Chapter 18

Research in aquaculture

18.1 Research in aquaculture

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Scientific research in aquaculture has always represented a solid base for its sustainable development. law No 41/1982 (repealed when Legislative Decree 154/2004 came into force) expressly envisaged the development of applied scientific and technological research into marine fisheries and aquaculture in marine and brackish waters, as being useful for achieving the objectives of the law itself. The interventions were organised into three-year fisheries and aquaculture plans. Article 7 of law No 41/1982 envisaged that, alongside the studies relating to the assessment of living resources, priority should also be given to those concerning aquaculture in marine or brackish waters.

The three-year fisheries plans, from the time that the Law 41/1982 was first applied, envisaged a consistent programme of research activities, including also those aimed at aquaculture development. The second three-year fisheries and aquaculture plan (that came into effect with the Ministerial Decree of 4 August 1988) constituted a shift in that direction, in as much as it highlighted, for the first time ever, the need to develop research on aquaculture. In fact, the defining aquaculture as a "young" sector was an admission that it had not "got under way with advanced cognitive bases, because at that time, no single multidisciplinary research organisation formed of various components had yet been developed". Describing the national aquaculture scenario, the plan defined research as "a key factor", but highlighted how the "lack of State-funded research centres to carry out applied research" had up to that point been "without doubt a delaying factor".

Hand in hand with the maintaining of a high level of priority and as proof of the determination in programming to proceed in this pathway, research in aquaculture (beginning with the second and in the subsequent three-year plans) recorded a marked growth as evidenced by the increase in the number of projects (table 18.1) and the amount of total funding, with a peak in the fourth plan.

Table 18.1 - Number of projects and funding per three-year plan. Source: MiPAAF (Ministry of Agriculture, Food and Forestry Policy) research data, General Directorate for Marine Fisheries and Aquaculture.

Three-year plan	No. of aquaculture projects	Total funding (€)	Average funding (per project - €)	% of total no. of projects funded
I (1984-86)	25	1,139,820.45	45,592	31
II (1988-90)	35	2,688,158.11	76,804	34
III (1991-93)	50	5,685,673.94	113,713	43
IV (1994-96)	162	12,618,591.32	77,892	57
V (1997-99)	103	8,672,860.00	84,202	55
VI (2000-02)	103	8,670,165.25	84,176	48

The fifth three-year plan (1997-1999) contained an admission that “research in aquaculture has been strongly diversified” and that “a system of research has been put together that is coherent with the needs of our aquaculture, which is currently the most diversified in Europe”. It went to highlight that “in this sense it has been agreed to create a strongly coordinated widespread research action”. The sixth three-year plan (2000-2002) stated that “research in aquaculture has found a support and coordination system in the national plan that generated a real national research network involving all the regulatory components of both public and private institutions”.
There was also an increase in funding allocated to research in aquaculture in relation to the total amount of funding from the three-year plans (figure 18.1). From 2002 many of the interventions already funded for the area of living resources were included in the national programme for the collection of fisheries data on the basis of Regulation (EC) 1543/2001, then replaced by Regulation (EC) 199/2008, and thus were no longer funded in the three-year plans.
Extremely positive effects on aquaculture planning were obtained with the implementation of research in aquaculture. Networks were created among research teams which still represent an important point of reference. In addition to being used for drawing up the policies and guidelines for aquaculture development, the results have also supported aquaculture growth. The correct transfer of scientific knowledge contributed to keeping operators briefed and rendering them more knowledgeable of management policies and legislative decisions.
During the implementation of the three-year plans the encouragement of higher coordination and a multidisciplinary approach, based on the precise expression of the Coordinating Committee for scientific and technological research applied to fisheries and aquaculture (Article 6 of Law 41/1982), was constructively received by the operational units involved. These teams favoured the establishment of networks of public and private research institutions working in close collaboration. The results of the research projects highlighted that this organisation prevented overlaps, repetitions or gaps in research, with a more rational use of allocated funds.
The coordinated research teams, which came into operation with the fourth three-year plan, worked on off-shore mariculture, mollusc farms, pathologies of cultured species, animal nutrition, feeds, product quality, aquaculture technologies and shellfish culture. Coordinated research teams were also established to deal with the topics highlighted in the Code of Conduct for Responsible Fisheries (Article 9, FAO 1995), such as the quality of the culture environment, animal welfare, interactions between aquaculture and the environment, genetics.
Beginning with the fifth three-year plan and still operating today, a coordinated team was established with different units carrying out research activities on the bluefin tuna farming - a topic that is still very relevant today. Given the extremely positive outcomes of the early experimental phases, during the fifth and sixth three-year plans, research on bluefin tuna farming continued. The work, also undertaken within the framework of the activities promoted by the ICCAT resulted in a broader knowledge of the reproductive biology and the development of a responsible and sustainable culture of this species. Experimental protocols were developed that were transferable to commercial farms, whilst also focussing on nutritional quality and safety.
Research projects on potential new indigenous species to be used in aquaculture took on a particular significance within the sixth three-year plan. The programme was developed and implemented to increase Italian aquaculture production, while establishing responsible methods for operating. New areas of knowledge were acquired in relation to the production of new species, without neglecting the impact on biodiversity on various scales. Eleven research teams participated to the projects on new species, such as bluefin tuna and porgy.

