheries Science 68, 706-708.

Hoque M.M., Takemura A. and Takano K., 1998. Annual changes in oocyte development and serum vitellogenin level in the rabbitfish *Siganus canaliculatus* (Park) in Okinawa, southern Japan. Fisheries Science 64(1), 44-51.

Hoque M.M., Takemura A., Matsuyama M., Matsuura S. and Takano K., 1999. Lunar spawning in *Siganus canaliculatus*. Journal of Fish Biology 55, 1213-1222.

Ibeas C., Cejas J.R., Fores R., Badia P., Gomez T. and Hernandez A.L., 1997. Influence of eicosapentaenoic to docosahexaenoic acid ratio (EPA/DHA) of dietary lipids on growth and fatty acid composition of gilthead seabream (*Sparus aurata*) juveniles. Aquaculture 150, 91-102.

IFFO. The healthiest omega-3s EPA & DHA are found mainly in fish oil and fishmeal. A guide to the long chain omega-3 fatty acids, EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) in fish oil. International Fishmeal and Fish Oil Organisation, www.iffo.net.

IUCN, 2004. Mediterranean marine aquaculture and environment. IUCN Centre for Mediterranean Cooperation, World Conservation Union, Barcelona, 29-30 April 2004.

Jerez S., Samper M., Santamaria F.J., Villamandos J.E., Cejas J.R. and Felipe B.C., 2006. Natural spawning of greater amberjack (*Seriola dumerili*) kept in captivity in the Canary Islands. Aquaculture 252, 199-207.

Jug-Dujakovic J., Dulcic J. and Katavic I., 1995. Embryonic and yolk-sac larval development of the sparid *Dentex dentex* (Linnaeus, 1758). Fisheries Research 24, 91-97.

Kalinowski C.T., Robaina L.E., Fernandez-Palacios H., Schuchardt D. and Izquierdo M.S., 2005. Effect of different carotenoid sources and their dietary levels on red porgy (*Pagrus pagrus*) growth and skin colour. Aquaculture 244, 223-231.

Kaushik S.J., Coves D., Dutto G. and Blanc D., 2004. Almost total replacement of fish meal by plant protein sources in the diet of a marine teleost, the European seabass, *Dicentrarchus labrax*. Aquaculture 230, 391-404.

Kelly P.D., Chu F., Woods I.G., Ngo-Hazelett P., Cardozo T., Huang H., Kimm F., Liao L., Yan Y.L., Zhou Y., Johnson S.L., Abagyan R., Schier A.F., Postlethwait J.H. and Talbot W.S., 2000. Genetic linkage mapping of zebrafish genes and ESTs. Genome Research 10, 558-567.

Kissil G.W., Lupatsch I., Stafanou D., Georgiou G. and Kyriacou Y., 2002. Development of practical feeds for the mass cultivation of white rabbitfish (*Siganus rivulatus*). Report submitted to Research Promotion Foundation of Cyprus.

Klaoudatos S.D., Iakovopoulos G. and Klaoudatos D.S., 2004. *Pagellus erythrinus* (common pandora): a promising candidate species for enlarging the diversity of aquaculture production. Aquaculture International 12, 299-320.

Klimogianni A., Koumoundouros G., Kaspiris P. and Kentouri M., 2004. Effect of temperature on the egg and yolk-sac larval development of common pandora, *Pagellus erythrinus*. Marine Biology 145, 1015-1022.

Kolkovski S. and Sakakura Y., 2004. Yellowtail kingfish, from larvae to mature fish – problems and opportunities. In: Cruz Suarez L.E., Ricque Marie D., Nieto Lopez M.G., Villarreal D., Scholz U. and Conzalez M., 2004. Advances en Nutricion Acuicola VII. Memorias del VII Simposium Internacional de Nutricion Acuicola. 16-19 Noviembre, 2004. Hermosillo, Sonora, Mexico.

Komatsu T., Nakamura S. and Nakamura M., 2006. A sex cord-like structure and some remarkable features in early gonadal sex differentiation in the marine teleost Siganus guttatus (Bloch). Journal of Fish Biology 68, 236-250.

Koumoundouros G., Carrillo J., Divanach P. and Kentouri M., 2004. The rearing of common dentex *Dentex dentex* (L.) during the hatchery and on-growing phases. Aquaculture 240, 165-173.

