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Enhancing Innovation and Sustainability in the Adriatic Aquaculture

MANUAL OF EUROPEAN SEA BASS AND GILTHEAD SEA BREAM SAFETY, QUALITY AND HEALTH BENEFITS

Hrvatski veterinarski institut

1933

Zagreb

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FOREWORD

AdriAquaNet (*Enhancing Innovation and Sustainability in Adriatic Aquaculture*) is an Interreg Italy-Croatia V-A 2014–2020 project which falls into Priority Axis 1 "Blue innovation – *Enhance the framework conditions for innovation in the relevant sectors of the blue economy within the cooperation area*".

The main goal of the project is to strengthen sustainable aquaculture in the Adriatic Sea by transferring advanced knowledge and new technologies throughout the aquaculture supply chain, from production management on the farm to marketing the processed product. The project is conceived to intervene in three aspects of the value chain:

1. Improvement of the farming procedure through innovative feed formulas and feeding procedures to improve the quality of fish and to conserve the environment, while implementing energy-saving technologies;
2. Implementing a new approach to health and welfare management through vaccination against bacterial diseases and the application of natural products for treatments;
3. Developing guidelines for fish consumers by assessing fish safety and quality, sensory and nutritional properties and health benefits and eventually, presenting these facts through a comprehensive marketing campaign to consumers of the Adriatic region.

The "Manual of European Sea Bass and Gilthead Sea Bream Safety, Quality and Health Benefits" is a starting document intended to educate stakeholders, including producers and consumers, on how to evaluate the safety, quality, and benefits of farmed fish consumption. It is an outcome of the activities undertaken within Work package 5. Improving the quality and marketing of fresh and processed fish, task 5.1. Determination of the farmed fish quality and shelf-life safety. The authors are partners in the AdriAquaNet project, as leading experts in a particular field of safety and fish quality from both side of Adriatic Sea, Italy and Croatia, and they are listed in alphabetical order:

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However, the appearance of this handbook would never have taken this form without the selfless help of sea bass and sea bream farmers, partners in AdriAquaNet, Orada Adriatic, Ltd., Cres and Kukuljanovo and Friškina, Ltd., Split, who, in addition to participating in all research activities, also left beautiful photographs. We are especially grateful to Mr David Skoko for the photos of the meals prepared from sea bass and sea bream farmers.

Dr. **Snježana Zrnčić**, Croatian Veterinary Institute

1. INTRODUCTION

Snježana Zrnčić,

Croatian Veterinary Institute, Laboratory for Fish Pathology

Emilio Tibaldi i Marco Galeotti,

University of Udine, Dept. of Agricultural, Food, Environmental and Animal Sciences

The Mediterranean diet, inscribed in 2013 on the Representative List of the Intangible Cultural Heritage of Humanity by UNESCO, is defined as follows: "*The Mediterranean diet involves a set of skills, knowledge, rituals, symbols and traditions concerning crops, harvesting, fishing, animal husbandry, conservation, processing, cooking, and particularly the sharing and consumption of food. Eating together is the foundation of the cultural identity and continuity of communities throughout the Mediterranean basin. It is a moment of social exchange and communication, an affirmation and renewal of family, group or community identity. The Mediterranean diet emphasizes values of hospitality, neighbourliness, intercultural dialogue and creativity, and a way of life guided by respect for diversity. It plays a vital role in cultural spaces, festivals and celebrations, bringing together people of all ages, conditions and social classes...*"

One of the very important foodstuffs of the Mediterranean diet is fish and fish products. A necessary precondition for the safety and quality of fresh fish and fish products is that fish originates from the high-quality and controlled marine environment.

The Interreg Italy-Croatia financed project "AdriAquaNet" is focused on marine aquaculture, as a very important economic activity carried out in the Adriatic Sea, both along the Italian and Croatian coasts. Aquaculture is one of the fastest-growing productive sectors among the animal food sectors, so much so that it has outstripped fishery production in recent years. In other words, today more than one fish out of two in the world is produced on the farm (FAO, 2018). Although less significant in Europe and western countries than in Asia, this trend has also characterised the Adriatic coast countries and in particular, Croatia where the marine aquaculture of European sea bass (*Dicentrarchus labrax*) and Gilthead sea bream (*Sparus aurata*) has now achieved significant production targets. This growth reflects in and is the result of recent developments on the fish market and, on the demand side, it also results from significant evolution and changes in consumer food habits that are increasingly orienting their market choices towards modern, functional and healthy food items.

The rapid growth of production volume and the spread of new sites for aquaculture, has accentuated the sensitivity of public opinion and decision-makers as to the environmental effects and the socio-economic sustainability of aquaculture growth, in the broader and sometimes competitive perspective of integrated coastal zone management. The growing aquaculture industry also raises consumer concerns about the overall quality of farmed fish compared to the fishery counterpart in terms of appearance, health attributes of fish flesh, and seafood safety.

These issues are driving the strategies of all actors involved in aquaculture production and supply chains, which are firmly engaged in developing and adopting environmentally friendly solutions for fish farming and healthy and nutritious strategies to improve fish quality and assure consumers.

For all these reasons, the main activities of the AdriAquaNet project aiming to achieve comprehensive improvement of marine aquaculture in the Adriatic region are directed towards implementing:

i) new fish feeds and feeding protocols to improve fish quality and limit pollution of the marine environment;
ii) waste management systems for reducing pollution and techniques for improving the environmental and energetic sustainability of fish farming;
iii) new vaccines and natural compounds for the control of infectious diseases and to promote fish health, and to provide consumers with "healthy and safe" fish;
iv) innovative and functional marketing systems to increase the awareness of the quality of both fish species.

The project activities will result in the development of new solutions or adopting existing solutions to ensure fish farm sustainability. AdriAquaNet will increase the environmental sustainability of fish farming by designing new fish feeds that will result in healthier fish of better quality for consumers, lowering the unfavourable impacts of farming on the environment, and achieve more economic production. An innovative anaerobic treatment of wastes from intensive aquaculture facilities and the fish hatchery will be tested, obtaining biogas that is useable as renewable energy (both thermal and electrical), and making the safe use of wastes as organic fertilisers possible to reduce their environmental impacts.

AdriAquaNet will also promote fish health and simultaneously provide safer products to consumers by proposing novel solutions to limit disease outbreaks caused by bacterial and parasitic pathogens. Instead of treating bacterial infections using antibiotics, AdriAquaNet will produce and test specific autologous vaccines to prevent disease outbreaks. To treat parasitic infestations, pyrethrins extracted from *Chrysanthemum cinerariifolium* will be proposed as a novel, biocompatible, readily available, economic, antiparasitic substance. A new probiotic formulation obtained from Adriatic sea bass and sea bream microbiome, as well as marine natural products (MNPs) from invertebrates or microalgae, will be proposed as innovative solutions with antimicrobial activity and immunostimulant properties to replace the use of synthetic drugs or additives, thereby avoiding antibiotic resistance development and environmental pollution. Eventually, avoidance of chemical treatments will have several benefits for the economy of fish farming, quality of environment, and the safety and quality of the final product. Moreover, new Operational Welfare Indicators will be proposed to be easily used by fish farmers to assess sea bass/sea bream welfare.

New packaging solutions will be evaluated to increase the shelf-life of fresh fish and fish products, and consequently to raise SME competitiveness.

All the innovative solutions tested and implemented through AdriAquaNet will result in a good quality, market-size fresh fish and the project is endeavouring to define the quality of the two most important farmed fish species in the Mediterranean, European sea bass (*Dicentrarchus labrax*) and Gilthead sea bream (*Sparus aurata*). The quality of farmed sea bass and sea bream will be analysed and proved by the determination of specific physico-chemical and microbial indices, and identified based on the results of analysis nutritional quality of fish products.

Based on the chemical composition and content of particular fatty acids, the health indices of Adriatic sea bream and sea bass consumption will be calculated. Additionally, to fill the market demand for ready-to-use fish products and to enrich the offer of fish products prepared from sea bass and sea bream, new products such as sea bass and sea bream burgers and smoked fillets will be developed.

Marketing and promoting these aspects among the population will be performed to increase and broaden consumption of healthy fish/high-quality fish products produced in Croatia and Italy.

Generally, there is a growing demand for seafood due to its high quality and nutritional performance. Fish is a high-quality food, offering a source of macro- and micronutrients essential for the normal functioning of the human body. It is also easily digestible and protein rich in amino acids and omega-3 fatty acids. A mutual WHO and FAO (2011) consultation on the benefits and risks of fish consumption concluded that there is convincing evidence of the health benefits of fish consumption, such as a reduction in the risk of cardiac death, and improved neurodevelopment in infants and young children when fish is consumed by the mother before and during pregnancy. There are also numerous studies demonstrating the health attributes of fish due to long-chain omega-3 PUFAs and other nutrients, such as proteins, selenium, iodine, vitamin D, choline and taurine which can be difficult to obtain from other sources.

It is known that safety is a fundamental criterion and a very important issue, since fish are highly perishable products. Special attention should be dedicated to the procedures of fresh fish manipulation from catching out to consumption. Fish farms are immersed in the marine environment, hence fish can also contain certain toxic contaminants such as metals (mercury, arsenic, cadmium, lead), organ halogenated compound (PCBs, dioxins, organochlorine insecticides) due to environmental pollution or even antimicrobials used to combat diseases during farming.

Nevertheless, safety and quality are very easy to control in fish farming to minimise the risks while maintaining the benefits (Costa, 2007). The safety and quality of fish can be measured by sensory, chemical, biochemical, physicochemical and microbiological methods and this manual is conceived as a guideline for farmers, HoReCa and the general public and clearly shows all the possible risks and benefits of farmed fish. It is also a useful document intended to teach consumers how to determine the sensory properties of fish and how to understand which specific ingredients determine fish quality. They will also become acquainted with the health indices of fish consumption and its beneficial impacts on human health.

After this introductory chapter, the manual is divided into four sections:

The first section "Safety of fresh fish" includes information on fish microbiological and chemical safety, providing readers with detailed information on microbiological safety and possible spoilage processes, bacterial species present during these processes, the legislative background for control, and scientific arguments for the microbiological safety of sea bass and sea bream. Moreover, clear recommendations are given for handling fish during the catching out, packing, selling and buying on the market, and before and after cooking to avoid contamination and spoilage. In the same section, fish safety also includes a description of the possible chemical contaminants in fresh fish and their products, and their impact on human health.

The second section "Sensory features of fresh fish" coaches readers on quality assessment parameters such as colour, aroma and texture of fish meat. These parameters represent important factors that influence the appropriateness of fish for various methods of culinary processing and use by the food industry.

The third section, "Nutritional quality of fresh fish" informs about the chemical composition of fresh fish and gives data on particular components, such as proteins, fatty acid composition and their ratios, as well as the content of vitamins, and macro- and micronutrients.

The fourth and final section considers all previous sections and offers scientific arguments on the benefits of fish in human nutrition.

The authors hope that this manual will enable professionals and the general public to obtain useful information about the safety and quality of Adriatic sea bass and sea bream, and that it will contribute to raising awareness on the benefits of consumption of these two species. Indirectly, it will be a document that will contribute to the enhancement of sea bass and sea bream consumption.

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1.1. Properties of European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*)

Giuseppe Comi,

University of Udine, Department of Food Sciences

Cage of market sized sea bream

European sea bass (*Dicentrarcus labrax*) and Gilthead sea bream (*Sparus aurata*) are characterised by having a bony skeleton and therefore belonging to the superorder of bony fishes, Teleostei. Both species are supplied by the fishing industry and by marine fish farming. In Europe, Directive 91/67 EC allows their farming in the open sea. Sea bass and sea bream are among the most widespread fish species in the world, thriving in coastal marine waters with different temperature regimes as long as they are not below 4°C, and tolerating wide salinity ranges. Both species have proven to be commercially important, given their appreciation as a food and their predisposition to domestication. These factors have contributed to the development of specific aquaculture for both species, especially in the Mediterranean area (Italy and Croatia). The first breeding of these species dates back to 1958 in Japan, and has undergone great progress over the years: the FAO reported that from 1986 to 2006, the increase in this activity was 10% per year, and it is still a fast-growing sector.

European sea bass belongs to the *Moronidae* family. It is characterised by a tapered and slightly compressed body, with a very robust head and two very different dorsal fins. The mouth has dense dentition, allowing it to catch and eat even prey larger than itself. Pigmentation is not homogeneous: the belly is completely white and the rest of the body is silver-grey, with a darker shade on the back. A peculiar characteristic of this fish is the brown speckling that covers the flanks. On the other hand, the fins are nearly colourless, although sometimes they may have some shades of black. The measurements of sea bass vary, ranging from 45 cm to over one meter in length. Weight also varies, and in some individuals, can even exceed 15 kg.

European sea bass *Dicentrarcus labrax*

The gilthead sea bream belongs to the *Sparidae* family, and it is characterised by a very convex head, rounded muzzle and small eyes. The lower jaw is slightly shorter than the upper jaw. On the front of each jaw, there are 4–6 large canine teeth, followed by 3–5 sets of upper and 3–4 lower molariform teeth. The body is oval and depressed. The main colour is silver, with a band of golden reflections in the centre between the two eyes. The dorsal fin is bluish-grey, with a darker median longitudinal band, it is long and unique with 11 spiny and 12–13 soft rays. It has a shorter anal fin, caudal fin, pectoral fins and two minor fins on the sides. Scales are absent on the muzzle, preorbital and interorbital. The lateral line includes 75–85 scales. The back is bluish grey and the silversides with thin longitudinal grey lines. A black and a golden band are interposed between the eyes. The scapular region is black, this colour continues on the upper part of the operculum, the edge of which is reddish. The maximum length of sea bream is 70 cm, but most commonly ranges between 20 and 50 cm; it can reach a weight of about 10 kg.

Gilthead sea bream *Sparus aurata*

Both species are solitary or form small aggregations and are sedentary; mainly carnivorous, feeding on molluscs, crustaceans and other fish.

2. SAFETY OF FRESH FISH

2.1. Microbiological quality of fresh fish

Giuseppe Comi,

University of Udine, Department of Food Sciences

Fishing out European sea bass

2.1.1. Characteristics of the meat of European sea bass and gilthead sea bream

The meat of both sea bass and sea bream, like of all fish products, is highly perishable. Refrigeration is the best storage method, although the fish product has a limited shelf-life. Due to the chemical composition, the low content of connective tissue (< 2%), the low acidity of the tissues (pH 6.5), the richness of protein and non-protein nitrogen, both species have a maximum shelf-life of 8 days if stored at 4°C or maintained either on flake ice or in polystyrene boxes with flake ice.

The freshness of fish products, in particular of sea bass and sea bream, is determined by inspection using the visual methods proposed by Council Regulations (EC) No 33/99 and Council Regulation (EC) No 2406/96 as shown in Table 2.1.1. The appearance, texture, and smell are determined and based on visual observations of the product or a lot of products. Based on these characteristics, a product or lot of products is then classified into different categories of freshness.

Table 2.1.1. Freshness test

Inspection, visual and olfactory test			
Appearance:	**State of:**	**Odour:**	**Category**
skin	meat	gills	extra
eye	vertebral column	skin	A
gills	peritoneum	abdominal cavity	B
colour of the meat			not allowed for consumption
vertebral column			
organs			

Council Regulation (EC) No 853/2004 requires checking a representative batch of sea bass and sea bream. Council Regulation (EC) No 2074/2005 specifies the requirement of food business operators regarding pests, and it defines visible pests and what is meant by visual inspection. The examination is visual, without the aid of optical magnification tools and under good light conditions for the human eye and, if necessary, also by candling, i.e., observing the fillets or flatfish against the light. It is performed on gutted fish on the abdominal cavity, on the livers and gonads if they are intended for human consumption, or on the fillets, depending on the type of processing.