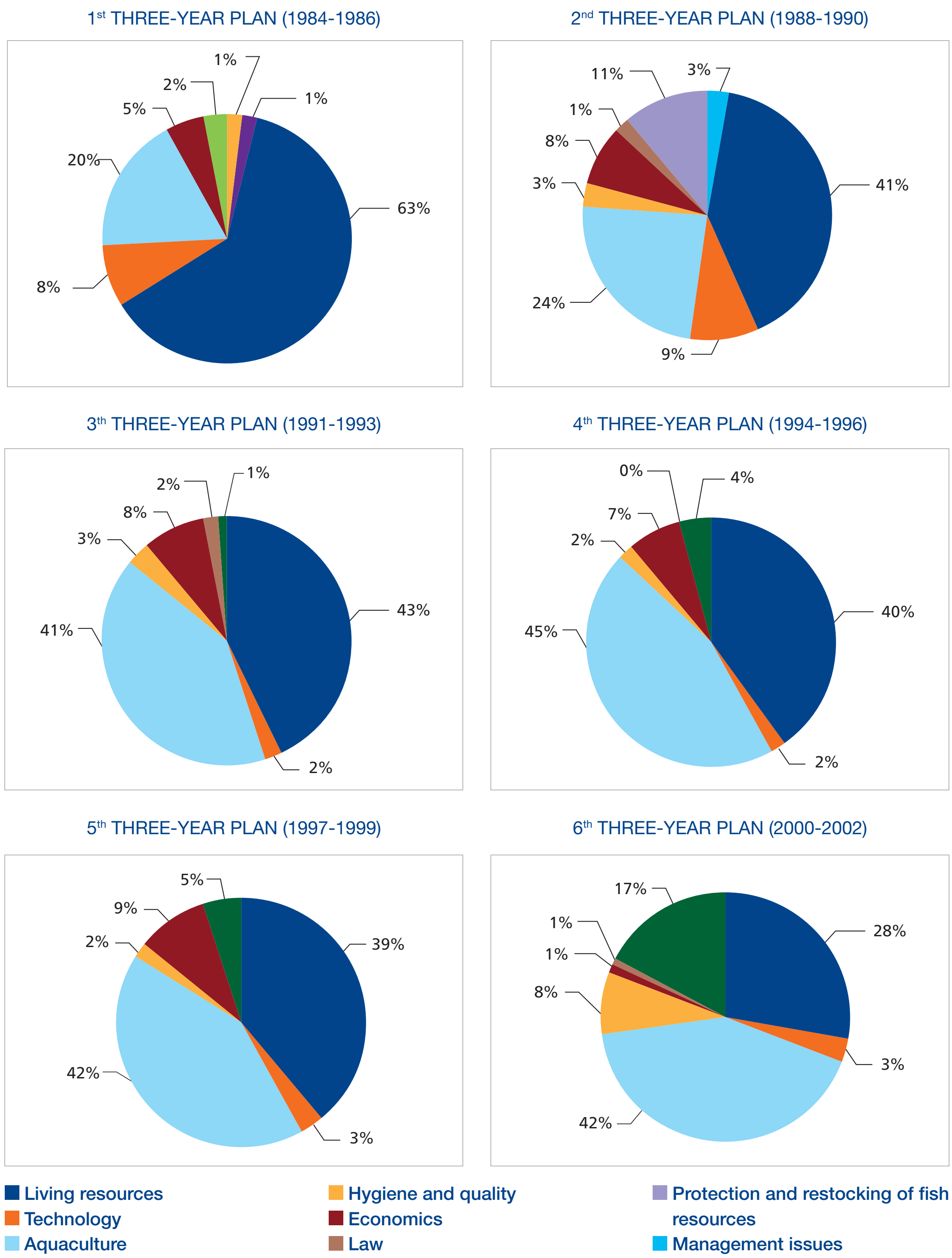

Figure 18.1 - Distribution of financial resources for scientific research by subject area (contributions allocated) – Three-year fisheries and aquaculture plans in marine and brackish waters (Source: MiPAAF research data, D.G. Marine fisheries and aquaculture).