Kousoulaki K., Miliou E., Apostolopoulou M. and Alexis M.N., 2007. Effect of feeding intensity and feed composition on nutrient digestibility and production performance of common pandora (*Pagellus erythrinus*) in sea cages. Aquaculture 272, 514-527.

Kozul V., Skaramuca B., Glamuzina B., Glavic N. and Tutman P., 2001. Comparative gonadogenesis and hormonal induction of spawning of cultured and wild Mediterranean amberjack (*Seriola dumerili*, Risso 1810). Scientia Marina 65(3), 215-220.

Lam T. J., 1974. Siganids: their biology and mariculture potential. Aquaculture 3, 325-354.

Lando, D., Pongratz, I., Poellinger, L., Whitelaw, M.L., 2000. A redox mechanism controls differential DNA binding activities of hypoxia-inducible factor (HIF) 1 alpha and the HIF-like factor. J. Biol. Chem. 275, 4618-4627.

Loir M., Le Gac F., Somarakis S. and Pavlidis M., 2001. Sexuality and gonadal cycle of the common dentex (*Dentex dentex*) in intensive culture. Aquaculture 194, 363-381.

Luo, J., Shibuya, M., 2001. A variant of nuclear localization signal of bipartite-type is required for the nuclear translocation of hypoxia inducible factors (1 alpha, 2 alpha, and 3 alpha). Oncogene. 20, 1435-1444.

Matusiewicz M., Dabrowski K., Volker L. and Matusiewicz K., 1994. Regulation of saturation and depletion of ascorbic acid in rainbow trout. Journal of Nutritional Biochemistry 5, 204-212.

Mazzola A., Favaloro E. and Sara G., 2000. Cultivation of the Mediterranean amberjack, *Seriola dumerili* (Risso, 1810), in submerged cages in the Western Mediterranean Sea. Aquaculture 181, 257-268.

Micale V., Garaffo M., Genovese L., Spedicato M.T. and Muglia U., 2006. The ontogeny of the alimentary tract during larval development in common pandora *Pagellus erythrinus* L. Aquaculture 251, 354-365.

Mihelakakis A., Yoshimatsu T. and Tsolkas C., 2001. Effect of feeding frequency on growth, feed efficincy, and body composition in young common pandora. Aquaculture International 9, 197-204.

Miller P.J. and Loates M.J., 1997. Fish of Britain & Europe. London, Harper Collins Publishers, 288 p.

Montero D., Robaina L., Caballero M.J., Gines R. and Izquierdo M.S., 2005. Growth, feed utilization and flesh quality of European sea bass (*Dicentrarchus labrax*) fed diets containing vegetable oils: A time-course study on the effect of a re-feeding period with a 100% fish oil diet. Aquaculture 248, 121-134.

Moran D., Smith C.K., Gara B. and Poortenaar C.W., 2007. Reproductive behaviour and early development in yellowtail kingfish (*Seriola lalandi* Valenciennes 1833). Aquaculture 262, 95-104.

Mourente G. and Bell J.G., 2006. Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (*Dicentrarchus labrax* L.) over a long term growth study: Effects on muscle and liver fatty acid composition and effectiveness of a fish oil finishing diet. Comparative Biochemistry and Physiology, Part B 145, 389-399.

Mylonas C.C., Papandroulakis N., Smboukis A., Papadaki M. and Divanach P., 2004. Induction of spawning of cultured greater amberjack (*Seriola dumerili*) using GnRHa implants. Aquaculture 237, 141-154.

Nakano T., Tosa M. and Takeuchi M., 1995. Improvement of biochemical features in fish health by red yeast and synthetic astaxanthin. Journal of Agricultural and Food Chemistry 43(6), 1570-1573.

Nilsson G.E. and Ostlund-Nilsson S., 2008. Does size matter for hypoxia tolerance in fish? Biological Reviews 83(2), 173-189.

Packer M.J. and MacDonald D.W., 2002. Business and biodiversity: existing threats – emerging opportunities. In: Business and Biodiversity: The Handbook for Corporate Action. Earthwatch Europe, IUCN and WBCSD.

Papandroulakis N., Mylonas C.C., Maingot E. and Divanach P., 2005. First results of greater amberjack (*Seriola dumerili*) larval rearing in mesocosm. Aquaculture 250, 155-161.