2.1.2. Gilthead sea bream and European sea bass spoilage

After fish death, spoilage occurs rapidly through numerous processes. Initially, spoilage happens due to tissue degradation followed by bacterial enzyme activity. Low molecular catabolites are produced by tissue enzymes. The catabolites from tissue enzymes and molecules naturally present in meat, such as urea, anserine, carnosine, free amino acids, inosine, ribose, creatine and trimethylamine oxide (TMAO, present only in sea fish) represent an adequate substrate for the proliferation of autochthonous microorganisms, located on the fish skin and in particular in the intestine and on the gills. Allochthonous microorganisms derive from the terrestrial environment,

Stunning/killing of European sea bass by ice-water slurry

from sewage or rivers. Contamination with exogenous bacteria also derives from humans, during fish handling onboard ships, harvesting from breeding cages, or fish processing. The level of contamination is closely linked to various factors such as the environment in which the animal lives, fishing season, water temperature, method of capture, manipulations that the animal undergoes on board, during processing in the factories, and during storage and sale. The muscle masses are initially sterile and further contaminated through evisceration, filleting or transformation by microorganisms present in the intestine, gills and skin. The same tools used during handling and environmental hygiene represent an important source of contamination. This is, however, variable and ranges between 10^3 and 10^5 colony-forming units per square centimetres (CFU/cm^2) on the skin and between 10^3 and 10^7 CFU/cm^2 in the muscles. Sea bass and sea bream are preferably contaminated by aerobic or aerobic-anaerobic Gram-negative bacteria such as *Pseudomonas, Moraxella/Acinetobacter, Shewanella* spp., *Proteus* spp., *Aeromonas* spp., *Flavobacterium*/*Cytophaga*, *Xanthomonas*, *Vi-*

brio marini, *Photobacterium* and Gram-positive bacteria *Corynebacterium, Micrococcus* and other cocci and lactobacilli as shown in Table 2.1.2. Gram-negative bacteria represent 80% of the total microbial population. In particular, the microbial population can change according to the water temperature. At temperatures below 12°C, there is a clear difference between skin microorganisms, mainly Gram-negative, and those of the intestine consisting of Gram-positive (coryneform, *Clostridium, Bacillus*). In any case, these are non-pathogenic, psychrotrophic species, as the typical microorganisms responsible for the sensorial spoilage of both fish species. The microbial population, acquired from the environment, consists mainly of enteric viruses (hepatitis A), yeasts, moulds and pathogenic bacteria (*Salmonella, Listeria monocytogenes, Vibrio cholerae*, *V. fluvialis, V. paraemoliticus, V. alginoliticus, V. vulnificus, E. coli* enterotoxigenic, enterotoxin strains of *Staphylococcus aureus, Bacillus cereus* and *Clostridium botulinum*). It also includes spoilage microorganisms such as *Pseudomonas* spp., Enterobacteriaceae (*E. coli, Yersinia enterocolitica, Proteus mirabilis, Klebsiella* spp.). Their concentration, strictly dependent on the degree of water pollution, increases with the spring/summer season when urban waste increases due to tourism activities.

Table 2.1.2. List of environmental spoilage bacteria

Microorganisms	Origin	Activity
Pseudomonas	E	S
Shewanella	E	S
Moraxella/Acinetobacter	E	S
Photobacterium	E	S
Aeromonas	E	S/P
Vibrio	E/H	S/P
Salmonella	H/A	S/P
Listeria monocytogenes	H/A	P
Escherichia coli	P/A	S/P
Bacillus	E	S
Clostridium	E	S/P
Staphylococcus aureus	H/A	P
Enteric virus	H	P

Legend: H: human; A: Animal; E: Environmental; S: Spoilage; P: Pathogen

Pathogens can also derive from direct human contamination, occurring during fish manipulation. During storage, *Pseudomonas, Shewanella* and *Photobacterium, Moraxellae*/*Acinetobacter* and *Flavobacterium* can reach concentrations of 10^8 and 10^9 CFU/g within 10–12 days, degrade sulphur amino acids (cysteine, methionine), and

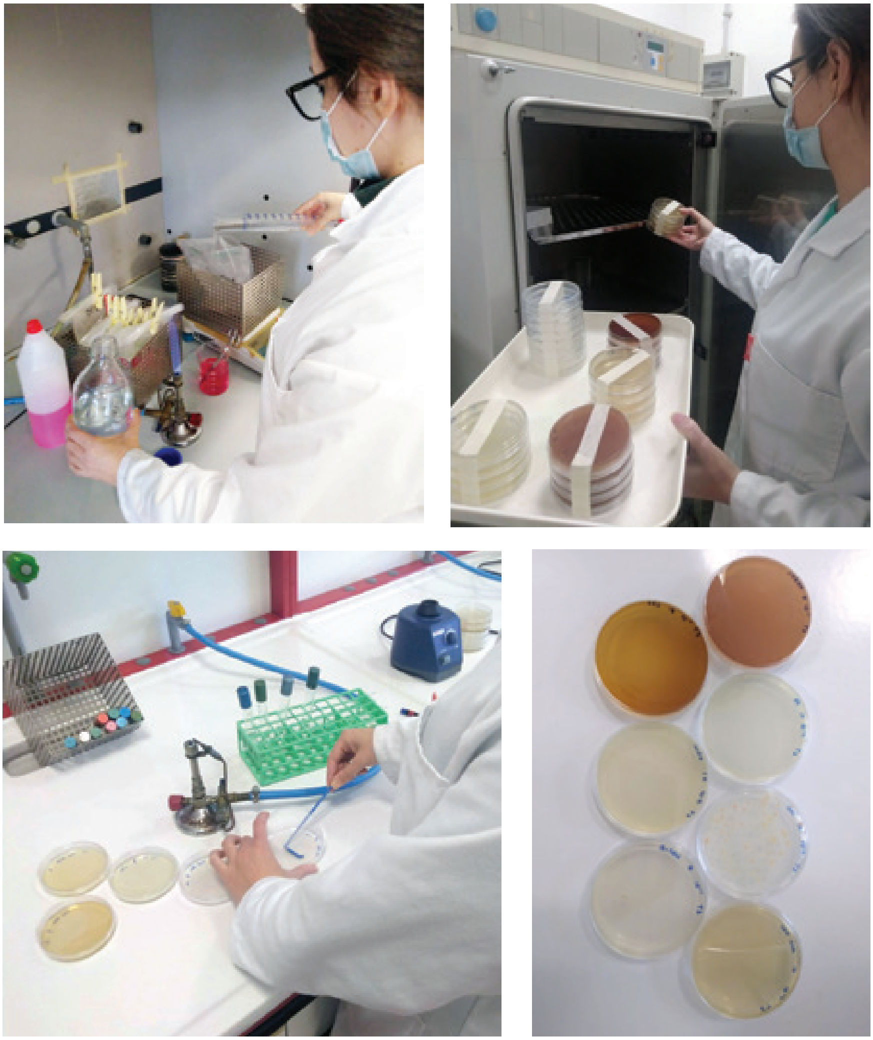
Sorting and packaging sea bream

produce typical spoilage molecules such as hydrogen sulphide, dimethyl sulphide and methyl mercaptan.

Generally, the spoilage of fish products includes two phases:

1. The first is the autolytic type produced by endogenous enzymes, which predispose the substrate for bacterial growth.
2. The second is typically of bacterial origin, and is supported by the moisture content of meat and the high pH value. It occurs faster than in mammalian meats. Anaerobic glycolysis, which begins with the death of the animal, produces lactic acid, which does not lower the pH below 5.8. After anaerobic glycolysis, endogenous enzymes and intestinal proteases are activated, causing softening of the tissues, hydrolysing proteins and nucleic acids and oxidising non-protein nitrogen compounds. The same ATP is degraded to uric acid (ATP (adenosine triphosphate) » ADP (adenosine diphosphate) » AMP (adenosine monophosphate) » IMP (inosinonophosphate) » inosine » hypoxanthine and xanthine » uric acid). The rate of spoilage depends on the fish product and while IMP gives the smell of fresh fish, hypoxanthine gives the smell of spoiled fish. As a result, short-chain peptides, amino acids and other non-protein nitrogen molecules are formed and transformed into sulphur compounds and ammonia by bacterial activity. During spoilage, phospholipids are also degraded by both endogenous and bacterial lipases and transformed into free fatty acids.

Both bacterial and endogenous enzymes can degrade TMAO (trimethylamine oxide) from fish muscle producing TMA (trimethylamine) even at chill storage temperature.



Microbiological analysis of fish

When the oxygen level in muscle is depleted, many spoilage bacteria utilise TMAO as a terminal hydrogen acceptor thus allowing them to grow in anoxic conditions (Ashie et al., 1996).

Pseudomonas spp. and *Shewanella putrefaciens* are examples of bacteria transforming TMAO into TMA (trimethylamine), DMA (dimethylamine) and FA (formaldehyde): indeed, *Shewanella putrefaciens* and *Shewanella baltica* use TMAO as an anaerobiotic electron acceptor as stated in Table 2.1.3. The lactic acid bacteria and micrococci, which preferably grow on carbohydrate substrates, produce lactic acid, acetic acid, ethanol, hydrogen sulphide, thiols, mercaptans, dimethyl sulphides, and indole, creating urinary odours in fish meat. The smell of spoiled fish also derives from the presence of amino valeric acid, amino valeric aldehyde and piperidine. Amino acids can also be decarboxylated to biogenic amines by *Hafnia alvei, Proteus* spp., *Pseudomonas* spp., *Shewanella putrefaciens* and *Morganella morganii*. Biogenic amines, in particular, can be found in mackerel (tuna, mackerel, etc.), because of the high concentrations of free amino acids present in their dark muscles. Histamine, putrescine and cadaverine are the main biogenic amines present in dark muscles causing a risk for the consumer as they are toxic at concentrations above 400 mg/kg.

Tablica 2.1.3. Spoilage microorganisms, precursors and metabolites

Specific spoilage microorganisms	Precursors	Precursors
Shewanella putrefaciens	TMAO – Amino acids ATP-Proteins	TMA, H_2S, CH_3SH, $(CH_3)_2S$, Hypoxanthine
Photobacterium phosphoreum	TMAO- ATP	TMA, Hypoxanthine
Pseudomonas spp.	Amino acids, Alcohols, Organic acids- Lipids	Ketones, Aldehydes, Esters, Sulphur compounds-H_2S
Vibrionaceae	TMAO – Amino acids	TMA, H_2S
Hafnia/Proteus/Pseudomonas/ Shewanella	Amino acids	Biogenic amines- Histamine

Legend: TMAO (trimethylamine oxide); TMA (trimethylamine); H_2S (hydrogen sulphide); CH_3SH (methanethiol); $(CH_3)_2S$ (dimethyl sulphide); ATP (adenosine triphosphate)

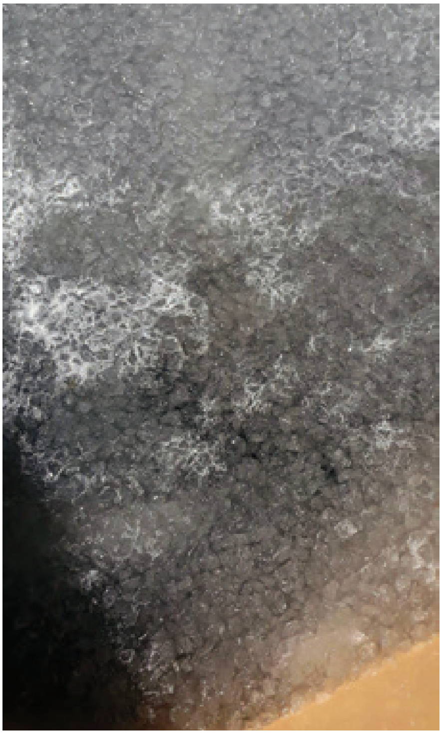
Sea bass and sea bream packed in a modified atmosphere (MAP) or vacuum-packed and stored at 4°C may be spoiled by previously described (*Shewanella* spp.) and micro-aerophilic microorganisms such as *Lactobacillus* spp., *Pediococcus pentosaceus*, *Micrococcaceae* and *Brochothrix thermosphacta*. Their activity is highlighted by the production of sulphur and ammonia compounds, by slight acidification and by a whitish patina.

Microbiological analyses applicable to fresh fish products concern the search for pathogens such as *L. monocytogenes*, *Salmonella* and *E. coli*. *Salmonella* spp. have to be absent in 25 g of sample. Council Regulation (EC) No 2073/05 proposes the criterion of acceptability as regards the presence of histamine in fish. The analysis has to be performed in 9 sample units (U.C.) and the fish is accepted if the value in 7 U.C. is less than 100 mg/kg and in 2 U.C. between the concentrations of 100 and 200 mg/kg. However, sea bream and sea bass do not present these biogenic amines.

Despite the high number of pathogenic and spoilage microorganisms present on fish products, we believe that the real dangers for the consumer are represented by intentionally or accidentally added molecules, algal biotoxins and parasites, which have become globalised and can be found in caught or farmed fish at any latitude. While parasites (*Anisakis simplex*) represent a danger for the consumption of raw or battered fish, algal biotoxins such as paralytic shellfish poisoning (PSP), amnesic shellfish poisoning (ASP), diarrheic shellfish poisoning (DSP) and heavy metal contamination must be considered emerging dangers in maximum exposure limits (MEL). Their presence is regulated by Commission Regulation (EC) No 853/04 (Biotoxin limits) and Commission Regulation (EC) No 1881/06 (Heavy metal contamination limits).

2.1.3. Consideration of the safety of farmed sea bass and sea bream

Farmed sea bass and sea bream are considered exceptionally safe for the following reasons:



Water-ice slurry for stunning the fish

1) hygienic quality is checked at every stage of production, and
2) the HACCP system is applied in each production phase: farming, fishing out, slaughtering and shipping.

HACCP includes regular controls of many different parameters during the production cycles:

1. Feed Control (presence of pathogenic microorganisms and toxic chemicals, presence of GMOs,);
2. Hygienic sanitary control of the environmental sea as the sea area where farm cages are situated. The special attention is paid to control of:
   a) Urban and industrial effluents. A fish farm should be situated either in an area of low urbanisation or effluents should be treated before release to the sea,

   b) Control of the water for the presence of pathogenic microorganisms of human and/or animal origin,
   c) Control for the presence of heavy metals, pesticides and other molecules toxic for humans and animals;
3. Control of feed quality and a balanced diet to avoid stress on farmed animals and ensuing disease onset;
4) Control of antibiotic molecules use - respect the withdrawal period before fishing out;
5) Vaccination against the most devastating pathogens (where possible);
6) Following good aquaculture practices, including the control of harvesting/fishing out:
   a) use of clean and disinfected equipment,
   b) handling fish during fishing out in a manner that avoids stress and the spread of intestinal parasites to the muscle masses,
   c) evisceration (if required) avoiding leaks of faecal material and contaminating the carcasses,
   d) Single carcass washing with drinking water or clean seawater;
7) control of packaging in boxes or Styrofoam boxes:
   a) use of clean and disinfected containers,
   b) use of flake ice made of clean drinking water or seawater;
8) Shipping control:
   a) Vehicle temperature control,
   b) Delivery to point of sale within 24 hours.

Control of all the above phases enables us to obtain a healthy product as observed from the following experimentation.

2.1.4. Analysis results confirming the high hygienic quality of farmed sea bass and sea bream

Analysed samples were gutted sea bass and sea bream. Analysed sea bass weighed about 474–578 g and sea bream 404–440 g. Samples of both species were packed under vacuum and stored at 4 ± 2°C during 12 days. The under vacuum technology was applied by an Orved VM53 vacuum machine (Italy). On days 0, 3, 6, 9 and 12, three specimens of each species were subjected to microbial and physico-chemical analysis, such as moisture content, pH, total volatile basic nitrogen (TVB-N), thiobarbituric acid-reactive substances (TBARS) as a marker of lipid peroxidation, and rancidity value. The results are shown in Tables 2.1.4., 2.1.5., 2.1.6. and 2.1.7.