Looking at another operational aspect of aquaculture activity, research projects were also carried out on coastal lagoons restocking with certified juveniles, always as part of the sixth three-year plan, involved a large coordinated team of operational units (13). Research scientists turned their attention to the study and prevention of the negative effects of restocking, using a new technology for mass production of wild-like juveniles and pursuing the certification of the material for restocking on a genetic, morphological and behavioural basis. All of this allowed, as an applicative result, responsible restocking actions to enhance fisheries. Legislative Decree 154/2004, regarding “Modernisation of fisheries and aquaculture in accordance with Article 1, Paragraph 2 of Law 38/2003”, which repealed Law 41/1982, took into account the distribution of responsibilities between the State and the administrative regions as per the amendment to Title V of the Constitution.
This reform ratified the legislative as well as the regulatory power of the Regions as far as aquaculture is concerned, something that had not been included in the list in Article 3 of the aforementioned law (issues for which the State had sole responsibility), nor among those in the shared legislation, for which the establishing of the fundamental principles rests with the State. The same legislative decree introduced the criterion of sustainability as the basis for integrating the measures for the protection of aquatic resources and the environment and the protection of commercial activities. It also envisaged that those interventions that fall under national jurisdiction (guidance and policies) should be listed in the national fisheries and aquaculture programme. Among the objectives of the programmes, the regulation envisages the “development of applied scientific research in fisheries and aquaculture” aimed at “sustaining the pursuit of the objectives envisaged by the national programme”. Particular reference was made to: the pursuit of the “durability of fish resources for present and future generations and safeguarding of biodiversities”; the “sustainable development of fisheries and aquaculture and related activities”; the “safeguarding of the consumer in terms of traceability of fish products, enhancing the quality of national production and transparency of information”.
On the basis of these the “National Fisheries and Aquaculture Programme for the year 2005” and the “First three-year fisheries and aquaculture programme 2007-2009” both envisaged redirecting research in aquaculture towards, guidance and coordination of the policies associated with aquaculture.
With regard to the above, in the implementation of the first three-year programme, extended to all of 2011 with Law 10/2011 (conversion of the “thousand extensions” decree), particular attention focussed on issues of national interest aimed at formulating guidelines.
The coming into effect of Regulation (EC) 834/2007 and subsequent modifications led to the pinpointing of the area of organic aquaculture and the establishment of a new team of tightly coordinated research units, taking into account the need for research to support the guidelines for organic aquaculture at a national level. The research programme entitled “Concerted Action for identifying the scientific contributions to the development of organic aquaculture in Italy” was a coordinated project in which eleven national research institutes were involved. The overall objective consisted of creating an expertise network, based on the model of concerted European actions, that was capable of summarising the available know-how and developing the appropriate scientific analysis to support the development of aquaculture and the validation of culture protocols that would have an effect on the four areas recognised as essential for ensuring sustainability in aquaculture: environment and biodiversity, animal welfare, food safety and quality and social responsibility. To set out future research priorities, the Communication from

the Commission to the European Parliament and the Council No 162 dated 8 April 2009 entitled "Building a sustainable future for aquaculture - A new impetus for the Strategy for the Sustainable Development of European Aquaculture" invited all Member States to guarantee advanced levels of research and technology for the production of food products and equipment, to maintain a strong competitive edge in research and to increase investments in the research sector associated with aquaculture and maintain the level of excellence in the field of research. Some of the key issues and priorities indicated by the European Commission had already been taken into consideration by the Italian Government during the implementation of the three-year plans and the first national fisheries and aquaculture programme. The provisions included in the communication led to the encouragement and supporting of further development of research in aquaculture so that this would responsibly free the potential for development and respond to the growing requests for the consumption of fish products, thus enabling a reduction in the overexploitation of wild stocks.
Even the document relating to the reform of the Common Fisheries Policy (EC Communication No 417 of 2011) refers specifically to the need to promote "an aquaculture that is sustainable, competitive and diversified, supported by the more advanced results in research and technology" through the drawing up of "national strategic plans" by Member States.
All of this demonstrates the need to sustain adequate research in support of a sustainable development of aquaculture.

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- Regulation (EC) 834/2007 of the Council of 28 June 2007 on the organic production and labelling of organic products and repealing Regulation (EEC) No 2092/91.
- Regulation (EC) 967/2008 of the Council of 29 September 2008, amending Regulation (EC) No 834/2007 of 26 February 2011, of conversion of Decree-law No 225, 29 December.

18.2 Some innovations in research

Cataudella S.

Recently the MiPAAF published "*La ricerca scientifica a supporto della pesca e dell'acquacoltura*" (Unimar, 2011). Such activity was carried out during the extension of the results of the projects from the 5th and 6th Fisheries and Aquaculture three-year plans. This publication contains a synthesis of the research projects, divided into topics (improvement of fishing gear, new candidate species for aquaculture; restocking in natural environments; diseases prophylaxis and animal welfare in aquaculture; food quality in fish products; economic and ecological sustainability of capture fisheries and aquaculture). For each topic, several experts wrote a synthesis, very useful to evaluate the research activities which were carried out and to make the results and recommendations from these projects available to the general public.
Selected innovative research activities in aquaculture, that can be transversally used in research projects with a high interest also for capture fisheries, are summarized in the following paragraphs. For complete information please refer to the above cited publication.

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18.2.1 Shape studies

Costa C., Pulcini D., Cataudella S.

Shape studies on fish represent a useful issue in both ecological and evolutionary framework (Pulcini *et al.*, 2008), and also a powerful and low-cost applied tool for selection practices being empirically performed by aquaculturists. In Italy the first experiences of such methods were applied to the common carp (Corti *et al.*, 1988) and European sea bass reared at different conditions (Loy *et al.*, 1993; Loy *et al.*, 1996). Shape description and analyses are ready references to study and classify organisms which require a tool to graphically and geometrically represent shape and its variation and to measure shape, analyzing it quantitatively.
The methodologies used to quantitatively study the shape of an organism (or a part of it) could be summarized into three main approaches (Costa *et al.*, 2011) (figure 18.2):

- Traditional morphometry (based on single measures and ratios);
- Geometric morphometry (based on the geometric relationships among points of biological or structural homology – landmarks);
- Outline methods (based on algorithms which synthesize the external shape of an object).