Parazo M.M., 1990. Effect of dietary protein and energy level on growth, protein utilization and carcass composition of rabbitfish, *Siganus guttatus*. Aquaculture 86, 41-49.

Pavlidis M., Greenwood L. and Scott A.P., 2004. The role of sex ratio on spawning performance and on the free and conjugated sex steroids released into the water by common dentex (*Dentex dentex*) broodstock. General and Comparative Endocrinology 138, 255-262.

Perez-Jimenez A., Cardenete G., Morales A.E., Garcia-Alcazar A., Abellan E. and Hidalgo M.C., 2009. Digestive enzymatic profile of *Dentex dentex* and response to different dietary formulations. Comparative Biochemistry and Physiology, Part A 154, 157-164.

Poli B.M., Parisi G., Zampacavallo G., Iurzan F., Mecatti M., Lupi P. and Bonelli A., 2003. Preliminary results on quality and quality changes in reared meagre (*Argyrosomus regius*): body and fillet traits and freshness changes in refrigerated commercial-size fish. Aquaculture International 11, 301-311.

Quemener L., Suquet M., Mero D. and Gaignon J.L., 2002. Selection method of new candidates for finfish aquaculture: the case of the French Atlantic, the Channel and the North Sea coasts. Aquatic Leaving Resources 15, 293-302.

Rahman M.S., Takemura A. and Takano K., 2000a. Lunar synchronization of testicular development and plasma steroid hormone profiles in the golden rabbitfish. Journal of Fish Biology 57, 1065-1074.

Rahman M.S., Takemura A. and Takano K., 2000b. Annual changes in ovarian histology, plasma steroid hormones and vitellogenin in the female golden rabbitfish, *Siganus guttatus* (Bloch). Bulletin of Marine Science 67(2), 729-740.

Rahman M.S., Takemura A. and Takano K., 2001. Lunar synchronization of testicular development and steroidogenesis in rabbitfish. Comparative Biochemistry and Physiology, Part B 129, 367-373.

Rehulka J., 2000. Influence of astaxanthin on growth rate, condition, and some blood indices of rainbow trout, *Oncorhynchus mykiss*. Aquaculture 190, 27-47.

Roo J., Hernandez-Cruz C.M., Borrero C., Schuchardt D. and Fernandez-Palacios H., 2010. Effect of larval density and feeding sequence on meagre (*Argyrosomus regius*; Asso, 1801) larval rearing. Aquaculture 302, 82-88.

Rytkonen, K.T., Vuori, K.A.M., Primmer, C.R., Nikinmaa, M., 2007. Comparison of hypoxia-inducible factor-1 alpha in hypoxia sensitive and hypoxia tolerant fish species. Comp. Biochem. Physiol. Part D, 177-186.

Saeed M.O., Alamoudi M.M. and Al-Harbi A.H., 1987. A *Pseudomonas* associated with disease in cultured rabbitfish *Siganus rivulatus* in the Red Sea. Diseases of Aquatic Organisms 3, 177-180.

Sandnes K. and Braekkan O.R., 1981. Ascorbic acid and the reproductive cycle of ovaries in cod (*Gadus morphua*). Comparative Biochemistry and Physiology 70A, 545-546.

Sandnes K., Ulgenes Y., Braekkan O.R. and Utne F., 1984. The effect of ascorbic acid supplementation in broodstock feed on reproduction of rainbow trout (*Salmo Gairdneri*). Aquaculture 43, 167-177.

Santamaria C.A., Marin de Mateo M., Traveset R., Sala R., Grau A., Pastor E., Sarasquete C. and Crespo S., 2004. Larval organogenesis in common dentex *Dentex dentex* L. (Sparidae): histological and histochemical aspects. Aquaculture 237, 207-228.

Saoud I.P., Kreydiyyeh S., Chalfoun A. and Fakih M., 2007. Influence of salinity on survival, growth, plasma osmolarity and gill Na^+-K^+-ATPase activity in the rabbitfish *Siganus rivulatus*. Journal of Experimental Marine Biology and Ecology 348, 183-190.

Saoud I.P., Mohanna C. and Ghanawi J., 2008a. Effects of temperature on survival and growth of juvenile spinefoot rabbitfish (*Siganus rivulatus*). Aquaculture Research 39, 491-497.

Saoud I.P., Ghanawi J. and Lebbos N., 2008b. Effects of stocking density on the survival, growth, size variation and condition index of juvenile rabbitfish *Siganus rivulatus*. Aquaculture International 16, 109-116.