Tablica 2.1.4. Microorganisms detected in gutted sea bass packed under vacuum and stored at 4 ± 2°C

Microorganism	Time (days)				
	0	3	6	9	12
Total viable count	3,7 ± 1,2[a]	5,7 ± 0,4[b]	6,0 ± 0,2[b]	7,4 ± 0,1[c]	8,0 ± 0,4[d]
Enterobacteriaceae	1,4 ± 0,1[a]	3,5 ± 0,3[b]	3,8 ± 0,3[b]	4,3 ± 0,6[b]	5,8 ± 0,1[c]
***Pseudomonas* spp.**	2,4 ± 0,7[a]	2,0 ± 0,2[a]	2,0 ± 0,3[a]	2,1 ± 0,1[a]	2,2 ± 0,1[a]
E. coli	< 10[a]	2,7 ± 0,2[b]	2,9 ± 0,1[b]	< 10[a]	3,6 ± 0,6[c]
Total Coliforms	1,6 ± 0,1[a]	3,5 ± 0,1[b]	3,3 ± 0,2[b]	3,5 ± 0,1[b]	5,1 ± 0,2[c]
***Clostridium* H_2S+**	< 10	< 10	< 10	< 10	< 10
Lactic acid bacteria	< 10[a]	3,7 ± 0,4[b]	4, 7± 0,2[c]	6,0 ± 0,3[d]	6,1 ± 0,7[d]
Enterococci	< 10[a]	< 10[a]	< 10[a]	2,9 ± 0,3[b]	3,4 ± 0,7[b]

Legend: Data represent the means ± standard deviations of the total samples; Mean with the same letters within lanes (following the values), considering every single parameter, are not significantly different (P< 0.05). Analyses were conducted in triplicate on three different samples per each sampling point. Data log CFU/g; < 10 CFU/g.

Table 2.1.4. reports the fate of the microbial flora in sea bass samples stored at 4 ± 2°C based on the results of microbiological analysis. All microorganisms, except *Pseudomonas* spp., grew during storage. As expected, the psychrotrophic species grew better than the mesophilic strains. The Total Viable Count increased over 8 log CFU/g, whereas Enterobacteriaceae strains and total coliforms almost 6 log CFU/g. *E. coli* seemed to grow, but considering it is a mesophilic strain, there is the possibility that this was not real growth, though this depended on the samples. Also, Lactic Acid Bacteria and Enterococci grew and it is likely that the vacuum stimulated their growth. Vice versa, *Pseudomonas* spp. did not increase because of the vacuum, since they are strongly aerobic.

Vacuumed sea bass

The significance of growth is demonstrated by the different superscript letters (a,b,-c,d) present in Table 2.1.5. Values with different letters are significantly different and demonstrate to be real growth. Microbial growth was confirmed by the increase of TVB-N, which reached values about 39 mg N/100 g as shown in Table 2.1.5.

Tablica 2.1.5. Physico-chemical values of gutted sea bass packaged under vacuum and stored at 4 ± 2°C

Parameter	Time (days)				
	0	3	6	9	12
Moisture	79,5 ± 0,3[a]	77,6 ± 0,9[b]	76,3 ± 0,9[b]	77,2 ± 2,0[b]	76,6 ± 0,8[b]
pH	6,16 ± 0,03[a]	6,03 ± 0,09[a]	6,06 ± 0,07[a]	5,91 ± 0,01[a]	6,03 ± 0,04[a]
TVB-N	12,9 ± 0,3[a]	11,0 ± 3,5[a]	21,0 ± 0,9[b]	31,5 ± 1,3[c]	39,0 ± 1,2[d]
TBARS	1,6 ± 1,2[a]	2,4 ± 1,2[a]	2,8 ± 0,5[a]	2,4 ±0,6[a]	2,6 ± 0,3[a]

Legend: Moisture %, TVB-N- Total volatile basic nitrogen mg N/100g; TBARS: nmol malonaldehyde/g. Data presented as means ± standard deviations of the total samples; Mean with the same letters within lanes (following the values), considering every single parameter, are not significantly different (P< 0.05). Analyses were conducted in triplicate on three different samples per each sampling point.

This value indicates at 12 days, spoilage has just started, and is slightly higher than the limit of 35 mg N/100g proposed by the Commission Regulation (CE) No 853/2004 and Commission Regulation (CE) No 854/2004. The TBARS and pH values changed but could not be considered real change (Table 2.1.5). These changes were likely sample-dependent, as they were different at each analytical time. In this case, the significance of the data are demonstrated by the different letters (a,b,c,d) shown in Table 2.1.6. Values with different letters are significantly different and demonstrate that microorganisms increase metabolites representing initial freshness decay. *L. monocytogenes* and *Salmonella* spp. were not detected in any samples. Finally, despite the microbial fate and the TVB-N values, the products must be accepted since no unacceptable odour was detected.

Table 2.1.6. presents the fate of the microbial population in sea bream samples stored at 4 ± 2°C. As in sea bass, all microorganisms except for *Pseudomonas* spp. grew during storage. As expected, psychrotrophic bacteria grew better than mesophilic strains. The Total Viable Count increased over 8 log CFU/g, whereas Enterobacteriaceae strains and Total Coliforms over 5 log CFU/g. Also, *E. coli* seemed to grow, but considering it is a mesophilic strain, there is the possibility that this was not real growth depending on the samples. Lactic Acid Bacteria and Enterococci also grew and their growth was likely stimulated by the vacuum.

Tablica 2.1.6. The fate of microorganisms in sea bream packed under vacuum and stored at 4 ± 2°C

Microorganism	Time (days)				
	0	3	6	9	12
Total viable count	2,3 ± 0,1[a]	2,3 ± 0,2[a]	4,5 ± 1,5[a]	5,4 ± 0,2[a]	5,5 ± 0,9[a]
Enterobacteriaceae	2,1 ± 0,3[a]	2,0 ± 0,1[a]	2,6 ± 0,3[a]	2,3 ± 0,1[a]	4,9 ± 0,4[a]
Pseudomonas spp.	< 10[a]	< 10[a]	2,1 ± 0,1[a]	2,2 ± 0,3[a]	2,1 ± 1,1[a]
E. coli	< 10[a]	< 10[a]	1,9 ± 0,8[a]	2,0 ± 0,9[a]	4,5 ± 0,8[a]
Total Coliforms	< 10	< 10	< 10	< 10	< 10
***Clostridium* H_2S+**	< 102[a]	< 102[a]	2,4 ± 0,7[a]	2,0 ± 0,1[a]	5,5 ± 0,4[a]
Lactic acid bacteria	< 102[a]	< 102[a]	2,0 ± 0,1[a]	2,0 ± 0,2[a]	2,0 ± 0,1[a]
Enterococci	2,3 ± 0,1[a]	2,3 ± 0,2[a]	4,5 ± 1,5[a]	5,4 ± 0,2[a]	5,5 ± 0,9[a]

Legend: Data represent the means ± standard deviations of the total samples; Mean with the same letters within a lane (following the values), considering every single parameter, are not significantly different (P< 0.05). Analyses were conducted in triplicate on three different samples per each sampling point. Data log CFU/g; < 10 CFU/g.

Vice versa, *Pseudomonas* spp. did not increase because of the vacuum since they are strongly aerobic. The significance of growth is demonstrated by the different letters (a,b,c,d) present in Table 2.1.7. Values with different letters are significantly different and demonstrate real growth. Microbial growth was confirmed by an increase of the TVB-N, which reached values about 35 mg N/100 g as shown in Table 2.1.7.

Tablica 2.1.7. Physico-chemical values of gutted sea bream packaged under vacuum and stored at 4 ± 2°C

Parameter	Time (Days)				
	0.	3.	6.	9.	12.
Moisture	75,3 ± 0,1[a]	75,6 ± 0,3[a]	76,1 ± 0,2[b]	76,2 ± 0,3[b]	76,0 ± 0,2[b]
pH	6,1 ± 0,1[a]	6,0 ± 0,1[a]	6,1 ± 0,1[a]	5,9 ± 0,0[a]	6,0 ± 0,1[a]
TVB-N	12,3 ± 0,2[a]	11,3 ± 1,5[a]	22,0 ± 0,3[b]	33,2 ± 0,3[c]	35,0 ± 112[d]
TBARS	1,2 ± 0,8[a]	2,2 ± 0,9[a]	2,4 ± 0,3[a]	2,6 ± 0,3[a]	2,7 ± 0,2[a]

Legend: Moisture %, TVB-N- Total volatile basic nitrogen mg N/100g; TBARS: nmol malonaldheyde/g. Data represent the means ± standard deviations of the total samples; Mean with the same letters within lanes (following the values), considering every single parameter, are not significantly different (P< 0.05). Analyses were conducted in triplicate on three different samples per each sampling point.

This value indicates that spoilage has started, but according to the Commission Regulation (CE) No 853/2004 and 854/2004, the product could be accepted since it is under the limit of 35 mg N/100g. The TBARS and pH values changed but this could not be considered real change. The change was likely sample-dependent, and were always different at each analytical time. *L. monocytogenes* and *Salmonella* spp. were never detected in all samples. Despite the microbial fate and the TVB-N values, the products must be accepted, considering there was no unacceptable odour.

2.1.5. Fish safety

It is possible to conclude that farmed sea bass and sea bream have a high hygienic quality value, and consequently are safe for human consumption.

Gilthead sea bream ready for sale

When consumers buy fresh fish, they primarily evaluate the sensory properties by evaluating the appearance, the state of the meat and the smell. However, both producers and the supermarket check quality through microbial and physico-chemical analyses that certify the safety and overall quality of the fish. The analysis results should be compared to the values reported below. As shown in Table 2.1.8., the total viable count must be less than 8 log CFU/g product and spoilage microorganisms must be less than 3-4 log CFU/g at the end of the shelf-life. Moreover, the safety and the freshness of a fish must be evaluated by molecules produced by bacteria during storage, in particular by determination of total volatile nitrogen (TVB-N) and the rancidity index (TBARS). The TVB-N and the TBARS of fresh fish should not exceed 35 mg N/100 g product and 5 nmol malonaldehyde/g products, respectively.

Tablica 2.1.8. Popis pokazatelja kvalitete ribe

Parameters	Log CFU/g
Total viable count	< 8,0
Enterobacteriaceae	< 4,0
Pseudomonas spp.	< 2,0
E. coli	< 2,0
Total Coliforms	< 4,0
Clostridium H_2S+	< 10
Lactic acid bacteria	< 4,0
TVB-N	< 35 mg N/100 g
TBARS	nmol malonaldehyde/g

Fish safety must also continue from farm to fork. The producers, sellers and consumers must manage fish to limit every risk. Proper handling and storage are essential in reducing the risk of food-borne diseases and ensuring a quality product. As pathogenic microorganisms are not visible on the fish, producers must handle fish cautiously. Different microorganisms such as *Salmonella* and *E. coli* are sometimes present on fish and can cause food-borne diseases. The handling of fish is of the utmost importance, and different stages are described in the HACCP system and should be applied in the following order.

Contamination prevention. After harvesting, a clean working environment is essential in the prevention of contamination. Hands must be washed thoroughly before and after handling raw fish. All the apparatus of the work area and utensils are sanitised with soap and disinfectants. Dirty utensils are not used. During work, they are frequently sanitised to prevent cross-contamination.

Storage. After killing, fish are quickly eviscerated and washed with fresh drinking water. A good washing can eliminate microorganisms at a level of 0.5 log CFU/g. Then the fish must be cooled to 4°C; fresh products (gutted fish) are placed in boxes in alternating layers of fish and cubed ice. Alternatively, fish are packed in plastic film under vacuum, skim film or in a modified atmosphere. They are quickly sent to market, ensuring that the temperature never exceeds 4°C. In each case, the shelf-life must not exceed 9–12 days.

Handling Market Fish. Fresh fish should be purchased immediately before leaving the market to avoid exposure to higher temperatures. At the market, unpacked fish must be placed on ice, and the ice should be changed every day. Even better is to pack the ice into the plastic bag to prevent any leakage. Fish need to be kept at a temperature under 4°C to maintain quality.

Handling at home. After buying, fish should be taken home as soon as possible and kept refrigerated. However, it is suggested that fresh fish be consumed within a maximum two days of purchase.

During cooking and serving, fish must be handled properly to prevent contamination. All utensils must be properly sanitised and changed.

The time and temperature of cooking must permit the centre of the fish to reach 75°C for almost 5 minutes. This ensure that all pathogenic microorganisms are killed and the product is safe.

After cooking, fish must be kept at a temperature over 65°C until eating. Alternatively, it may be cooled to less than 4°C and recooked before eating. In each case, it is better to eat cooked fishes within two days.

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2.2. Possible chemical contaminants in fish

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Automatic feeding of fish in cages

2.2.1. Introduction

Fish can be contaminated by chemical residues originating from the environment in which they live. In Europe, there has been a high number of seizures of fish products contaminated by heavy metals and organic molecules (veterinary drugs, antibiotics, pesticides) of environmental origin and microbial histamine. Wild fish, living along the coasts, are even more contaminated by these pollutants. Farmed fish, on the other hand, are less contaminated due to the application of strict Good Aquaculture Practices and strict controls on the use of permitted and prohibited drugs (i.e., chloramphenicol).

Fish products can be contaminated by numerous chemical compounds and molecules of natural origin, deriving from urban and industrial pollution and farming practices. The main chemical compounds posing a risk for human health include heavy metals (mercury, lead, cadmium), organic organochlorine compounds and poly-bisphenols, dioxins, insecticides, antibiotics used for farming, and specific toxic metabolites of some fish. The contaminants, deriving from industrial activities in the aquatic environment represent a risk for humans when they reach high levels in aquatic animals and/or when the exposure to fishery products is long-lasting. The potential danger of contaminants for humans and other living beings lies in their ubiquity, persistence, toxicity and in their lipophilia.

Tablica 2.2.1. Limits for certain chemical contaminants in fish product (EC, 2006; FDA, 1998; *Canadian Food Inspection Agency*, 2007)

Substance	EU (mg/kg)	US (mg/kg)	Fish product	Canada (mg/kg)	Fish
Arsenic (As)		76 – 86	C- Mb	3,5	**Fish protein**
Cadmium (Cd)	0,05 – 1	3 – 4	C- Mb		**All fish**
Lead (Pb)	0,3 – 1,5	1,5 – 1,7	C- Mb	0,5	**Fish protein**
Methylmercury	0,5 – 1,0	1,00	all	0,5 – 1,0	**All (excluding swordfish; sharks)**

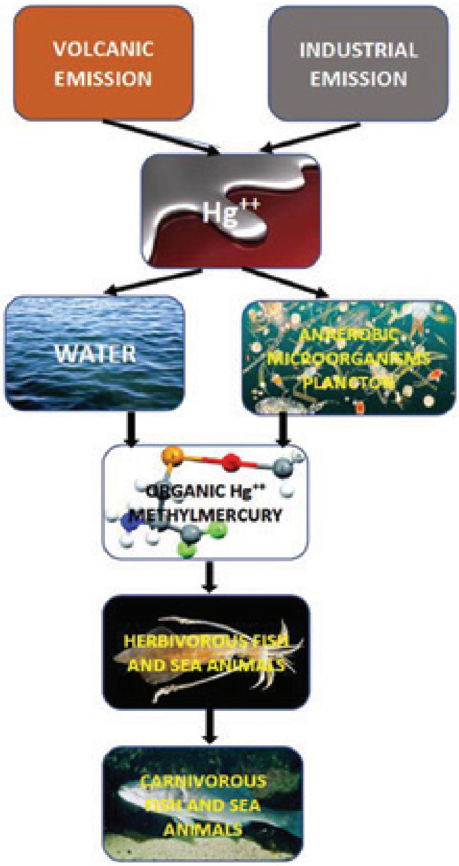
Legend: C: Crustaceans; Mb: Lamellibranch molluscs

The presence of contaminants in marine animals depends on the geographical location, species, size, type of food and the solubility and persistence of the compound in water. Once in the environment, toxic contaminants can undergo biomagnification, i.e., they are concentrated in the highest levels of the food chain and/or bioaccumulation. The concentration of the compound increases throughout life in the muscle mass or organ of the fish, and so old animals are more contaminated than young ones. However, in humans, the risk of acute pathology is reduced or very low. Serious diseases (neurological damage, cancer and teratogenic effects) occur only by continuous exposure and in those countries where the diet is mainly based on fish products. Further risks are found in young people or children, whose diet is based on the consumption of fish oil. To avoid the consumption of fish containing high levels of toxic chemical compounds, several international committees for the safety of fishery products have indicated useful interventions to reduce risks, in particular to check the degree of water pollution and ensure continuous monitoring of fish products in line with the proposed acceptability limits. The European Community by Council Regulation (EC) No 1881/2006 of 19 December 2006 defined the maximum levels of certain contaminants in food products. Consequently, it adjusted the limits for arsenic, cadmium, lead and mercury in fish in relation to the limits set by the Codex Alimentarius, and reaffirmed the need to reduce the presence of lead in food. It confirmed a provisionally tolerable weekly intake (PTWI) of 1.6 mg/kg of body weight of mercury and methylmercury in food, whereas methylmercury constitutes more than 90% of the total mercury in fish and seafood.