In aquaculture these incruent techniques base their principles on the tight links among growth, shape, functions, performances and rearing conditions. The latter can influence individual morphology or particular structure shape caused by the phenotypic plasticity and eco-phenotypic response which characterize fish species. Through the study of relationships between growth and shape it is possible to identify the pathway of morphological modifications (growth trajectories) in the different species in order to answer to the questions on shape changes velocity, if a relation between the larval stage and ecology occurs, or if culture conditions modify shape with respect to the wild.
Rearing conditions resulted to be relevant for the onset of morphological anomalies assessed

with shape analyses (Boglione & Costa, 2011), determining a performance reduction and consequently an economic loss for aquaculture companies. Through geometric morphometrics and outline methods it was possible to morphologically discriminate between lots reared at different conditions visualizing external shape differences. It was moreover possible to build statistical models able to quantify shape quality with respect to a wild reference (figure 18.8). This approach, once integrated in an industrial opto-electronic on-line processing system, could allow an indirect increase of high quality fish productions.

Shape methods can also be successfully applied in species identification. Recent studies demonstrated the high efficiency (99%) in discriminating the clams *Ruditapes decussatus* and *Ruditapes philippinarum* (Costa *et al.*, 2008) using the elliptic Fourier analysis on shell outlines.

Form could also be used to estimate fish size and weight. A recent study funded by the European Commission demonstrated that complex shape descriptors (such as elliptic Fourier coefficients) could increase the precision in weight estimation if compared with traditional morphometric methods (log of body length). Several projects funded by the Italian Ministry of Agriculture, Food and Forestry Policies (MiPAAF) and carried out by the University of Rome “Tor Vergata” led to the construction of a dual camera system prototype to count and size Mediterranean bluefin tuna during transfer from the purse-seine to the culture cages. Couples of images acquired by the opto-electronic dual camera system were processed by an artificial neural network and estimated fish lengths (Costa *et al.*, 2009) (figure 18.9). Another application concerns the count and measure of tunas from aerial images applying a custom image analysis procedure.

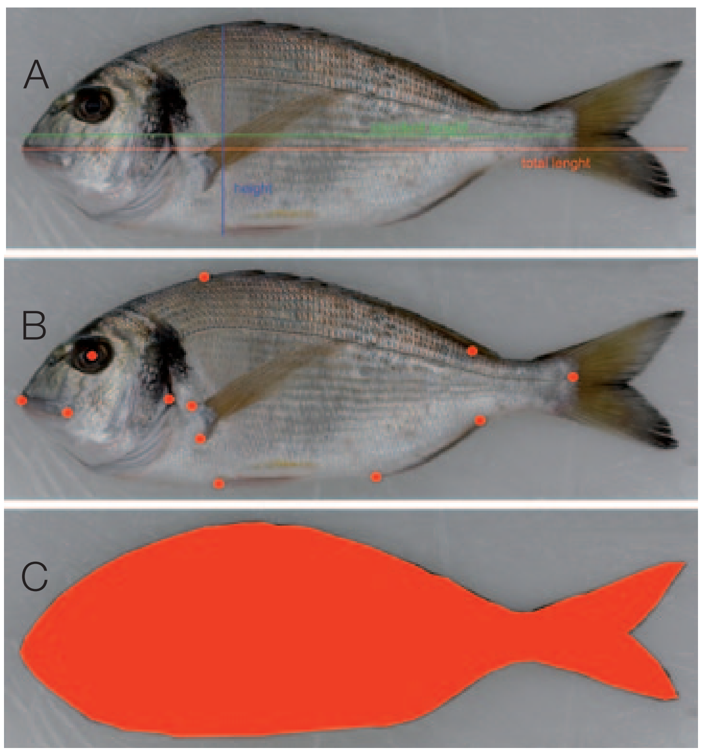

Figure 18.2 - Examples of morphometric measurements on gilthead sea bream (*Sparus aurata*). (A) Biometric measurements (lengths, heights); (B) homology points (landmarks, in red) selected on the fish body; (C) body outline (in red).