Saoud I.P., 2010. Culture potential for marbled spinefoot rabbitfish (*Siganus rivulatus*). The Practical Asian Aquaculture 1(1), 15-17.

Schiavone R., Zilli L. and Vilella S., 2008. Sex differentiation and serum levels of sex steroids in meagre (*Argyrosomus regius*). Comparative Biochemistry and Physiology, Part A, 151, S16-17.

Sfakianakis D.G., Koumoundouros G., Divanach P. and Kentouri M., 2004. Osteological development of the vertebral column and of the fins in *Pagellus erythrinus* (L. 1758). Temperature effect on the developmental plasticity and morpho-anatomical abnormalities. Aquaculture 232, 407-424.

Sitja-Bobadilla A., Pena-Llopis S., Gomez-Requeni P., Medale F., Kaushik S. and Perez-Sanchez J., 2005. Effect of fish meal replacement by plant protein sources on non-specific defence mechanisms and oxidative stress in gilthead sea bream (*Sparus aurata*). Aquaculture 249, 387-400.

Skalli A. and Robin J.H., 2004. Requirement of n-3 long chain polyunsaturated fatty acids for European sea bass (*Dicentrarchus labrax*) juveniles: growth and fatty acid composition. Aquaculture 240, 399-415.

Skalli A., Hidalgo M.C., Abellan E., Arizcun M. and Cardenete G, 2004. Effects of the dietary protein/lipid ratio on growth and nutrient utilization in common dentex (*Dentex dentex* L.) at different growth stages. Aquaculture 235, 1-11.

Soitamo, A.J., Rabergh, C.M.I., Gassmann, M., Sistonene, L., Nikinmaa, M., 2001. Characterization of a hypoxia-inducible factor (HIF-1α) from rainbow trout. J. Biol. Chem. 276, 19699-19705.

Stephanou D. and Georgiou G., 1999. Recent experiences on the culture of rabbitfish *Siganus rivulatus* in Cyprus. Cahiers Options Mediterranennes 47, 295-301.

Storebakken T., 2002. Atlantic salmon, *Salmo salar*. In: Nutrient requirements and feeding of finfish for aquaculture, Webster C.D. and Lim C.E., CAB International, 2002, p. 79-102.

Suzer C., Saka S. and Firat K., 2006a. Effects of illumination on early life development and digestive enzyme activities in common pandora *Pagellus erythrinus* L. larvae. Aquaculture 260, 86-93.

Suzer C., Firat K. and Saka S., 2006b. Ontogenic development of the digestive enzymes in common pandora *Pagellus erythrinus*, L. larvae. Aquaculture Research 37, 1565-1571.

Tacon A.G.J., Rausin N., Kadari M. and Cornelis P., 1990. The food and feeding of marine finfish in floating net cages at the National Seafarming Development Centre, Lampung, Indonesia: rabbitfish, *Siganus canaliculatus* (Park). Aquaculture and Fisheries Management 21, 375-390.

Tacon A.G.J. and Metian M., 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: Trends and future prospects. Aquaculture 285, 146-158.

Tejera N., Cejas J.R., Rodriguez C., Bjerkeng B., Jerez S., Bolanos A. and Lorenzo A., 2007. Pigmentation, carotenoids, lipid peroxides and lipid composition of skin of red porgy (*Pagrus pagrus*) fed diets supplemented with different astaxanthin sources. Aquaculture 270, 218-230.

Terova, G., Saroglia, M., Papp, Z.Gy. & Cecchini, S., 1998a. Ascorbate dynamics in embyos and larvae of sea bass and sea bream, originating from broodstocks fed supplements of ascorbic acid. Aquaculture International, 6, 357-367.

Terova, G., Saroglia, M., Papp, Z.Gy. & Cecchini, S., 1998b. Dynamics of collagen indicating amino acids, in embryos and larvae of sea bass (Dicentrarchus labrax) and gilthead sea bream (*Sparus aurata*), originated from broodstocks fed with different vitamin C content in the diet. Comparative Biochemistry and Physiology, Part A, 121, 111-118.