The main groups of chemical residues transmitted to humans by fish product consumption include heavy metals, organic molecules (veterinary drugs, antibiotics, pesticides) of environmental origin and histamine of microbial origin. Heavy metals in fishery products include mercury, lead and cadmium, and have been shown to represent a real and the highest risk to humans.

2.2.2. Mercury

Mercury is present in the environment and derives from both natural sources (volcanic activity) and human activities, such as production processes that causes its emission to the atmosphere, soil, sediments and marine waters. These emissions derive from combustion processes and various industrial productions (chlorine-alkali production, batteries, measuring instruments, catalysts) or mining activities. Some estimates state that some 40,000 or 50,000 tonnes of mercury are released into the atmosphere and 4,000 tonnes into the sea each year. This contamination includes natural emissions deriving from volcanic eruptions and seismic movements and the movement of historical mercury deposits, both of natural and anthropogenic origin.



Mercury cycle

Elemental mercury is toxic to humans both by ingestion and by inhalation, but the real risk lies in its organic form, methylmercury, which is the main form of exposure for humans. This molecule, produced by microorganisms that develop in the soil or sediments, is taken up by aquatic organisms and transferred to the aquatic food chain with bioaccumulation.

Every form of mercury (ionic, metallic, organic) bioaccumulates, but its organic form accumulates the most due to its high absorption by the intestines of animals, including humans. The animal intestines absorb mercury in its methylated form (95%) and only 2% of the total is absorbed in ionic form (Hg^{++}) or salts. Mercury tends to accumulate throughout the fish life and consequently, the highest levels are observed in predatory fish (tuna, sharks, swordfish).

Methylmercury has a half-life of 60–120 days in humans and 2 years in fish. However, its bioaccumulation is continuous, and therefore the decay is not observable, even following the stationing of fish products in waters devoid of this compound. Its inorganic form is easily disposed of, but in the sea, it is quickly methylated by bacterial and plankton activity. Methylmercury is a toxic compound for humans because it binds to the SH groups (glutathione and proteins) and inactivates enzymes, produces alterations at the mitosis level, at the chromosomal level with cellular and neuron damage, and produces demyelination of axons with paraesthesia, incoordination, tremors and epileptic seizures. The target of mercury is mainly the brain. It is also a teratogenic agent, and crossing the placental barrier it can cause foetal damage and mental retardation in newborns. Accordingly, in 2004 the US Food and Drug Administration (FDA) and Environmental Protection Agency (EPA) issued simple recommendations addressed to the highest risk population group, in particular, stressing that childbearing, pregnant and lactating women and children, should not consume predator fish, such as sharks, swordfish and large mackerels that can contain high levels of methylmercury. The risk is related to the chemical form of mercury, the dose, exposure time, route of exposure and the age and health of the exposed person. In Europe, the legal limits of mercury in fish products are 1 mg/kg (mg/kg) in a small number of fish species and genera comprising the largest predators and 0.5 mg/kg in other fishery products. The mercury content of several fish species in the Adriatic area was less than 0.5 mg/kg, which represent the maximum permitted level. Consequently, sea bass and sea bream caught or bred in the Mediterranean are compliant with these limits. Therefore, a diet based on the consumption of these fish is in line with the recommended intake level for methylmercury (PTWI 1.6 μg/kg body weight), as established by Joint FAO/WHO Expert Committee on Food Additives (JEFCA) in 2006 and based on the development of neurotoxicity in the most sensitive species (human species).

2.2.3. Lead

Lead is a metal distributed worldwide by humans through the metallurgical industry and in leaded gasoline. It is present in both the inorganic and organic form (tetraalkyl lead) and its concentration in marine sludge, in marine waters and fish depends on the proximity of industrial and urban discharges, with concentrations ranging between 5 and 50 ng/L in seawater. In fish products the organic form prevails, which derives from water contamination and is estimated to be between 5 and 500 μg/kg. Fish caught in "open" seas contain low concentrations of lead (2–10 µg/kg), while those caught in "closed" seas, such as the North Sea or the Baltic Sea, generally contain higher quantities (20–50 mg/kg). Fish accumulate lead mainly in the bones, and less in soft tissues, heart, gonads and gastrointestinal system. Therefore, since the skeletal system of fish is not consumed, the risk for humans is low. There is a potential danger for small fish, which are eaten whole and not gutted. However, it has been observed that lead can also be present in the muscle masses of fish in areas highly polluted by agricultural and industrial waste. Lead has an average half-life of 30 years in the bone cortex and only 4-6 weeks in soft tissues and blood. Also, in humans, it is localised at the skeletal (cortical and transecular), renal, pulmonary and central nervous system levels. Continued exposure to low doses produces chronic effects such as hypertension and chronic kidney disease with increased urinary excretion of low molecular weight proteins and lysosomal enzymes. Children constitute a particular risk group because the gastrointestinal absorption of the metal is much higher than in adults; in early childhood and children, lead exposure impacts the development of the nervous system, producing detectable cognitive deficits and producing abnormalities in the urinary tract. Currently, the CDC (Centre for Disease Control and Prevention) recommends for children a limit of 10 µg lead/100 mL, and although there are no safety thresholds, it emphasises primary prevention of exposure to lead from all sources. Lead is determined by the measure of the enzymatic activity of aminolaevulinic acid dehydratase (ALAD) in the blood, which in children should not exceed 2.5 µg/100 mL. Regarding the intake of lead, JECFA (1999) indicated a PTWI of 0.025 mg/kg body weight. Given its low concentration in food, lead has negligible effects on the neurobehavioral development of children. However, it stressed the control of the presence of this metal in the air and the human environment. The legal limits for lead in food within the EEC are set out in Council Regulation (EC) No 1881/2006 of 19 December 2006. The maximum levels for fishery products are shown in Table 2.2.2.

Tablica 2.2.2. The maximum level of lead in fishery products

Fishery products	Maximum levels (mg/kg wet weight)
Fish Meat	0,30
Crustaceans, except for dark meat of the crab, the head and thorax of the lobster and similar large crustaceans (*Nephropidae, Palinuridae*)	0,50
Bivalve molluscs	1,5
Cephalopods (without viscera)	1,0

Fishery products are not important for lead exposure, as confirmed by recent research data. Scientific literature data show lead levels in Adriatic fish products below the detection limit (0.005 mg/kg) in the majority of samples and a maximum value of 0.03 mg/kg. Higher levels (0.045 mg/kg) depend on the fishing period but in general, confirms its low risk in both caught and bred fishery products.

2.2.4. Cadmium

Cadmium is one of the most dangerous metals for human health. It is present at the level of the earth's crust together with zinc. Humans contaminate the environment through mining (zinc and lead extraction) and industry (galvanic electroplating, pigment production, alkaline accumulators and nickel-cadmium batteries, ceramic production, engraving and printing processes, plastics, gold working).

Cadmium is present in the air, in the soil, in the waters of industrial areas and also in some foods, both animal and vegetable origin, and in tobacco. Cadmium contaminates food by dry or wet deposition on crops located near the emission source, by wastewaters, by phosphate fertilisers with high concentrations of cadmium, by sludges from waste treatment; by food containers, such as glazed ceramics. However, the level of food contamination is low; only molluscs and crustaceans can have high concentrations even when living in uncontaminated areas. Despite the fact that it is a recently acquired contaminant, cadmium is widely distributed in the aquatic environment and bioaccumulates in all animals that live in these ecosystems. Cephalopods, molluscs and crustaceans accumulate cadmium especially at the level of the viscera and in the hepatopancreas, while fish accumulate it in the muscles. However, in fish products, cadmium levels are lower than those encountered in other animals. Directive 2005/87/EC, relating to undesirable substances in animal feed reminds that "the accumulation of cadmium in animal tissues depends on the concentration in the diet and the exposure times".

Humans absorb cadmium via the respiratory and gastrointestinal tracts with food, air and water. Absorption by inhalation is highest in occupational exposure in high risk industries and by smokers. Tobacco is an important source of cadmium in heavy smokers. In case of chronic exposure to cadmium, the effects on humans include kidney damage, which occurs after exceeding the 200–240 µg/g limit in wet cortical tissue, anaemia, mild entity, hypertension, liver dysfunction and damage of the bone tissue. Cadmium carcinogenicity seems to be derived from inhalation through tobacco.

In any case, real risks could be observed in monotonous feeding populations living in polluted areas, or fed with offal, or mineral deficient (especially zinc) diets, or in individuals in particular para-physiological condition. The PTWI is 0.007 mg/kg body weight (JECFA, 2005).

The average levels observed in Mediterranean fish are estimated as low, with the lower concentrations ranging from 0.25 to 20.2 g/kg. The maximum levels for fishery products are shown in Table 3 (Council Regulation (EC) No 1881/2006 of 19 December 2006).

Tablica 2.2.3. The maximum level of cadmium in fishery products

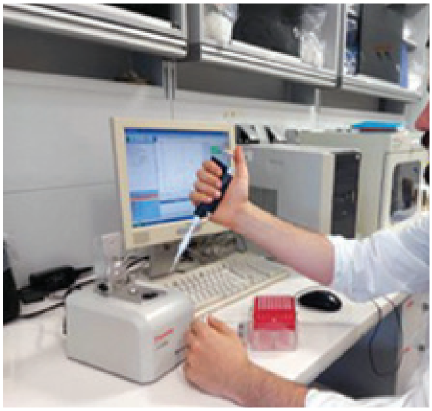
Fresh fishery products	Maximum levels (mg/kg wet weight)
Meat	0,050
Meat: Anchovy (*Engraulis species*); Palamita (*Sarda sarda*); Common banded bream (*Diplodus vulgaris*); Eel (*Anguilla anguilla*); Mullet (*Mugil labrosus labrosus*); Suro or horse mackerel (*Trachurus* sp.); Luvaro or emperor fish (*Luvarus imperialis*); Sardine (*Sardina pilchardus*); Sardines of the genus *Sardinops* (*Sardinops* sp.); Tuna and tuna (*Thunnus* sp., *Euthynnus* sp., *Katsuwonus pelamis*); Sole cuneata (*Dicologoglossa cuneata*)	0,10
Swordfish Meat (*Xiphias gladius*)	0,30
Crustaceans, except for dark crab meat, lobster head and thorax and large similar crustaceans (*Nephropidae e Palinuridae*)	0,50
Bivalve molluscs	1,0
Bivalve molluscs	1,0

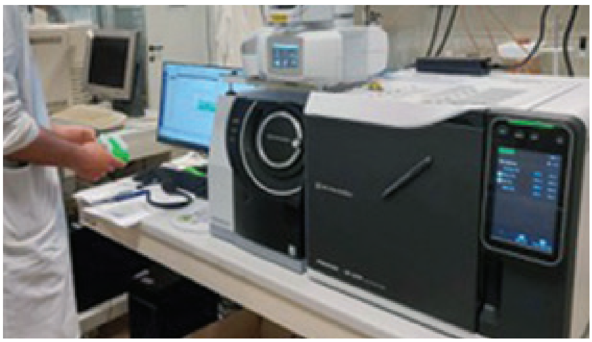
2.2.5. Pesticides (*Phytopharmaceuticals*)

Pesticides are compounds used mainly in agriculture against animals, plants or weeds and are dispersed in the environment generating pollution. From the terrestrial ecosystem, they are rinsed into freshwater and marine ecosystems, where they are absorbed and accumulated by individuals in the food chain at the level of lipid, muscle and viscera tissue. The active ingredients of pesticides including alpha hexachlorocyclohexane (alpha-BHC), gamma-BHC malathion, chlorpyrifos, isodrin, endosulfan, dieldrin, p,p’- dichlorodiphenyltrichloroethane (DDT), clordanic compounds, aldrin, endrin, isodrin, heptachlor epoxide, t-dichlorodiphenyldichloroethane (t-DDD) and t-dichlorodiphenyldichloroethylene (t-DDE), have always been isolated from different foodstuffs, including fish products, with variable concentrations ranging from 0.1 to 18 μg/kg. In different aquatic ecosystems, it has been observed that the most involved fish include carp, crabs, cod and molluscs. The nature and concentration of pesticides appears to be highly correlated to the area and season of fish capture. Theoncentrateion increases with the size, weight, lipid content and with the organ (ovary), whereas the muscles are less contaminated. The bioaccumulation of DDT and its metabolites never exceeds the maximum levels in the edible components of fish and in any case, despite considering the long half-life of this molecule, a constant decrease has been observed over the years.

2.2.6. Dioxins and PCBs

The term “dioxins” usually indicates a group of 75 polychlorinated dibenzodioxin (PCDD) and 135 polychlorinated dibenzofuran (PCDF) congeners, 17 of which raise most toxicological concerns. Polychlorinated biphenyl (PCBs) (similar dioxins) make up a group of 209 different congeners; among these, 12 congeners have toxic effects similar to those of dioxins. The main sources of PCDD and PCDF are the combustion processes, such as waste incineration, and metal smelting and refining operations, while PCBs were produced until 1985 for industrial purposes for a wide range of uses (chlorophenols for the impregnation of construction timber). Unpredictable sources are old transformers and capacitors, which can contain several kilograms of PCBs and hundreds of milligrams of PCDD/F. Dioxins cause a wide variety of toxic and biochemical effects on experimental animals. Given the high number of congeners belonging to the two classes (dioxins and PCBs), it is impossible to issue provisions for each substance so toxicity is expressed in “Toxic equivalents” (TEQ) as the concentration of





Analytical control of toxic substances in fish tissue

the reference substance capable of generating the same toxic effects. Furthermore, to add the toxicity of the various congeners, the concept of the “toxic equivalency factor” (TEF) has been introduced to facilitate risk assessment and regulatory control. TCCD has shown to have immunotoxic, teratogenic, and carcinogenic action on animals and has been included in Class I (human carcinogens) by the International Agency for Research on Cancer (IARC) since 1997. The European Union has established a tolerable weekly intake (TWI) for PCDD/F of 14 pg TEQ/kg pc/week (Scientific Committee on Food, 2001). Since the toxic action of dioxins, furans and dioxin-like PCBs in the human body is similar, in 2006 the EU set maximum limits for the sum of the residues of these groups of substances. Food with a high fat content is the main source of exposure for humans to PCBs and dioxins. It is estimated that among food, about 15% of dioxins and dioxin-like PCBs are taken up with fish. The presence of PCBs and dioxins in fish derives not only from their presence in the environment, but above all from the use of contaminated commercial feed in farming. Fish species, size and age influence the bioconcentration of the organochlorine compound. However, the concentration does not seem related to the lipid content, but instead to the length and mass of the particular fish product.

Tablica 2.2.4. Dioxins and dioxin PCBs expressed TEQ (1) according to Commission Regulation (EC) No 1881/2006 of 19 December 2006)

Fish products	Limits	
	Sum of Dioxins (OMS-PCDD/F-TEQ) [(1)]	**Sum of Dioxins and PCB dioxins (OMS-PCDD/F-PCBTEQ) [(2)]**
The muscle of fish and fishery products and their derivatives, excluding eel. [(3)] The maximum level applies to crustaceans, excluding dark meat of the crab and those of the head and chest of the lobster and similar large crustaceans (*Nephropidae e Palinuridae*).	4.0 pg/g fresh fish	8.0 pg/g fresh fish

[(1)] Dioxins [sum of polychlorine dibenzo-para-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF), expressed in toxicity equivalents of the World Health Organization (WHO) using WHO-TEF (equivalent toxicity factors)], and sum dioxins and dioxin-like PCBs [sum of PCDDs, PCDFs and polychlorinated biphenyls (PCBs), expressed as WHO toxicity equivalents, using WHOTEFs]. WHO-TEF for human risk assessment based on the conclusions of the WHO meeting held in Stockholm (Sweden) from 15 to 18 June 1997 [Van den Berg et al., 1998; Toxic Equivalency Factors (TEFs) for PCBs, PCDDs, PCDFs for Humans and Wildlife. *Environmental Health Perspectives*, 106 (12), 775].

[(2)] Upper limit concentrations: upper bound concentrations are calculated assuming that all the values of the various congeners below the limit of quantification are equal to the limit of quantification.

[(3)] If the fish are intended to be consumed whole, then the maximum content applies to the whole fish.