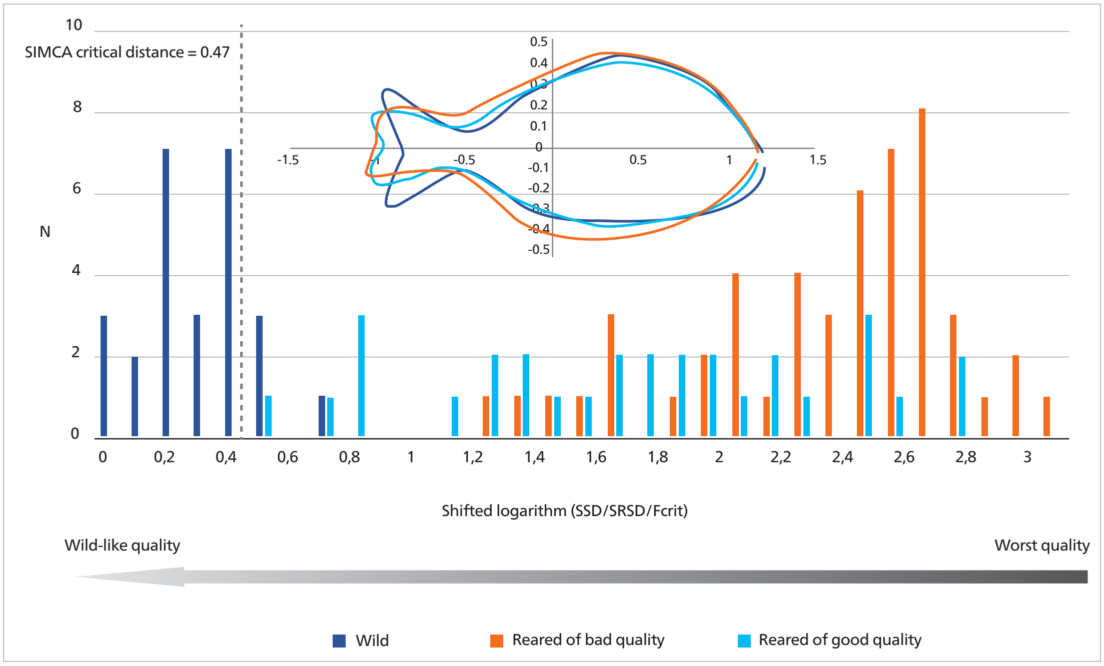

Figure 18.3 - Example of a model (SIMCA - Soft Independent Modelling Class Analogy) based on the external profile of wild and reared at different densities gilthead sea bream. In the middle of the figure the mean configuration of each group is represented. N = number of samples (Source: Project MiPAAF "Sistemi innovativi per la tracciabilità della filiera ittica").

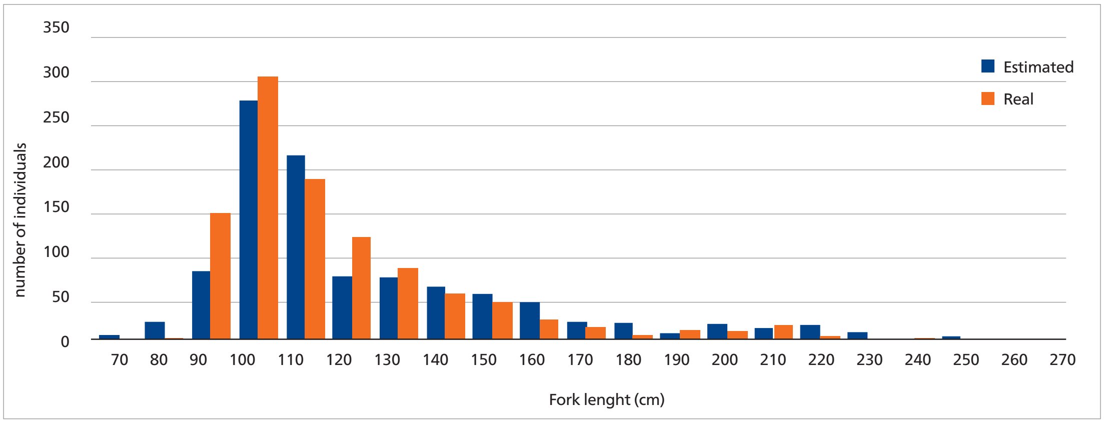

Figure 18.4 - Number and lengths of bluefin tuna assessed during transfer to culture cages with a semi automated Artificial Neural Network tool on June 28th 2005 near Alicudi (Eolian Islands). (Source: Project MiPAAF "Innovazioni tecnologiche per l'acquacoltura responsabile del tonno rosso: raccolta e analisi di immagini per applicazioni morfometriche alla valutazione quantitativa delle catture trasferite, tecnologie per il controllo della biologia riproduttiva e delle potenziali emissioni di gameti spontanee e indotte in mare" - 6C138).

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18.2.2 Research in aquaculture: monitoring the morphological quality of reared larvae and juveniles

Boglione C., Cataudella S.

Since the first marine fish larvae (figure 18.5) rearing trials, scientists realized that hatchery produced larvae showed differences in shape and pigmentation from wild conspecific ones (Cataudella S. & Bronzi P. (eds), 2001). As the first produced larvae were obtained from captive wild breeders, the observed differences were attributed to confined rearing conditions and human handling.

Figure 18.5 - Bluefin tuna larva (*Thunnus thynnus* L., 1758) at hatching (photo by C. Boglione).

Anomalous individuals (figure 6-7) are still present today in reared lots, with frequencies varying according to species, developmental stages and/or rearing technology. The monitoring of skeletal anomalies in reared European seabass and gilthead seabream carried out in a research project supported by the Italian Ministry of Agriculture, Food and Forestry Policies enhanced that the rate of severely deformed fishes (i.e. with skeletal anomalies that affected body shape) ranged from 15% (hatchery lot) to 44% (commercial size lot) in seabass, and from 16% (hatchery lot) to 35% (pre-ongrowing lot) in seabream.