Terova, G., Rimoldi, S., Chini, V., Gornati, R., Bernardini, G., Saroglia, M., 2007a. Cloning and expression analysis of insulin-like growth factor I and II in liver and muscle of sea bass (*Dicentrarchus labrax*, L) during long-term fasting and refeeding. J. Fish. Biol. Vol 70 sb, 219–233.

Terova, G., Rimoldi, S., Larghi, S., Bernardini, G., Gornati, R., Saroglia, M., 2007b. Regulation of progastricsin mRNA levels in sea bass (*Dicentrarchus labrax*) in response to fluctuations in food availability. Biochem. Biophys. Res. Commun. Vol 363/3, 591-596.

Terova G., Rimoldi S., Cora S., Bernardini G., Gornati R. and Saroglia M., 2008. Acute and chronic hypoxia affects HIF-1α mRNA levels in sea bass (*Dicentrarchus labrax*). Aquaculture 279, 150-159.

Thresher R.E. and Kuris A.M., 2004. Options for managing invasive marine species. Biological Invasions 6(3), 295-300.

Torrissen O.J., 1989. Pigmentation of salmonids: interaction of astaxanthin and cantaxanthin on pigment deposition in rainbow trout. Aquaculture 79, 363-374.

Tsukaguchi H., Tokui T., Mackenzie B., Berger U.V., Chen XZ., Wang Y., Brubaker R.F. and Hediger M.A., 1999. A family of mammalian Na^+-dependent L-ascorbic acid transporters. Nature 399, 70-75.

Valdes P., Garcia-Alcazar A., Abdel I., Arizcun M., Suarez C. and Abellan E., 2004. Seasonal changes on gonadosomatic index and maturation stages in common pandora *Pagellus erythrinus* (L.). Aquaculture International 12, 333-343.

Vassallo-Agius R., Watanabe T., Imaizumi H., Yamazaki T., Satoh S. and Kiron, V., 2001a. Effects of dry pellets containing astaxanthin and squid meal on the spawning performance of striped jack *Pseudocaranx dentex*. Fisheries Science 67, 667-674.

Vassallo-Agius R., Imaizumi H., Watanabe T., Yamazaki T., Satoh S. and Kiron, V., 2001b. The influence of astaxanthin supplemented dry pellets on spawning of striped jack. Fisheries Science 67, 260-270.

Vassallo-Agius R., Watanabe T., Satoh S., Kiron V., Imaizumi H., Yamazaki T., and Kawano K., 2001c. Supplementation of paprika as a carotenoid source in soft-dry pellets for broodstock yellowtail *Seriola quinqueradiata* (Temminck & Schlegel). Aquaculture Research 32 (Suppl. 1), 263-272.

Verakunpiriya V., Mushiake K., Kawano K. and Watanabe T., 1997. Supplemental effect of astaxanthin in broodstock diets on the quality of yellowtail eggs. Fisheries Science 63(5), 816-823.

Virani N.A. and Rees B.B., 2000. Oxygen consumption, blood lactate and inter-individual Fvariation in the gulf killfish, *Fundulus grandis*, during hypoxia and recovery. Comparative Biochemistry and Physiology, Part A 126, 397-405.

Waagho R., Thorsen T. and Sandnes K., 1989. Role of dietary ascorbic acid in vitellogenesis in rainbow trout (*Salmo gairdneri*). Aquaculture 80, 301-314.

Yeldan H. and Avsar D., 2000. A preliminary study on the reproduction of the rabbitfish (*Siganus rivulatus* (Forsskal, 1775)) in the Northeastern Mediterranean. Turkish Journal of Zoology 24, 173-182.

Yildiz M., Sener E. and Timur M., 2007. Effects of variations in feed and seasonal changes on body proximate composition of wild and cultured sea bass (*Dicentrarchus labrax* L.). Turkish Journal of Fisheries and Aquatic Sciences 7, 45-51.

Yousif O.M., Kumar K. and Ali A.A., 2005. Growth performance, feed utilization, survival and body composition of rabbitfish *Siganus canaliculatus* raised at two different stocking densities in sea net cages. Emirate Journal of Agricultural Science 17(2), 14-22.

Zaki M.I., Massoud A.A., Madkour G.A., El-Fiky N.K. and Elmesiry G.E., 1994. The cyclic changes in the pituary gland and gonads of *Siganus rivulatus* from the Red Sea (Hurghada Area). Journal of Kau. Marine Science 7, 271-284, special issue, Symposium on Red Sea Marine Environment.