However, the literature data are variable and depend on the type of fish product and the catch area. For example, in trout feeds, average values of PCBs were found to be less than 10 μg/kg, and trout and fish fed with these feeds had concentrations of PCBs lower than 0.8 μg/kg. The strategies employed to reduce the concentrations of these contaminants seem effective. The search for PCDDs, PCDFs, PCBs and DDTs in feed for the feeding of salmonids has shown a significant decrease compared to previously published values. An example of dioxin concentrations in food is shown in Table 3.5 (1881/2006/EC for dioxins and PCBs-dioxins). For organochlorine plant protection products (DDT, DDD, chlordane, aldrin, dieldrin, lindane, hexachlorobenzene), the limits are between 0.001 and 0.1 mg/kg (DM 27 August 2004 for plant protection products).

2.2.7. Veterinary drugs

Antibiotics are widely used in aquaculture to fight bacterial diseases. Consequently, it is possible to find their residues in muscles and this can represent a real risk for the consumer. The intensive development of aquaculture has led to an increase in diseases caused by various pathogenic bacteria, including *Aeromonas hydrophila, A. salmonicida, Edwarsella tarda, Photobacterium damselae* subsp. *piscicida, Vibrio anguillarum, V. salmonicida, Yersinia ruckeri*. These diseases are increased by overcrowding of animals in cages or breeding tanks. The use of antibiotics, directly added to water or feed, represents the best method to combat microbial diseases in farmed fish, considering that there is a low number of effective vaccines available. Therefore, antibiotics are widely used for the purpose of prophylaxis and for the treatment of diseases and to avoid economic losses, but they should never be used as an easy alternative to good aquaculture practices. On the market, it can be possible find numerous drugs useful in the prevention and control of pathologies of aquatic farmed animals and where their use is allowed, specific doses and withdrawal periods are defined to prevent residues presence in fish edible parts that reach the consumer. Table 2.2.5. shows the antibiotics permitted for use in aquaculture.

Fish are poikilothermic animals, and their metabolism leading to the degradation of the active ingredients of antibiotics is closely linked to the water temperature in which they live, so the withdrawal period will vary based on temperature. In some cases, it is measured as degree-days. For example, for amoxicillin, the withdrawal period is 150 degree-days, which corresponds to 10 days at a water temperature of 15°C. However, the calculation of the withdrawal time must be verified for different fish species, as they have different metabolisms at different temperatures. In case of availability of a defined suspension time, according to Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products, the minimum suspension time adopted for fish must be 500 degree-days. Currently, in the aquaculture of the temperate waters of Europe and North America, there is a large reduction in the use of antibiotics for prophylaxis or treatment, due to the wide use of vaccines that are harmless and produce no residues or resistance like antibiotics. In particular, vaccines are available only for finfish. The main danger deriving from the use of antibiotics is residues, as in addition to having a direct effect on humans, they can also select for resistant microorganisms. Antibiotic resistance can be harmful to both the fish and especially for humans, in which these residues can produce allergies, toxic effects, changes in the human intestinal flora and acquisition of drug-resistance in pathogens for humans (WHO, 1999). Drug resistance is observed in all fish pathogenic microorganisms and affects all antibiotics. The irresponsible use of antibiotics in aquaculture can generate their dispersion in the environment, inducing resistance in bacteria contaminating sediments and wild aquatic animals living close to farming areas. Due to the relatively small number of antibiotics registered for use in aquaculture, rotation is not permitted during repeated treatments to avoid antibiotic resistance, which is likely to occur. Considering that the use of antibiotics is unfortunately necessary for fish farming, international programmes for the control of their use and their residues are needed. These controls include the use of approved antimicrobials and above all the control of sale and use in farming. To limit the damage due to the irresponsible use of antibiotics, the European Union with the Council Regulation (EEC) No 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin, has recommended the duration and concentrations of antibiotics which can be used for farmed fish, and other countries have imposed similar regulations. It should be highlighted that the use of antibiotics in aquaculture is intended only for therapeutic purposes. The drugs registered in Italy for specific employment in aquaculture are currently: 1) chlortetracycline; 2) oxytetracycline; 3) amoxicillin; 4) flumequine; 5) sulfadiazine + trimethoprim; 6) bronopol. The first five principles are molecules included in Council Regulation (EC) No 2377/90, and have defined maximum residual limits (Table 2.2.5).

Tablica 2.2.5. Antibiotics in aquaculture (FAO *Fisheries Technical Paper*, 444)

Group of antimicrobials	Composition
Sulfonamides	Sulfamerazine; Sulfadimidine; Sulfadimetossina[1]
Potentiated Sulfonamides	Co-trimazine/Sulfatrim[1,2,3] (a combination of trimethoprim e sulfadiazine)
Tetracyclines	Chlortetracycline; Oxytetracyclines[1,2,3,4]
Penicillin (ß-lactams)	Ampicillin[4]; Amoxicillina[2,4]; Benzyl penicillin[3]
Quinolones	Ciprofloxacin; Enrofloxacin; Norfloxacin; Oxolinic Acid[2,3,4]; Perfloxacin Flumequinina[3,4]; Sarafloxacin[2]
Nitrofurans	Furazolidone
Macrolides	Eritromicina[4]; Spiramycin
Aminoglycosides	Gentamicin
Other antibiotics	Chloramphenicol; Florfenicol[1,3,4]; Tiamefenicol[4]; Tiamulin; Nalidixic acid; Milozacina

[1]allowed in Canada (http://www.syndel.com/msds/canada_approved.htm); [2]Authorized in the UK (Alderman and Hastings, 1998); [3] Permitted use in Norway (Alderman and Hastings, 1998); [4] Permitted use in Japan (Okamoto, 1992)

Tablica 2.2.6. Antibiotics approved for use in Italian aquaculture (www.acquacoltura.org)

Antibiotic	Limit	Fish
Chlortetracycline; Oxytetracycline	100 µg/kg (meat + skin)	Eel, cyprinids
Amoxicillin trihydrate	50 µg/kg (meat + skin)	Salmonids – Marine fish
Flumequine	600 µg/kg (meat + skin)	Salmonids
Sulfadiazine + Trimethoprim	100 µg/kg (meat + skin)	Salmonids

The aquaculture products of the Mediterranean Sea are healthy concerning antibiotic residues, as their use is reduced and the withdrawal times are always higher than the 500 degree-days and above all abide by the provisions of Directive 2006/193/EC of 6 April 2006, in regards to the Implementation of Directive 2004/28/EC on the Community code of veterinary medicinal products, which requires breeders (owners or keepers) to register (art.79): date, identification of the veterinary medicinal product, batch number, quantity, name and address of the supplier of the medicinal product, identification of the animals undergoing treatment, start and end date of treatment.

2.2.8. Histamine

Biogenic amines represent substances produced by various microorganisms (Enterobacteriaceae, such as *Morganella morganii, Klebsiella oxytoca* and *Hafnia alvei*) through the decarboxylation of amino acids, present in free form in food. Histamine derives from the decarboxylation of histidine, an amino acid particularly abundant in the musculature of dark fish meat. It is a toxic biogenic amine formed in fish following its deterioration. In humans, it produces an intoxication known as "mackerel syndrome" or Histamine Fish Poisoning (HFP), an acute syndrome caused mainly by the consumption of fish products, containing high levels of histamine and probably other vasoactive amines and/or others compounds. In fish, a histamine level less than 5 mg/100 g is considered safe, levels of 5–20 mg/100 g are considered as possible causes of intoxication, levels of 20 to 100 mg/100 g as probably toxic and levels higher than 100 mg/100 g as toxic. The toxin is not present when the fish is caught but is subsequently produced by bacteria of enteric origin producing histidine decarboxylase when the rules for maintaining a controlled temperature (<5°C) and correct hygienic handling are not respected during the evisceration and processing phases. Also, the contaminated fish cannot be rehabilitated during cooking, sterilisation or other conservative treatments, since histamine is thermostable, and reaches the consumer with high amine contents. It is probable that during improper storage, significant quantities of histamine can be produced in originally healthy fishery products exposed to contaminating microflora after opening the package. Sea bass and sea bream do not contain high amounts of histidine and other free amino acids as in mackerels. Consequently, they are not at risk for the presence of histamine and other biogenic amines. Moreover, Council Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs requires testing for histamine presence only in mackerels (*Scombridae*, *Clupeidae*, *Engraulidae*, *Coryphenidae*, *Scomberesocidae*, *Pomatomidae* families), and the following limits: 9 Sample Units (U.C.) must be analysed, of which 7 must have a histamine content less than 100 mg/kg; 2 U.C. can have a content higher than 100 mg/kg, but lower than 200 mg/kg and no values observed may exceed the limit of 200 mg/kg.

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3. SENSORY FEATURES OF FRESH FISH

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Sea bream in a breeding cage

3.1. Introduction

Quality parameters for fish and seafood include species-specific parameters, and descriptive parameters intended to meet consumer expectations of the sensory profile of the food item. These parameters include the health and safety aspects, nutritional value, functional characteristics, and fulfilment of standards for nutritional composition, appearance, packaging, labelling and shelf-life of a given product. All of the above can be quantified and are used as quality assessment parameters.

The colour, aroma and texture of fish meat are important factors that influence the appropriateness of fish for different culinary methods and uses by the food industry. These factors are species-specific and have a substantial impact on seafood market value. The share of proteins, fats, vitamins and minerals affects not only the nutritional value of a given product but also its sensory profile. Various non-proteinic nitrogen compounds may also influence not only food safety and its appropriateness for further use, but also its sensory features. The biological state of the fish will also affect muscle protein content and indirectly the sensory features of the fish meat.

3.2. Freshness

Freshness is the primary attribute used for the assessment of market value of a number of fish species, and largely contributes to fish quality. Fish is an easily spoiling foodstuff that can be stored only for a short period. Fish and shellfish just caught have a vivid and intense colour, silver or other that is typical to the species. The gills are bright red, the body is firm and elastic, with the typical aroma of algae or mussels. Right after catching, biochemical changes of saccharides, nitrogen compounds and fats commence; due to the chemical oxidation of fats, these freshness-related features gradually deteriorate and are replaced by signs of rotting. In fish and marine vertebrates, the initial loss of original freshness is primarily caused by catabolic reactions taking place on nucleotides and saccharides, followed soon after by the degradation of nitrogen compounds and the hydrolysis and peroxidation of fat.

Sea bass samples on first day of freshness examination

Sea bass samples fifth day of freshness examination

Sea bream samples first day of freshness examination

Sea bream samples fifth day of freshness examination

In that stage, the ongoing reactions are mostly catalysed by endogenous enzymes. Later, the decay witnessed during storage can almost exclusively be attributed to microbiological activity. The rate at which fish loses its freshness and becomes spoiled is controlled by external factors related to fish species, the degree of parasite infestation, initial bacterial contamination, and handling and storage of fish both onboard the ship and onshore.

Fish freshness can be determined via sensory assessment, chemical identification of biochemical reaction products, or measurement of physical characteristics of fish meat.

3.3. Sensory evaluation of fish

Sensory evaluation can be defined as a scientific discipline that evokes, measures, analyses and interprets the responses to the characteristics of products as perceived by the senses. Therefore, sensory evaluation can be defined as the use of one or more of the give senses (sight, smell, taste, touch and hearing) to the end of adjudicating or giving an opinion on certain quality aspects. Fish and fish product-related aspects that can be adjudicated using these senses are listed in Table 3.1. It should be pointed out, however, that fish quality goes far beyond mere edibility. The latter undoubtedly represents the most important overall quality component, but is largely influenced by the manner of fish storage (keeping on ice vs freezing). When assessing fish quality, one should also bear in mind other quality aspects, such as the value of a given fish, its appropriateness for processing, its size suitability and the presence/absence of damage and/or tainting.

Tablica 3.1. Some aspects of fish and fish product quality and the senses engaged in their assessment

Sense	Aspect of quality
Sight	General appearance and condition, size, shape, physical blemishes, colour, gloss, identity
Smell	Freshness, off-odours and off-flavours, taints, oiliness, rancidity, smokiness
Taste	Freshness, off-tastes and flavours, taints, oiliness, rancidity, smokiness, astringency, the primary tastes of acidity, bitterness, saltiness, sweetness
Touch (by finger or mouth)	General texture, hardness, softness, elasticity, brittleness, roughness, smoothness, grittiness, gumminess, fluidity, wetness, dryness, crispness, the presence of bones
Hearing	Brittleness, crispness

Sensorial changes of appearance, smell, texture and taste can be perceived by the human senses. When it comes to fresh fish assessment, sensory techniques have major advantages due to their rapidness, reliability and non-destructiveness, so that no expensive analytical equipment is required. All these techniques require is continuous panellist training supervised by the senior panellist, and the utilisation of fish samples of known freshness. If performed adequately, sensorial techniques provide swift, accurate and unique information on food under consideration. They allow for direct measurement of perceived features and therefore provide information that contributes to the better understanding of consumer response.

Sensory evaluation of whole raw fish carried out onshore and at fish auctions, as well as during storage on ice before processing, includes the assessment of appearance, texture and smell. A typical course of spoilage of fish kept on ice can be outlined using sensory assessment and divided into four stages:

- in stage 1, the fish is fresh and has a delicate taste,
- in stage 2, its typical smell and taste gradually vanish,

- in stage 3, spoilage signs can be noted,
- in stage 4, the fish can be described as spoiled and rotten.

Texture changes are apparent upon catching when rigour commences. The muscles become hard and rigid; following this, the rigour ceases and the muscles relax and become flaccid, but are not as elastic as they were previously.

Techniques to assess fish freshness are applied throughout the fishery chain “from hook to cook”, with sensory analysis thereby taking place in various fish processing stages. In general, such an assessment is entrusted with trained assessors/panellists given clear instructions. When it comes to the recruitment, selection and training of panellists, the procedures are stipulated by international guidelines and norms.

Numerous raw fish assessment protocols have been developed over the past 50 years. The Torry Research Station developed the first modern and detailed technique of white fish sensory assessment. Sensorial features included in their protocol are overall appearance, smell and texture of fish and fish meat, belly covers included. Given that the common market standard applicable to fishery products aims to make the trade easier for both producers and consumers under product quality improvement, and given that fishery products are unprocessed and sold either fresh or cooled, their quality is greatly predetermined by their freshness, which is adjudicated using organoleptic testing.

3.4. EU scheme - raw fish

In the EU, the technique that represents the current state-of-art when it comes to raw fish quality assessment, and is therefore most commonly used and most recommended in both industrial settings and during inspections, is the EU protocol laid down under the Council Regulation (EC) No 2406/96, enforced on November 29, 1996. Under this Regulation, the common market standard is defined by two categories, i.e., fish freshness and fish size.

Freshness is defined based on special evaluation of five product groups (white fish, oily fish, sharks, cephalopods, and crustaceans) and divided into three categories (extra, A, and B); should the fish be inadequate for human consumption, it is categorised as “unacceptable”. In a nutshell, anatomic sites that should be inspected organoleptically during product freshness assessment vary based on the product group but encompass the skin, skin mucosity, eyes, gills, opercula (gill covers) and peritoneum (in fish lacking viscera). One assesses the overall appearance and smell, the smell of the gills and the abdominal cavity, as well as the meat and its firmness. Each category descriptive of fish freshness determined at a given anatomic site targeted by organoleptic assessment is described verbally in terms of its colour, appearance, meat structure, or smell. Freshness ratings for white fish (haddock, cod, saithe, pollack, redfish, whiting, ling, hake, Rays bream, anglerfish, pouting and poor cod, bogue, picarel, conger, gurnard, muller, plaice, megrim, sole, dab, lemon sole, flounder, scabbard fish) relative of the specific appraisal criteria are shown in Table 3.2.