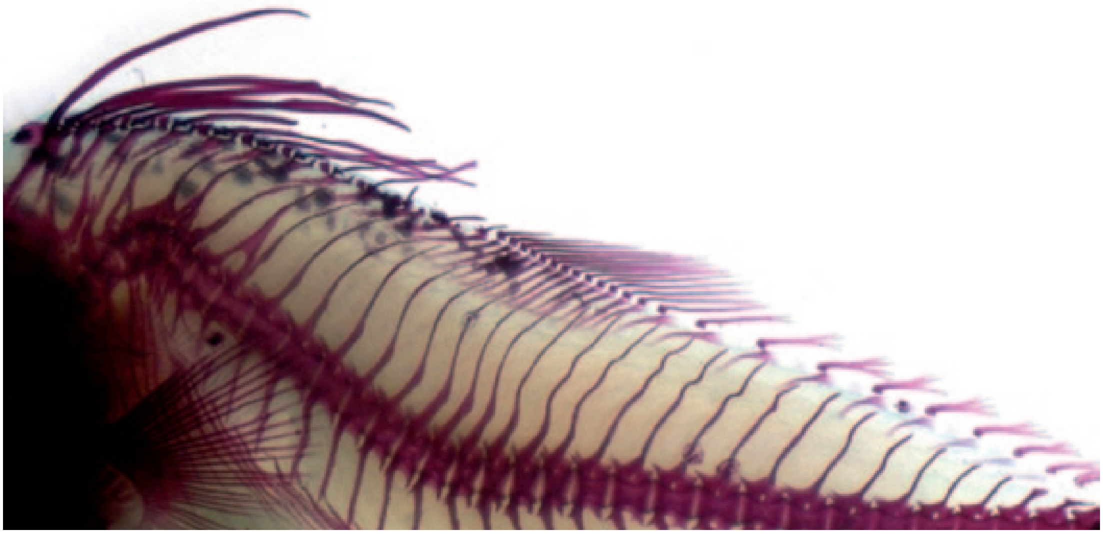

Figure 18.6 - Reared bluefin tuna postlarva (SL = 27.3 mm) showing several severe vertebrae anomalies. Staining: Alizarin S red (bone tissue) and Alcian blue (cartilage) (photo by M. Marroncini).

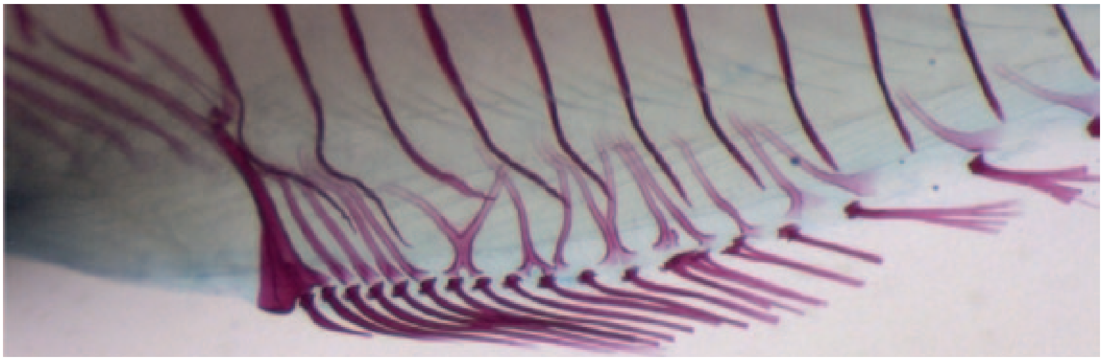

Figure 18.7 - Reared bluefin tuna postlarva (SL = 28.6 mm) showing anomalous inner supports (pterygophores) of anal fin. Staining: Alizarin S red (bone tissue) and Alcian blue (cartilage) (photo by M. Marroncini).

Severely deformed fish can induce diffidence in consumers regarding aquaculture products. These fish have to be culled out before they reach the market, thus reducing the profitability of aquaculture production. Furthermore automatic fillet processing is impaired by the presence of vertebral anomalies, thus lowering the economic input of processing deformed fishes. During the rearing cycle, deformed fish grow slowly, as locomotion and feeding are impaired by severe skeletal anomalies; they are weaker than unaffected fish and more subject to disease and parasites. Consequently they cannot be considered as fish in welfare condition. Repeated grading are necessary all along the productive cycle in order to cull out deformed fish because they can arise all along the rearing cycle, with economic (extra work) and biological (handling stress) effects.