Tablica 3.2.a. The EU protocol applicable to the white fish freshness assessment

	Criteria			
	Freshness category			Not admitted (1)
	Extra	A	B	
Skin	Bright, iridescent pigment (save for redfish) or opalescent; no discolouration	Pigmentation bright but not lustrous	Pigmentation in the process of becoming discoloured and dull	Dull pigmentation (2)
Skin mucus	Aqueous, transparent	Slightly cloudy	Milky	Yellowish grey, opaque mucus
Eye	Convex (bulging); black, bright pupil; transparent cornea	Convex and slightly sunken; black dull pupil; slightly opalescent cornea	Flat; opalescent cornea; opaque pupil	Concave in the centre; grey pupil; milky cornea (2)
Gills	Bright colour; no mucus	Less coloured; transparent mucus	Brown/grey becoming discoloured; thick, opaque mucus	Yellowish; milky mucus (2)
Peritoneum (in gutted fish)	Smooth; bright; difficult to detach from flesh	Slightly dull; can be detached from flesh	Speckled; comes away easily from flesh	Does not stick (2)
Smell of gills and abdominal cavity				(2)
whitefish other than plaice	Seaweedy	No smell of seaweed; neutral smell	Fermented; slightly sour	Sour
plaice	Fresh oily; peppery; earthy smell	Oily; seaweedy or slightly sweetish	Oily; fermented; stale, slightly rancid	Sour
Flesh	Firm and elastic; smooth surface (3)	Less elastic	Slightly soft (flaccid), less elastic, waxy (velvety) and dull surface	Soft (flaccid) (2); scales easily detached from skin, surface rather wrinkled

b. Extra criteria for headed anglerfish

Blood vessels (ventral muscles)	**Sharp outline and bright red**	**Sharp outline; darkening of the blood**	**Diffuse and brown**	**Totally (2) diffuse, brown and yellowing of the flesh**

(1) This column will apply only until a Commission Decision in taken establishing criteria for fish which is unfit for human consumption, pursuant to Council Directive 91/493/EEC

(2) Or in a more advanced state of decay

(3) Fresh fish prior to the onset of rigor mortis will not be firm and elastic but will still be graded in category Extra

To summarise, the sensory assessment of freshness of seafood includes an assessment of the outer appearance, smell and texture of raw fish. The technique discussed above provides fairly limited information on fish status, is not species-specific, and fails to acknowledge the differences between fish species. For the reasons stated above, the technique is most commonly exercised during auctions. Furthermore, this technique fails to provide any results based on which either fish age or its shelf life could be predicted. Decay is described in steps lacking continuity. The system has some serious flaws, especially when all sensorial features are not assessed using the same category.

3.5. The QIM method

In light of the above, a novel and improved system of the assessment of quality and freshness of seafood was designed, best described as swift, impartial and applicable to various fish species. The Quality Index Method (QIM) is underpinned by the protocol originally developed by the Tasmanian Food Research Unit (TFRU) operating under the umbrella of the Commonwealth Scientific and Industrial Research Organization (CSIRO), which is adaptable to any given fish species. However, the highest number of QIM protocols has been developed in Europe. To date, within the 2000–2020 timeframe, the protocols available in the scientific literature have been built for 49 different fish species, together with the respective storage conditions, QI range and estimated shelf life.

The QIM has several advantages, including the estimation of past and remaining on-ice storage time. The technique is based on characteristic changes taking place in raw fish, i.e., on well defined, characteristic changes in features descriptive of raw fish outer appearance (eyes, skin, gills, smell), and uses the scoring system spanning from “0” to “3” demerit (index) points. The points allocated to each characteristic are summed up and give an overall sensory score, known as the “quality index”. The latter increases with the duration of storage on ice in a linear fashion. The description of each point allocated to each parameter is given under the QIM protocol. The panellist is obliged to assess all parameters embraced by the protocol. Within the frame of the QIM protocol, fish outer appearance, its eyes, gills and texture are appraised. Furthermore, the gill smell is assessed, and in some species also the smell of the skin and skin mucosity. The colour of the blood and the fillets is assessed once the fish is eviscerated. To avoid biological differences in fish spoilage rate, at least 3, and not more than 10, fishes from each lot should be assessed.

Tables 3.3. and 3.4. show the QIM scheme for raw gilthead sea bream and sea bass, respectively. Huidobro et al. (2000) were the first to propose the QIM protocol applicable to raw gilthead sea bream and decapitated eviscerated gilthead sea bream, while Campus et al. (2011) developed the QIM protocol applicable to decapitated gilthead sea bream packaged in a modified atmosphere. Although the QIM protocols have been developed to serve industrial purposes, studies aiming to develop consumer QIM protocols (Consumer - Quality Index Method; C-QIM) have also been carried out. C-QIMs is a consumer-oriented tool aiming to assist consumers in their decision to purchase fish in a shop or at the fishmonger. The use of this tool can help a consumer to buy a product of high sensorial quality and to learn more about food quality and its variations.

Tablica 3.3. Metoda određivanja indeksa kvalitete (QIM) primjenjiva na sirovu komarču

	Attribute	Description	Score
Appearance	Skin	Very bright Bright Dull	0 1 2
	Slime / mucus	Clear-transparent Slightly cloudy / cloudy	0 1
Flesh	Elasticity	Elastic Marked by pressure	0 1
Odour	Odour	Fresh Neutral Fishy Off-odours	0 1 2 3
Eyes	Clarity	Clear translucent Slightly opaque Opaque, bloody	0 1 2
	Shape / Form	Convex Flat Concave	0 1 2
Gills	Colour	Bright, dark red Brownish red / discoloured	0 1
	Odour	Fresh, seaweed Neutral Fishy Off odours	0 1 2 3
		QIM score	**0-15**

Tablica 3.4. Quality Index Method (QIM) applicable to raw sea bass

	Attribute	Description	Score
Koža	Colour / appearance	Bright, iridescent pigmentation Rather dull, becoming discoloured (head) Green, yellowish, mainly near the abdomen	0 1 2
	Odour	Fresh seaweedy, neutral Cucumber, metal, hay Sour, dish cloth Rotten	0 1 2 3
	Texture	In rigor Finger mark disappear rapidly Finger leaves mark for 3 seconds	0 1 2
Eyes	Pupils	Clear and black, metal shiny Grey Matt, grey	0 1 2
	Form	Convex Flat Sunken	0 1 2
Gills	Colour	Blood red / orange Pale red, pink / light brown Grey-brown, brown, grey	0 1 2
	Mucus	Fresh, seaweed, neutral Metal, grass Sour, mouldy, dish cloth Rotten	0 1 2 3
	Sluz	Transparent Milky, clotted Brown, clotted	0 1 2
Flesh, fillets	Colour	Translucent, bluish Waxy, milky Opaque, yellow, brown spots	0 1 2
Viscera	Solution	Whole Beginning to dissolve Viscera dissolved	0 1 2
		QIM score	**0-22**

3.6. Torry scheme

When assessing the sensory properties of fish fillets, it is customary to cook the fillets and subsequently assess their smell and aroma. The technique most often used for the assessment of cooked fish freshness is the Torry scale, in some countries also employed by the fish industry and by fish product consumers. It represents a 10-point descriptive scale developed by the Torry Research Station to be used with lean, medi-

Table 3.5. Torry freshness score sheet applicable to iced white fish (raw cod)

Score	Eyes	Skin	Texture and Effect of Rigor Mortis	Flesh and Belly Flaps	Kidney and Blood	Gills		Score
						Appearance	Odour	
10	Bulging, convex lens, black pupil, crystal clear cornea	Bright, well-differentiated colours, glossy, transparent slime	Flesh firm and elastic. Body pre-rigor or in rigor	Cut surface stained with blood. Bluish translucency around backbone. Fillet may have rough appearance due to rigor mortis contraction	Bright red blood flows readily	Glossy, bright red or pink mucus	Initially very little odour increasing to sharp, iodine, starchy metallic odours changing to less sharp seaweedy, shellfish odours	10
9	Convex lens, black pupil with loss of initial clarity		Flesh firm and elastic. Muscle blocks apparent. In or just passing through rigor	White with bluish translucency, may be corrugated due to rigor mortis effect	Bright red, blood does not flow			9
8	Slight flattening or plane, loss of brilliance	Loss of brilliance of colour	Firm, elastic to the touch	White flesh with some loss of bluish translucency. Slight yellowing of cut surfaces of belly flaps	Slight loss of brightness of blood	Loss of gloss and brightness, slight loss of colour	Freshly cut grass. Seaweedy and shellfish odours just detectable	8
7							Slight mousy, musty or caprylic	7
6		Loss of differentiation and general fading of colours; overall greyness. Opaque and somewhat milky slime	Softening of the flesh, finger indentations retained, some grittiness near tail	Waxy appearance of the flesh, reddening around the kidney region. Cut surfaces of the belly flaps brown and discoloured	Loss of brightness, some browning	Some discoloration of the gills and cloudiness of the mucus	Bready, malty, beery yeasty	6
5	Slightly sunken, slightly grey pupil, slight opalescence of cornea						Lactic acid, sour milk or oily	5
4	Sunken, milky white pupil, opaque cornea	Further loss of skin colour. Thick yellow knotted slime with bacterial discoloration. Wrinkling of skin on nose	Softer flesh, definite grittiness	Some opacity, reddening along backbone and brown discoloration of belly flaps	Brownish kidney blood	Slight bleaching and brown discoloration with some yellow bacterial mucus	Lower fatty acid odours (e.g., acetic or butyric acids), composted grass, "old boots", slightly sweet, fruity or chloroform like	4
3							Stale cabbage water, stale turnips, "sour sink", wet matches	3

um fatty and fatty fish. Fresh taste and smell can be allocated up to 10 points, while spoiled fish can score up to 3 points. The description below 3 points is lacking, since fish scoring so low is not suitable for human consumption. Sensorial assessment of cooked samples also allows for the assessment of maximal storage time. The average score of 5.5 is viewed as the limit of acceptability for consumption. Research has shown a linear correlation between raw fish QI and cooked fillet assessment using the Torry scale, meaning that whole raw fish QIM can be assessed instead of the cooked samples sensory assessment. Furthermore, the QIM is swifter and used in the earlier stages of the production chain. Table 3.5. shows the Torry scheme for iced white fish - raw cod as a typical white fish representative.

3.7. QDA method

The Torry scale provides only limited information on changes of certain cooked fish characteristics during storage; however, the use of quantitative descriptive analysis (QDA) may provide much more detailed information. The QDA is a sensory analysis technique used to obtain a detailed description of the sensory profile of a given product, but also to determine its maximal shelf life. Within the QDA frame, all recognisable and detectable aspects of a product are described and listed by a trained panel guided by the senior panellist. While describing the product, the panellists compile a list of terms in form of a glossary. The list is then used for product assessment and the panellists quantify the sensorial aspects of the product using a non-structured scale for each of the sensory terms. Before engaging in an assessment that makes use of a non-structured scale to adjudicate each sensory parameter, panellists receive proper training. The terms used to describe the fish smell and aroma can be grouped into favourable and unfavourable sensory parameters, depending on whether fresh fish or fish close to the expiry date is described.

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4. NUTRITIONAL QUALITY OF FISH

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4.1. Introduction

Consumption of fish generally provides an important source for nutrition for consumers worldwide and makes a very significant contribution to a healthy diet. Information on the chemical composition of fish in respect to its nutritive value should also be compared with other sources of animal protein foods, such as red meat and poultry. Historically, the main effects of fish consumption have been attributed to the high content of valuable fatty acids, though new research is showing that other nutrients from fish have positive effects on human health.

In fresh fish, skeletal muscle is the largest edible tissue and its composition is considered to be the major quality aspect, while organoleptic properties, nutritional value and freshness are the main quality parameters for consumers. In the case of European sea bass and gilthead sea bream, skeletal muscle represents 44–58% and 34–48% of total body weight, respectively. Muscle is composed of fat, proteins, water, inorganic elements, volatile bases such as ammonia, trimethylamine and dimethylamine, trimethylamine oxide, free amino acids, urea, vitamins, carbohydrates and volatile compounds, such as alcohols, aldehydes, ketones and terpenes that contribute to fish aroma.

The chemical composition of fish varies greatly among species depending on age, sex, environment, and season. Variations in nutritional composition have even been found between individuals of the same species, age and sex, in the same catch. Individual differences in chemical composition can be significant and serve as an important factor in assessing the average quality of different types of fish. Fish is the only protein source that contains all the essential amino acids. However, fish contributes more to the human diet than just the high-quality protein they are so well known for. In addition to proteins, lipids are a major component of fish food and an excellent source of valuable micronutrients, vitamins, and minerals. Fish meat is generally considered to be a very valuable food by its nutritional properties, with high nutritional value in the beneficial amounts of protein, lipids and essential micronutrients.

Fish meat generally contains up to 80% water, 15–23% proteins, 0.7–20% fat, while carbohydrates are present at a very low share (<2%). Fish proteins are easily digested due to the lower proportion of collagen and have good recovery (93–98%) and chemical score. In addition to proteins, the nutritional value of fish meat is also linked to its lipid composition. Besides being a major source of omega-3 long-chain polyunsaturated fatty acids (n-3 LC PUFA), it is also characterised with a well-balanced amino acid composition, containing high proportions of taurine and choline. The minerals in fish include macro-minerals such as potassium, calcium and phosphorous, and also many microelements. Vitamins include the fat-soluble vitamins A, D, E, K and others. Fish should therefore be an integral component of the diet, preventing malnutrition by making these macro- and micro-nutrients readily available to the body.

This manual presents the basic nutritive composition, fatty acid profile and vitamin and mineral profile for different fish species in general. Some examples of these values were determined for sea bass and sea bream as the most commercially important species of farmed fish.

4.2. Basic nutritive composition

The importance of fish in providing easily digested protein of high biological value is well known. On a fresh-weight basis, fish contains about 18–20% protein and contains all the essential amino acids, including the sulphur-containing amino acids cysteine and methionine. A portion of fish provides one-third to one-half the daily protein requirement. This explains how fish plays an important role in meeting nutritional food security, especially in preventing protein-calorie malnutrition. In comparison to other sources of dietary animal proteins, consumers have a wide choice of fish as far as affordability is concerned, given the many varieties and species of fishes available. Also, in comparison to other sources of dietary protein of animal origin, such as chicken, lamb, pork and beef, the unit cost of production of fish is much cheaper.

However, the protein content of a fish species depends on biological and environmental circumstances (sex, age, sexual maturity, nutritive conditions, etc.). In general, the protein content of fish flesh, unlike fat content, is highly constant, independent of seasonal variations related to the feeding and reproductive cycles, and shows only small differences among species. Fat content can range from 1% in low-fat fish to 30% in higher-fat fish. In general, the fat content determines the value of the raw material and the taste, and according to the fat content in the meat, fish can be divided into several groups: lean fish whose fat content does not exceed 2–4%; semi-fatty fish with a fat content of 4–8%, fatty with a fat content of more than 8% and extra-fat with more than 15% fat. Sea bream and sea bass are classified as semi-fatty fish.

Fish is also divided into oily and white based on their fat distribution. Oily fish store fat in fat cells all over the body, while white fish stores fat in the liver and abdominal cavities. The percentage of fat in white fish is low, especially in the meat where it is about 1%, while 90% is made up of structural fats or phospholipids. Although the total content of highly unsaturated fatty acids in white fish is lower, it represents a higher proportion in the total fat in comparison to fatty fish (e.g., 37% in comparison to 17%). However, due to the overall increased fat content of farmed fish compared to wild fish, such products represent a valuable source of omega-3 fatty acids in the diet.

Whereas the average water content of the flesh of fatty fish is about 70%, individual specimens of certain species may be found with water content anywhere from 30–90%. The water in fresh fish muscle is tightly bound to the proteins in the structure. After prolonged chilled or frozen storage, the proteins are less able to retain all the water, and some of it, containing dissolved substances, is lost as a drip. Frozen fish that are stored at too high a temperature will produce a large amount of drip and consequently the quality will suffer. In live fish, the water content usually increases and the protein content decreases as spawning approaches. The amount of carbohydrate in fish muscle is generally too low to be of any significance in the human diet. In white fish, the amount is usually less than 1%, though the dark muscle of some fatty species may occasionally contain up to 2%. The ash content of the gills, head, and fishbone reflects the mineral content of different fish species.

Table 4.1. shows the proximate basic composition of farmed gilthead sea bass and sea bream obtained in our earlier studies on these species farmed at different locations in the Adriatic Sea.