The identification of the causative factors is not an easy task as different non-genetic causes may induce the same anomaly in different species and the same causative factor can determine different anomalies at different stages or in different species. The current wide knowledge on human osteopathology can only rarely be shifted to be used for lowering skeletal anomalies in reared fish: mammalian skeletal tissues are often categorised as either bone or cartilage but fish skeletal tissues include several types of bone, many different types of cartilage and many tissues types that are intermediate between connective tissue and bone, and between bone and cartilage (Hall & Witten 2007, Witten *et al*. 2010). The frame is complicated by the presence of differences in bone tissues not only among *Chondrichthyes* (cartilaginous fishes) and *Osteichthyes* (bony fish) but, in the latter, also between Chondrostei (e.g., sturgeons) and Teleosts and between Euteleosts (e.g. salmonids) and Neoteleosts (e.g., seabream, seabass and flatfish). The actual state of knowledge on causative factors of skeletal anomalies onset in reared fish has detected many genetic and non-genetic different causative factors, very often interacting synergycally.

In the last twenty years, the University of Rome "Tor Vergata" has carried a series of studies aimed at ameliorating the final quality of reared juveniles by using the larvae monitoring method, which is based on the assessment of the quantity and typology of morphological differences at skeleton level observed between farmed and wild juveniles, of the same species. A large dataset on meristic count and skeletal anomalies inspected in reared and wild finfish juveniles (European seabass, gilthead seabream, mullets, sharpsnout seabream, dusky grouper, pandora, common seabream, dentex, greater amberjack and bluefin tuna) has been created in our laboratory and used to enhance the direct effects of rearing conditions on anomalies onset and incidences. The peculiarity of larval monitoring is that the morphological quality reference used to assess the quality of reared fish is the skeletal pattern observed in wild conspecific fishes living in unpolluted water. This implies that the higher quality is obtained in juveniles reared in welfare conditions, which are able to determine the most *wild-like* phenotype (*wild-like* reared fish, Cataudella *et al.*, 2002). A more recent implementation of the methodology foresees the use of artificial intelligence tools in order to find correlation between different multiparametric and multilevel (anatomical, physiological, productive, genetic, nutritional, etc.) data and skeletal anomalies type and incidence (Russo *et al.*, 2010; Russo *et al.*, 2011).

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18.2.3 Molecular sciences in aquaculture

Saroglia M., Bernardini G., Terova G.

The XXI century aquaculture must cope with old and new issues which are connected to its sustainability. Among them, nutrition strategies, aiming at reducing fish meal (FM) and oil (FO) in favor of vegetable ones (VM and VO), stand out. Moreover, fish welfare, diseases prevention, market dynamics and related production strategies, product quality for human health, new species, certifications and traceability require new knowledge. In this context, there is a need of tools for an early monitoring of the physiological responses at the cellular-tissutal level to afford the assessment long before the mounting of any zootechnical and clinical evidence.
The ‘omic approach responds to these needs as it allows a monitoring of the response of genes during the whole life span of cultured fish, as well as the identification of early markers of freshness such as muscle proteins which are first degraded during the *post mortem* shelf life. The tools generated by the scientific revolution following the Human Genome Project (Venter *et al.*, 2001), being applicable on other species, became available to aquaculture as well (Gornati *et al.*, 2005). Furthermore, gene mapping and transcriptomic activity on fish and shellfish species have been performed in a number of research projects since the early 2000, leading to aquaculture-related functional genomics, nutrigenomics, proteomics and metabolomics (Saroglia & Liu, 2012). After a first period during which the molecular approach was available only in highly sophisticated research facilities, a reduction of the costs and protocol simplification, have introduced the ‘omic methodologies in most laboratories. As machines become faster, cheaper, smaller, and easier to use, more in-field applications for this technology in aquaculture are likely to be realized.
The first step, consisting on fish genes mapping, was completed in at least six species of fish, while dozens of such projects are in progress, for other fish species as well as mollusks and crustaceans interesting for aquaculture. The knowledge of gene sequences (structural genomics) allows to associate somatic responses to the transcriptional activity of specific genes (functional genomics) which in turn respond to stimulation of the cell originated by environmental agents, nutritional or internal body reactions. The transcriptional activity of genes can be quantitatively assessed by titration of the messenger RNA (mRNA) from sampled tissues, thus obtaining detailed early information about the animal response.
Recent developments in cutting-edge technologies such as proteomics could give better insight into the mechanisms involved in postmortem-related biochemical processes in fish, thus facilitating the identification of markers that can predict fish quality. Beyond, metabolomics provides an overall picture of the products of cellular metabolites, derived from the cascade of reactions

initiated by the transcription and followed by enzymatic synthesis, and with the subsequent reactions of the enzymes with their specific substrates.

By providing an integrated genomic-protein-axis that characterizes the metabolic cellular response to any kind of perturbation, the molecular approach allows to assess the animal reaction when the response is still elaborated by the cells, so long before the possibility to monitor it at the physiological, clinical or livestock levels. Among other numerous possible advantages, this enables to improve the nutritional strategy, prevent mortality, control reproduction processes, and monitor in vivo as well as post mortem quality.