Table 4.1. Basic chemical and mineral composition of sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) farmed in the Adriatic Sea (Pleadin et al., 2017)

Parameter	Sea bass	Sea bream
Water (%)	70,81 ± 3,28	70,16 ± 2,50
Protein (%)	19,22 ± 1,46	19,09 ± 0,33
Fat (%)	9,11 ± 3,06	10,48 ± 3,08
Ash (%)	1,21 ± 0,02	1,24 ± 0,07
Carbohydrates (%)	0,10 ± 0,01	0,08 ± 0,02

Distillation and titration in the assessment of protein content by Kjeldahl

4.3. Fatty acids

In general, there are three types of fatty acids (FAs): saturated (SFAs), monounsaturated (MUFAs) and polyunsaturated (PUFAs). PUFAs include omega-3 (also called ω-3 or *n*-3) and omega-6 (also called ω-6 or *n*-6) fatty acids. SFAs and MUFAs are synthesised endogenously, but PUFAs cannot be synthesised by humans from other components by any known biochemical pathways, and therefore must be obtained from the diet. Fish fats contain from 17 to 21% saturated and from 60 to 84% unsaturated fatty acids with five or six double bonds. The total content of PUFAs is slightly lower in freshwater fish (70%) than in marine fish lipids (about 88%). Fish fat is characterised by a low content of n-6 fatty acids, so in marine fish fat, the ratio of n-3 and n-6 is high and ranges from 5:1 to 10:1.

The results of studies have shown that the most strongly represented FA in many farmed fish species are oleic acid (C18:1n-9, OA), followed by linoleic acid (C18:2n-6, LA) and palmitic acid (C16:0, PA), but also that significant differences in the FA profiles were observed among the fish species. Fish has been acknowledged as an integral component of a well-balanced diet according to its content of n-3 long-chain PUFAs. Their well-known hypotriglyceridaemic effect in the body may be beneficial in terms of reducing the percentage of pro-atherogenic small low-density lipoprotein (LDL) particles, and perhaps by ameliorating the inflammatory processes associated with the metabolic syndrome seen in patients with diabetes mellitus or cardiovascular disease. The *n*-3 fatty acids that are important in human nutrition are α-linolenic acid (18:3, *n*-3; ALA), eicosapentaenoic acid (20:5, *n*-3; EPA), and docosahexaenoic acid (22:6, *n*-3; DHA). These three PUFAs have 3, 5 or 6 double bonds in a carbon chain of 18, 20 or 22 carbon atoms, respectively.

The human body cannot synthesise *n*-3 fatty acids *de novo*, but it can form 20-carbon unsaturated *n*-3 fatty acids (like EPA) and 22-carbon unsaturated *n*-3 fatty acids (like DHA) from the eighteen-carbon *n*-3 fatty acid α-linolenic acid. These conversions occur competitively with *n*-6 fatty acids, which are closely related chemical analogues derived from linoleic acid (LA). Both *n*-3 α-linolenic acid and *n*-6 linoleic acid are essential nutrients which have to be obtained from food. Synthesis of the longer *n*-3 fatty acids from linolenic acid within the body is competitively slowed by the *n*-6 analogues. Thus, the accumulation of long-chain *n*-3 fatty acids in tissues is more effective when they are obtained directly from food or when competing amounts of *n*-6 analogues do not greatly exceed the amounts of *n*-3.

Fish from intensive systems displayed the lowest levels of EPA, DPA and DHA, but the highest levels of n-6 fatty acids. It is common knowledge that the fatty acid composition in fish flesh reflects the dietary fatty acid profile. Hence, the high levels in n-6 fatty acids and the large variation in some flesh fatty acids, especially in linoleic acid, in fish reared in intensive systems suggest different degrees of dietary incorporation of plant sources. Over recent decades, fish nutrition research has devoted continued efforts into developing sustainable feeds that could provide adequate levels of long-chain n-3 fatty acids for human nutrition. The proximate fatty acid composition of farmed gilthead sea bass and sea bream from different locations in the Adriatic Sea is shown in Table 4.2. The proximate share of ALA, EPA, DHA, AA and LA as nutritional quality indices, respectively, obtained from the same fish samples, are shown in Tables 4.3. and 4.4.

Table 4.2. Fatty acid composition of sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) farmed in the Adriatic Sea (Pleadin et al., 2017)

Fatty acids (%)	Sea bass	Sea bream
SFA	23,93 ± 2,13	21,24 ± 3,25
MUFA	51,82 ± 2,45	54,66 ± 3,74
PUFA	24,25 ± 2,30	24,10 ± 1,38
Ukupne n-6	15,74 ± 2,06	16,87 ± 0,37
Ukupne n-3	8,51 ± 1,29	7,23 ± 1,48

The results are expressed as mean values ± standard deviation (relative FA amount - % of total fatty acids in edible muscle part); SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid

Table 4.3. Proximate shares of the most important fatty acids in sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) farmed in the Adriatic Sea (Peadin et al., 2017)

Fatty acids (g/100 g)	Sea bass	Sea bream
ALA	0,25 ± 0,04	0,30 ± 0,10
EPA	0,16 ± 0,04	0,11 ± 0,06
DHA	0,25 ± 0,08	0,21 ± 0,11
EPA + DHA	0,41 ± 0,11	0,32 ± 0,16
AA	0,02 ± 0,00	0,02 ± 0,01
LA	1,22 ± 0,17	1,52 ± 0,06

The results are expressed as mean values ± standard deviation (g of fatty acid/ 100 g edible muscle part); ALA, *alpha-linolenic acid;* EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AA, arachidonic acid; LA, linoleic acid

Tablica 4.4. Table 4.4. Mean values of nutritional quality indices in sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) farmed in the Adriatic Sea (Pleadin et al., 2017)

Parameter	Sea bass	Sea bream
n-3/n-6	0,55 ± 0,11	0,43 ± 0,09
PUFA/SFA	1,02 ± 0,17	1,15 ± 0,19
AI	0,35 ± 0,05	0,32 ± 0,07
TI	0,38 ± 0,04	0,35 ± 0,06
HH	3,34 ± 0,55	3,94 ± 1,03
FLQ	4,53 ± 1,25	3,06 ± 1,55

The results are expressed as mean values ± standard deviation; n-3, omega-3 fatty acids; n-6, omega-6 fatty acids; SFA, *saturated fatty acid*; PUFA, polyunsaturated *fatty acid*; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; AI, atherogenic index; TI thrombogenic index; HH hypocholesterolaemic/hypercholesterolaemic ratio, FLQ, flesh lipid quality

Gas chromatograph with flame ionization detection (GC-FID) for testing the composition of fatty acids

4.4. Minerals

Fish is a particularly good source of minerals such as potassium, phosphorus, sodium, calcium, and also some microelements. These minerals in fish are highly

Atomic absorption spectrometry (AAS) for mineral determination

bioavailable meaning they are easily absorbed. Their content variations are closely related to seasonal and biological differences such as species, size, dark/white muscle, age, sex and sexual maturity, catching or farming area, processing method, food source and environmental conditions such as water chemistry, salinity, temperature and concentration of contaminants. Performed studies have shown that the most important macro- and microelements in sea bass and sea bream are potassium, phosphorus, calcium, sodium, magnesium, iodine, iron, zinc, manganese and selenium (Table 4.5). Iron and zinc were the dominant elements among 14 minerals and constituted about 80% of the total trace mineral contents in farmed and wild sea bass and sea bream.

4.4.1. Potassium and phosphorus

Potassium is an electrolyte that interacts with sodium as one of the main electrolytes in the body to conduct nerve impulses and it also performs many other functions in the cells. Phosphorus plays an important role in the bones and in the cellular membranes as a component of the phospholipids that build the membrane lipid bilayer. It is also a component of many intracellular compounds, including nucleic acids, nucleoproteins and organic phosphates. A phosphorus deficiency leads to muscle disorders, metabolic acidosis, encephalopathy and alteration in bone mineralisation as well as cardiac, respiratory, neurological and metabolic disorders. In several publications, fish and seafood are suggested to be a good source of phosphorus with an average between 204 and 230 mg/100 g in fish.

4.4.2. Calcium

Calcium is required for bone formation and mineralisation as also for the normal functioning of muscles and the nervous system. Most calcium in the body is contained in the bones, but about 1% is used for nerve impulses and muscle contractions that sustain life and enable movement. Calcium participates in the protein structuring of RNA and DNA, so it affects the genetic structure and genetic mutations in the body's constant cellular replacement programme. The intake of calcium, phosphorus and fluorine is higher when small fish are eaten with their bones rather than when fish bones are discarded. A calcium deficiency may be associated with rickets in young children and osteomalacia (softening of bones) in adults and the elderly.

4.4.3. Zinc

Among the microelements, *zinc* plays an important role in growth and development, as well in the proper functioning of the immune system and healthy skin. It also has a role in cell division and growth, wound healing and the breakdown of carbohydrates, and is needed for the senses of smell and taste. Its deficiency is associated with poor growth, skin problems and hair loss among other problems. High-protein foods like meat and fish contain the highest amount of zinc, and it is easily absorbed from these sources. It was evidenced that oily fish provide a significant amount of zinc.

4.4.4. Iron

Iron is important in the synthesis of haemoglobin in red blood cells which is important for transporting oxygen to all parts of the body, and its deficiency is associated with anaemia, impaired brain function and in infants with poor learning ability and poor behaviour. Due to its role in the immune system, iron deficiency may also be associated with an increased risk of infection. Compared to other animal sources, although fish contain less iron than the amount found in red meat, iron in white fish is well absorbed and so is a useful source of iron.

4.4.5. Iodine

Iodine is important for hormones that regulate body metabolism and in children, it is required for growth and normal mental development. An iodine deficiency may lead to goitre (enlarged thyroid gland) and mental retardation in children (cretinism). Fish is one of the few reliable sources of iodine.

4.4.6. Magnesium

The main characteristics of *magnesium* are that it participates in more than 300 enzymatic reactions, and it is essential for the conversion of vitamin D to its biologically active form that then helps the body to absorb and utilize calcium.

4.4.7. Selenium

Selenium is a component of some enzymes that protect the body against oxidation damage (free radical damage). It is also necessary for the use of iodine in thyroid hormone production and immune system function. It has also been shown that selenium and selenite from fish are highly bioavailable and had a higher bioavailability than selenium from yeast. Fish is a particularly good source of selenium and a 100 g portion of fish could provide roughly half the daily recommended intake of this mineral.

Tablica 4.5. Mineral composition of sea bass and sea bream farmed in the Aegean Sea (Özden and Erkan, 2008)

	Sea bass (mg/kg)	Sea bream (mg/kg)
Potassium	4 601,03 ± 0,07	3 911,39 ± 0,08
Phosphorus	3 749,80 ± 0,41	3507,38 ± 0,03
Sodium	775,26 ± 0,15	291,14 ± 0,05
Calcium	616,50 ± 0,51	195,15 ± 0,40
Iodine	343,30 ± 0,03	517,00 ± 0,03
Magnesium	325,77 ± 0,05	219,41 ± 0,04
Iron	25,77 ± 0,04	224,68 ± 0,05
Zinc	2,89 ± 0,02	1,08 ± 0,05
Manganese	0,54 ± 0,09	6,47 ± 0,08
Selenium	0,29 ± 0,01	0,24 ± 0,03

4.5. Vitamins

The vitamin content of fish varies according to species, age, season and fishing localities and in the case of cultured fish also with feed. Seafood is known to contain vitamins A, D, E, thiamine, riboflavin, niacin, B6, pantothenic acid, B12 and negligible quantities of vitamin C. Very few results are generally available on the amounts of vitamins in fish, including in sea bass and sea bream. Some results obtained for the vitamin content in these two species are shown in Table 4.6..

4.5.1. Vitamin A

Vitamin A from fish is more readily available to the body than from plant foods. Among all the fish species, fatty fish contains more vitamin A than lean species. Studies have shown that mortality is reduced for children under five with a good vitamin A status. Vitamin A is also required for normal vision and bone growth. In the flesh of sea bass, vitamin A in some studies was not detected which was explained by the fact that this vitamin is more abundant in fish oil and liver. In mature fish, about 90% or more of the total vitamin A in the body is usually stored in the liver. As sun-drying destroys most of the available vitamin, other processing methods should be implemented to preserve them. Our studies found a mean vitamin A content of 12.9±6.6 μg/100 g in sea bass and 4.3±1.2 μg/100 g in sea bream.

4.5.2. Vitamin E

Naturally occurring *vitamin E* exists in eight chemical forms (α, β, γ and δ-tocopherol and α, β, γ and δ-tocotrienol), which have varying levels of biological activity. Of these, α-tocopherol has been the most studied form as it has the highest bioavailability and represents the most important lipid-soluble antioxidant that protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. This removes the free radical intermediates and prevents the oxidation reaction from continuing. Free-radical mechanisms have been implicated in the pathology of several human diseases, including cancer, atherosclerosis, malaria, rheumatoid arthritis, and neurodegenerative diseases. Authors reported that the vitamin E content in the edible parts of fish range from 0.2 to 270 mg/100 g wet weight. The mean vitamin E content determined in sea bass and sea bream from the Adriatic Sea in our studies resulted with values of 1.9±0.4 mg/100 g and 1.05±0.3 mg/100 g, respectively.

4.5.3. Vitamin D

Vitamin D present in fish liver and oils is crucial for bone growth since it is essential for the absorption and metabolism of calcium. It also plays a role in immune function and may offer protection against cancer. Oily fish is the best food source of unfortified vitamin D. The form of vitamin D found in fish is vitamin D3 (cholecalciferol), which is also the form produced in the skin from 7-dehydrocholesterol when exposed to ultraviolet light. Literature data show variations of vitamin D content in various species between 0.5 and 30 mg/100 g of fish muscle.

4.5.4. B vitamins

Fish is also a good source of the *B vitamins* and can provide a similar beneficial contribution to the diet for this group of vitamins as red meat. The B complex of vitamins is responsible for converting food to energy in the cells, and they assist with the function of nerve tissue. Pantothenic acid has been recognised as participating in the basic biochemical reactions of animal cells, constituting a part of coenzyme A. It occurs in the largest amount in the ovaries, generally followed by dark meat and the liver, but it is scarce in the white flesh. The level of riboflavin is highest in metabolically active tissues, in particular in fish-eye retina, melanin in the skin, dark meat and pelagic species. High niacin is thought to be due to the high amount of fat content in fish flesh so its content in oily fish was higher than that of lean meat. The content of folic acid, in general, is very low and body organs of fish such as the liver, kidney and spleen contain more folic acid than both white and dark meat.

Tablica 4.6. Vitamin content of sea bass and sea bream

Vitamin	Sea bass[a] (mg/kg)	Sea bream[b] (mg/kg)
Thiamine	0,46 ± 0,02	n.a.
Riboflavin	0,16 ± 0,01	n.a.
Folic acid	0,06 ± 0,00	n.a.
Niacin	12,00 ± 0,00	n.a.
Ascorbic acid	12,95 ± 0,05	n.a.
Pantothenic acid	3,20 ± 0,00	n.a.
Vitamin E	6,90 ± 0,10	3,10- 6,00
Vitamin A	n.d.	0,27- 0,60
Vitamin D	n.a.	0,98- 1,70

[a] sea bass (*Dicentrarchus labrax* L.,1758) cultured in the Black Sea (Kocatepe and Turan, 2012)
[b] sea bream (*Sparus aurata* L., 1758) cultured in the Black Sea during four seasons (Öztürk et al., 2019) n.a. – not analysed; n.d. – not detected

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5. BENEFITS OF FISH IN HUMAN NUTRITION

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5.1. Introduction

Nowadays, health is a mega-trend that determines consumer behaviours and has led to the development of a specific niche called LOHAS (Lifestyles of Health and Sustainability). This market segment consists of a well-educated, financially situated population with strongly positive attitudes towards healthy eating, sustainability and ecological initiatives.

Having a low-fat content and containing high-quality proteins and numerous micronutrients such as vitamins and minerals, fish is generally accepted as a vital component of a balanced and healthy diet. Due to its high nutritional value, fish is linked with positive benefits in many pathological conditions (i.e., cardiovascular diseases, obesity, metabolic syndrome, cancer, mental health, etc.).

A meal prepared of sea bass fillet

5.2. Fish consumption from the consumer perspective

World fish consumption has more than doubled since the first available data in 1961 (9.0 kg/capita/year) to stands at 20.4 kg/capita/year in 2017. The average annual increase has even exceeded that of meat and has surpassed population growth. However, fish consumption differs considerably across the world and regions due to various geographical, cultural and economic factors.

Fish consumption in the European Union has remained stable at around 23 kg per capita over the last four years (2014–2017), with notable differences among countries. Mediterranean countries, i.e., Portugal, Spain, France and Italy, are the most relevant in terms of *per capita* consumption (56.84, 42.47, 34.37, 29.8 kg/capita/year, respectively), which can be explained by their traditional dietary habits that include a variety of fish. Although Croatia belongs to the group of Mediterranean countries, its fish consumption is below the EU average, at only 18.7 kg/capita/year. Within countries, consumption varies depending on geographical location, i.e., consumption is typically higher in coastal areas and hinterland.

Fish consumption involves complex choices that are reinforced by a variety of drivers and barriers. The main drivers of fish consumption are a positive attitude towards eating fish and the perception of fish as a healthy food. The most important barriers seem to be a sensory dislike of fish, inconvenience, health risk concerns, insecurity in selecting and preparing fish, high price, and the unavailability of fish. As far as consumer preferences regarding fish quality attributes are concerned, most consumers seem to prefer wild, domestic, fresh and whole fish rather than farmed, imported, frozen and processed fish.

In addition to nutritional and toxicological aspects, there are two additional issues that affect fish consumption: the ecological and economic aspects. Ecological concerns are related to the depletion of wild fish stock with the added stress imposed by climate variability and habitat alteration, particularly for heavily overfished stocks that are more sensitive to climate variability. It is estimated that fish production will need to increase by 50% by 2050 if the basic protein requirements of the world's growing population are to be met and food security ensured around the globe.

From an economic perspective, fisheries and the fish processing industry, together with related businesses such as grocery and restaurants suppliers, are key determinants of the type, amount, and form of fish that people consume, by affecting the availability, desirability and cost of different fish. As fish consumption increases, so does the threat of overharvesting wild fish stock and unsustainable methods in aquaculture production. Changes in consumer behaviour, however, have great potential in enhancing the sustainability of wild fish harvesting and aquaculture. Accordingly, by encouraging greater consumer demand for more environmentally friendly fish options, it could be possible to impact the type of fish harvested and farmed.

5.3. Nutritional benefits of fish consumption

Fish is an integral part of several healthy dietary patterns, such as the Mediterranean diet, Nordic diet, or Dietary Approach to Stop Hypertension (DASH), associated with a lower risk of modern non-communicable diseases. Many of the beneficial effects of fish consumption derive from the different types of nutrients found abundantly in fish and how these nutrients interact. However, frequency, amount of consumption and fish type are important factors in estimating the extent of the positive effects of fish consumption on disease prevention and health promotion.

Beneficial nutrients in fish which are related to its positive impact on human health are proteins, taurine, vitamin D, n-3 fatty acids, selenium and iodine. The greatest differences in the content and concentration of nutrients can be found between fatty fish and lean fish. Fatty fish has been found to have higher levels of n-3 fatty acids and the fat-soluble vitamin D, while lean fish has greater concentrations of iodine and taurine.

A full meal made of sea bream

Fish proteins have been found to have a beneficial effect on satiety levels, with a slower decline in satiety resulting in reduced appetite and lower overall food intake. Fish proteins are of high biological value, as they contain all essential amino acids in the right proportions. They represent an excellent source of lysine and the sulphur-containing amino acids, methionine and cysteine. The amino acid composition in combination with bioactive peptides could explain the positive effects of fish proteins on lipid metabolism.

Fish is a good food source of taurine. Taurine is a non-protein amino acid with multiple functions as it supports neurotransmission in the brain, helps stabilise cell membranes and is involved in the transport of ions such as sodium, potassium, calcium and magnesium.

The marine long-chain polyunsaturated fatty acids (LC PUFA) from the n-3 class, eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3), are mainly present in fatty fish. Lean fish, however, is also a source of n-3 fatty acids as it contains approximately 260 mg n-3 per 100 g. The n-3 fatty acids can induce a broad range of biological effects, leading to improvements in blood pressure and cardiac function, endothelial function, arterial compliance and vascular reactivity, reduced neutrophil and monocyte cytokine formation, lipid and lipoprotein metabolism, and potent antiplatelet and anti-inflammatory effects.

Vitamin D, from the group of fat-soluble vitamins, is primarily important for calcium homeostasis but it also has an anti-inflammatory effect on human immune cells. Low vitamin D intake is also associated with unfavourable changes in the fatty acid profile.

Regarding minerals, fish is a valuable source of iodine and selenium. Iodine is important in ensuring normal thyroid function, through the production of hormones. Thyroid gland disorders can have a significant impact on thermogenesis, body weight, and lipolysis in adipose tissue. Hypothyroidism is often associated with weight gain, decreased thermogenesis and decreased metabolic rate. Selenium is a co-factor in antioxidant activities and thyroid hormone metabolism.

5.4. Positive health effects of fish consumption

In the modern Western diet, the intake of n-6 fatty acids is continuously increasing while the intake of n-3 fatty acids is decreasing. Consequently, the n-6:n-3 LC PUFA ratio has been steadily growing from the desired ratio of 1:1 to 15:1 or even higher. These changes are linked to the increased prevalence and risk of various diseases (obesity, cardiovascular disease and related chronic inflammatory diseases).

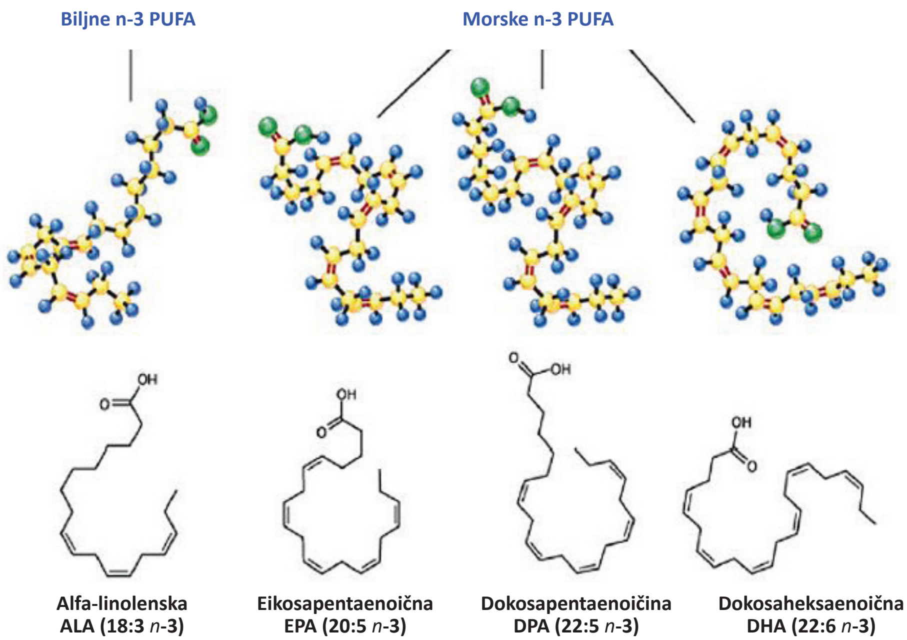
Since inflammation is an essential mechanism in human health and disease, chronic inflammation is one of the major causes of many chronic diseases (such as coronary heart disease, obesity, rheumatoid arthritis, cancer, diabetes and mental illness). Inflammation is a protective response triggered by pathogens or foreign bodies in, or injury to, host tissues. The outcome of this process depends on the balance between the presence of mediators and sensors that either amplify the inflammatory process or control the return to normal health. Excessive intake of n-6 LC-PUFA promotes the synthesis of pro-inflammatory eicosanoids, while a high intake of n-3 LC-PUFA versus n-6 LC-PUFA has anti-inflammatory effects. A high content of n-3 PUFA is often indicated to be the most important nutrient in fish since it can influence the production of lipid mediators from inflammatory cells, thus affecting the outcome of inflammatory processes. The anti-inflammatory and immunomodulatory effects of n-3 PUFA protect against many chronic diseases.

A meta-analysis of prospective cohort studies on the association of fish consumption and the risk of chronic disease has concluded that fish consumption is associated with a lower risk of all causes of mortality, cardiovascular mortality, heart failure, coronary heart disease, stroke, myocardial infarction, depression, and liver cancer. Fish consumption is independently associated with lower inflammatory markers among healthy adults consuming more than 300 g fish per week.

5.4.1. Cardiovascular disease

Studies investigating the health effects of fish consumption have gained prominence, following the observation that Greenlandic Innuits, who consume a diet characterised by the high intake of fat from oily fish and seal meat, have low rates of cardiovascular disease (CVD). This observation clashed with the perceived association between dietary fat and CVD at the time.

Cardiovascular disease, the leading cause of death globally, is responsible for more than 40% of the deaths from non-communicable diseases. There is strong evidence that replacing dietary saturated fatty acids (SFA) with higher PUFA intake is cardioprotective. In this light, long-chain polyunsaturated fatty acids from marine sources include the previously mentioned EPA, DHA, as well as docosapentaenoic acid (DPA) (22:5n-3), a long-chain n-3 PUFA metabolite of EPA. Since alpha-linolenic acid (ALA) (18:3n-3) is the plant-derived n-3 fatty acid and the share of its endogenous conversion into EPA, DHA and DPA is very limited, tissue and circulating EPA and DHA levels are primarily determined by their direct dietary consumption (Figure 23).



Structure of n-3 PUFA (Mozaffarian & Wu, 2011)

The beneficial effects of marine n-3 PUFA, important in reducing the risk of vascular disease, may include lowered plasma triacylglycerol, anti-inflammatory effects, reduced platelet reactivity and heart rate, and improved endothelial dysfunction, among others.

The biological effects of n-3 PUFA vary: EPA might be more important in atherothrombosis, while DHA probably may be important for anti-arrhythmic effects. Less is known of the biological effects of DPA.

Findings from follow-up studies have confirmed that marine n-3 PUFA may be associated with a lower risk of myocardial infarction and ischaemic stroke caused by atherosclerosis and peripheral artery disease. The adipose tissue content of marine n-PUFA is considered the gold standard biomarker of intake and metabolism of these fatty acids. Findings from clinical supplemental trials generally have confirmed moderate beneficial effects on cardiovascular endpoints. There has been controversy, however regarding the effectiveness of n-3 LC-PUFA in reducing myocardial infarction, arrhythmia, cardiac and sudden death, or stroke.

A systematic review of the effectiveness of fish consumption in reducing vascular risk factors, including cholesterol, triglycerides, blood pressure and inflammatory factors, suggests that oily fish consumption (ranging from 20 to 150 g per day) can lead to a moderately significant decrease in plasma triglycerides levels and an increase in high-density lipoprotein (HDL) levels.

5.4.2. Obesity and metabolic syndrome

Obesity is considered to be a metabolic disorder and its prevalence in developed countries has risen sharply in the past two decades. Low-grade inflammation is seen as a major factor in the development of obesity-related metabolic disorders. Obesity is linked to a higher risk of developing chronic morbidities, such as insulin resistance, hypertension and dyslipidaemia, which are major elements of metabolic syndrome. Given the effectiveness of n-3 LC-PUFA in reducing plasma triglyceride levels, a high intake could improve some previously mentioned obesity-associated metabolic syndrome features.

The term "metabolic syndrome" (MetS), traditionally also known as insulin resistance syndrome or syndrome X, is used to describe a cluster of different interrelated factors that directly increases the risk for type 2 diabetes mellitus and CVD. Metabolic syndrome is characterised by a presence of any three of given risk factors: hypertriglyceridemia, abdominal obesity, increased blood pressure, relatively low high-density lipoprotein cholesterol concentration, and elevated fasting glucose level. This syndrome significantly affects public health by increasing the risk of morbidity and mortality. It has been suggested that MetS is associated with a two-fold risk of CVD and as high as a five-fold increase in the risk of type 2 *Diabetes mellitus*. The data suggest that approximately one-third of adults in developed countries suffer from this syndrome. The exact aetiology of MetS has yet to be fully explained. Nevertheless, many cross-sectional or longitudinal studies suggest that MetS is strongly associated with insulin resistance, oxidative stress inflammation, endothelial dysfunction and risk of cardiovascular diseases.

In addition to caloric intake, the biomarkers of MetS are affected by several food components with multiple interactions and varying bioavailability, including fat, fish and n-3 fatty acids, proteins and certain vitamins (D, E, C). Results of cross-sectional and follow-up studies suggest that fish consumption may have a preventive role in MetS development and could improve metabolic health. Evidence suggests that the protective effects of fish consumption are not related only to the usually mentioned fatty fish, but that the consumption of lean fish could also significantly contribute to a lower risk of MetS. Intervention studies have confirmed a drop in MetS prevalence after the consumption of lean white fish, due to weight loss, reduced waist circumferences and blood pressure. Additionally, the consumption of one serving of lean fish per week has been associated with decreased triglycerides and increased high-density lipoprotein HDL levels. The effects of lean fish consumption have also been related to age and gender. Studies have confirmed more positive effects on older participants (60–70 years) while men benefit more compared to women. To achieve the effect of decreasing the risk of metabolic syndrome, one serving/week increment in fish consumption is needed.

5.4.3. Cancer

Epidemiological studies have confirmed a lower incidence rate of cancer in populations living in regions with a traditionally high consumption of fish oil rich in n-3 fatty acids. Polyunsaturated fatty acids of the n-3 class are considered to be anti-proliferative and/or apoptosis-inducing agents for cancerous cells. They can have anti-cancer effects on various mechanisms involved in cancer development. These include changing the composition of cell membranes and altering the activity of key proteins, enzymes, and transcription factors involved in apoptosis. Some of the effects of n-3 fatty acids may not be directly related to fatty acid molecules themselves but rather to their metabolites such as eicosanoids and lipid peroxides. Additionally, a favourable n-3:n-6 ratio with the predominance of n-3 over n-6 could act as an effective adjuvant for chemotherapy.

Due to their anti-inflammatory properties, n-3 fatty acids could have a protective effect against cancers that are highly related to inflammation such as liver cancer. Positive associations have been found also between fish consumption and the risks of gastric cancer and myeloid leukaemia, while further research is needed to confirm their influence on other cancers. An increment of 20 g/day fish consumption decreases the risk of gastrointestinal cancer by 2%, liver cancer by 6%, and brain cancer by 5%.

5.5. Cognitive function and mental health

The anti-inflammatory properties of n-3 fatty acids could also be one of the mediator pathways through which they can have an effect on improving mental health. Consumption of larger amounts of DHA appears to reduce the risk of depression, schizophrenia, bipolar disorder and mood disorders. On the other hand, it has been demonstrated that the loss of DHA from the nerve cell membrane can cause dysfunction of the central nervous system in the form of susceptibility to stress, anxiety, irritability, impaired memory and cognitive functions, dyslexia and extended reaction times. DHA also has an important role in healthy ageing, by helping to prevent macular degeneration, Alzheimer's disease, and other brain disorders while improving memory and strengthening neuroprotection in general. An EPA/DHA combination with a ratio >2 (EPA/DHA>2) can be considered as an effective treatment of major depressive disorders.

5.6. Recommendations for fish intake

As part of a healthy diet, the usual recommendation includes a weekly intake of at least two servings of various fish (approximately 240 g), including a portion of oily fish. This intake will provide a mean consumption of 250 mg EPA + DHA, especially when fish replace the consumption of less healthy food. The European Food Safety Agency has advised 250 mg of EPA + DHA, and reference values for the EPA + DHA RDI are typically in the 250–500 mg range.

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Adriatic mariculture supplies high-quality fish products to the local markets as well as the markets of the neighbouring countries. Aiming to ensure the further economic development of this sector based on environmental and social sustainability, scientists and producers on both sides of the Adriatic Sea; Italy and Croatia launched the project **“STRENGTHENING INNOVATION AND SUSTAINABILITY IN ADRIATIC AQUACULTURE” - ADRIAQUANET**.

ADRIAQUANET CONSORTIUM is composed of scientists from seven research institutions, four production organizations and breeders’ associations from Italy and Croatia. The activities were financed from the Interreg Italy-Croatia 2014-2020 program, until June 2022. The coordinator of the consortium is prof. Marco Galeotti from the the University of Udine, Italy.

THEY DEFINED **THREE MAIN GOALS TOGETHER:**

FISH FARMING: improvement of fish farming by introducing innovations in feeding technology and disposal of waste materials.

FISH HEALTH: strengthening resistance to diseases by applying new autogenous vaccines, probiotics and natural medicinal substances. The application of the principle of fish welfare is a strategic determinant in the prevention of the occurrence of diseases.

MARKETING: assessment of the quality of farmed fish with welfare principles in ecologically favourable conditions based on the analysis of hygienic, sensory and nutritional parameters and its promotion as the development and promotion of new fish products that will meet the needs of the market