Following the first Aquaculture Genome Workshop held in Dartmouth, Massachusett, in the fall of 1997, among the first in Europe, Monetti *et al.* (2002, 2003) reported the responses of candidate genes that may describe fish stress and welfare conditions, while Vilhelmsson *et al.* (2003) reported a proteomic study showing trout responding to different protein sources as FM and VM substitutions. It was only the fact that fish density biomass and nutritional changes cause the activation or deactivation of specific genes, then the substitution of fish meal and fish oil with plant products alter the framework of hepatic proteins, but it represented the beginning of a profound transformation in the methods of study. In Italy, starting from a research program as the Fifth Three-year Plan for Fisheries and Aquaculture (MiPAAF), resources were given to projects affording the omic approach on fish welfare, organic aquaculture, fish nutrition, new species. The feed-back consisted in a number of papers affording sea bass growth, welfare and nutrition (Terova *et al.*, 2005; 2007; 2008). Moreover, 1,229 clones from a cDNA library of sea bass (*D. labrax*) as well as 10,163 clones from cDNA libraries of liver, testis and ovary of bluefin tuna (*Thunnus thynnus*) were produced and deposited in the NCBI database (http://www.ncbi.nlm.nih.gov/), thus greatly expanding the number of genes from which to seek answers (Chini *et al.*, 2006, 2008). It arises, which provisional synthesis, a tool box of molecular markers containing the sequences of the genes that can be used to study the response of the fish to environmental factors and food, allowing inter alia a targeted approach to the nutrigenomic study (see Table 1 in Terova *et al.*, 2013). Later, a significant contribution to nutrigenomics became available with the identification of seabass intestinal oligopeptide transporter for (PepT1) (Terova *et al.*, 2009; Verri *et al.*, 2011; Sangaletti *et al.*, 2009).

Although the study of the activity of genes by functional genomics is now affordable by the majority of laboratories and kits are available for “on-field” applications, it is still complex and expensive to study what happens downstream. Nevertheless, following a project on organic aquaculture granted by MiPAAF, indicators of post-mortem fillet protein degradation, together with the influence of different slaughtering and conservation protocols, were identified by means of two-dimensional differential electrophoresis (2D-DIGE) and mass spectrometry (HPLC-MS), in B-diphosphate kinase and 2-phosphoglycerate mutase, as shown in figure 18.8.

In spite of metabolomics is being still less applied, it has been included by MIT’s Technology Review Boston among the top ten emerging technologies. With it, it is possible to describe the profile of the metabolites present in cells, tissues, organs and biological fluids. In aquaculture it may potentially provide basic indications for the formulation of specific diets, especially if associated with nutrigenomics. In Italy, metabolomics was proposed by Anedda (2009) for a study on the quality and traceability in gilthead sea bream, by Scano *et al.* (2009) for the study of lipids in the grey mullet bottarga (salted and dried egg roe), then by Savorani *et al.* (2010), to study the effect of different techniques of breeding and conservation. The cost of the instrumentation and the know-how required for experiments of Nuclear Magnetic Resonance (NMR) along with the chemometric complexity of data analysis discourage the metabolomic approach. Nevertheless, possibility to afford metabolomics with cheaper approaches as HPLC-MS are under study, so that these still existing obstacles could be overcome and metabolomics could assume an important role for research in aquaculture.

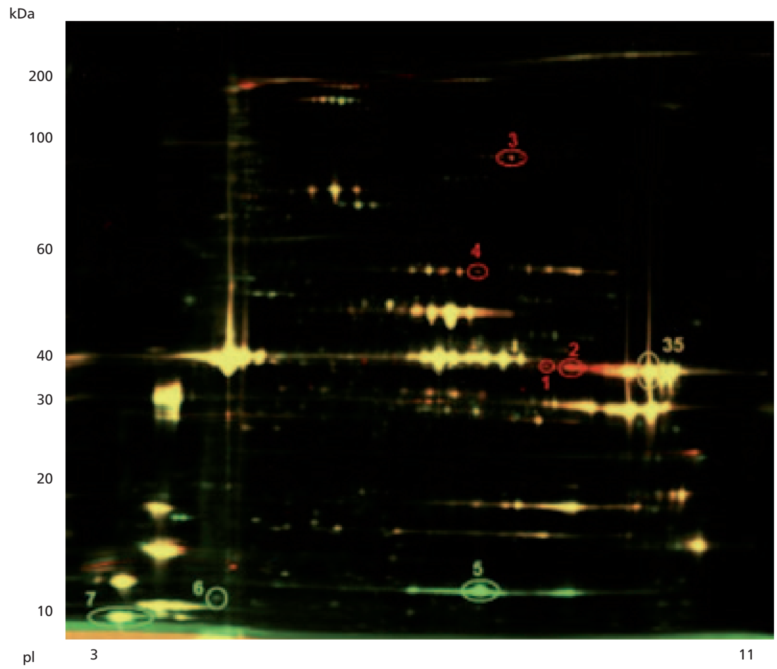

Figure 18.8 - Two-dimensional differential electrophoresis (DIGE) of the protein profile of muscle of sea bass just slaughtered and after 5 days of storage at a temperature of 1 °C or 18 °C. The green spots correspond to a reduction of the presence of the corresponding protein, while the red spots indicate an increase of the same protein. The overlapping of green and red (yellow spots) indicate substantial stability of the protein that migrated at the same spot (Terova *et al.* 2011).

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