



AdriAquaNet

Enhancing Innovation
and Sustainability in
Adriatic Aquaculture



TRAINING MATERIALS

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Workpackage 3 IMPROVING ENVIRONMENTAL SUSTAINABILITY OF FISH FARMING

Overview

Workpackage 3 IMPROVING ENVIRONMENTAL SUSTAINABILITY OF FISH FARMING

Researchers at the University of Udine have developed new feeds for the breeding of Mediterranean fish species, in order to replace unsustainable feeds, such as fishmeal and vegetable protein concentrates, with proteins derived from meat waste from the poultry industry and from insects. Furthermore, to evaluate the reduction in the environmental impact obtained using these new feeds, the researchers applied the FiCIM (Fish Cage Integrated Model) mathematical model developed by Bluefarm Srl, a Veneto-based company specializing in precision aquaculture. The model uses a range of data, such as fish growth, oxygen consumption and ammonia excretion rates, temperature, and so forth. Finally, the researchers verified that the economic performance of the new feeds is equivalent to that achieved using current commercial feeds and is expected to be improved further in the short term.

To make running an aquaculture company more efficient, researchers from the University of Udine have developed a new method for extracting energy from fish farm sludge by analyzing the biochemical methane potential (BMP) of the brackish effluents from the hatchery at the Azienda Agraria Ittica Caldoli, a project partner. The data indicate a high potential for biomethane production, comparable or even superior to that of other effluents, by-products or biomass currently used in the agricultural sector.

Working in the field, researchers from the Zooprofylactic Experimental Institute of the Venezie in Legnaro have experimented with the use of solar panels, heat pumps and photovoltaic systems by installing a small electric motor equipped with batteries rechargeable via solar panels on the boats used for the inspection of the offshore cages of the Friskina Ltd., a project partner company in Croatia. These engines are light, silent and do not pollute. Recordings with immersion cameras are underway to assess the impact of the lower acoustic stress on the well-being and growth of the animals in the cages.

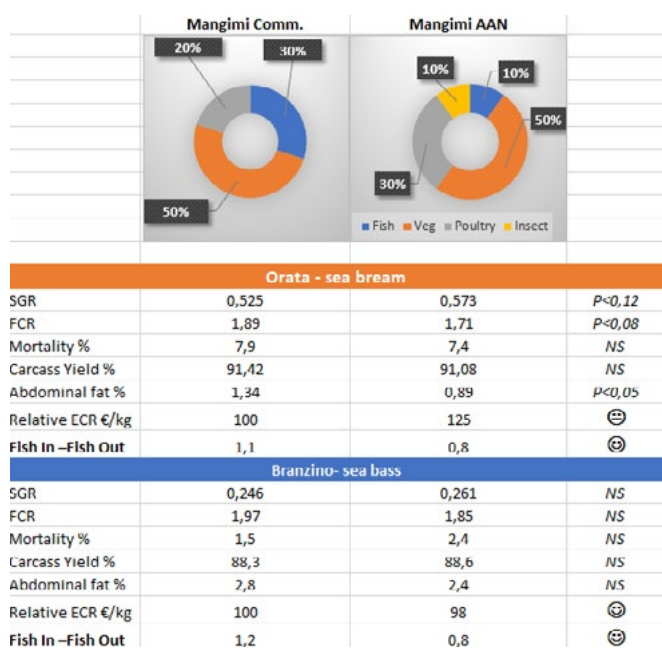
In an experiment carried out at the Azienda Agricola Ittica Caldoli, a project partner, and with the participation of the Industrial Engineering Department of the University of Padua, they have also examined the efficiency of a latest generation heat pump in water heating, contained in non-insulated fibreglass tanks, comparing it to the capacity of a classic system based on electric heaters. The data show a large performance gain using the heat pump, which will shortly be connected to a photovoltaic system.

A NEW GENERATION OF FEEDS FOR CULTURED MED-FISH SPECIES

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Novel approaches in the field of aquafeed formulation for marine fish, as proposed within the AdriAquaNet (AAN) project, were presented to stakeholders. They were first familiarised with the technical-scientific background of the future protein transition of aquafeeds, where the currently used unsustainable protein-rich feed ingredients, such as fish meals and plant-protein concentrates, need to be replaced by underutilized or innovative protein sources that comply with the principles of circular bioeconomy and have a low environmental footprint, such as poultry by-product and insect meals. We then described the zootechnical results, physiological response and environmental performance of the innovative aquafeed prototypes proposed and studied at laboratory scale in sea bass and sea bream within the AdriAquaNet (AAN) project, and compared them to the results obtained with conventional formula feeds. In particular, the advantages in terms of growth performance, animal welfare and environmental footprint that can be achieved with a new generation of feeds that combine poultry and insect (*H.illucens*) meals with minimal amounts of fish and vegetable proteins,

were highlighted. Finally, the operational results of the comparative tests between AAN and commercial feeds conducted on a farm scale in two Croatian farms, were presented and discussed with stakeholders. They substantially validated the results achieved on a pilot scale, confirming a trend towards better zootechnical outcomes and lower environmental footprint of the AAN prototype feeds compared to the currently used commercial preparations. Physical properties of pellets and their sinkability values were presented, as well as the results of organic disposition based on “on-farm” model simulations where the acceptable environmental impacts of AAN feeds were acknowledged. The economic performance of the AAN feeds has not been overlooked, as it was shown to be similar or slightly better than that achieved with current commercial feeds for sea bass but slightly worse for sea bream, although it will certainly improve if the current positive outlook on prices for the most expensive protein source of innovative feeds (i.e. insect meal) is maintained.

AN EXPERT TOOL TO MONITOR AND PREDICT FISH GROWTH AND WASTE LOAD DISPERSION FROM MARINE CAGES: BASIC THEORY AND PRACTICAL APPLICATION (EXAMPLES DRAWN FROM AAN FISH FARMS)

Pastres Roberto, Edouard Royer, Daniele Brigolin
 Bluefarm s.r.l. (UNIUD subcontractor)

The FiCIM, Fish Cage Integrated model is presented. This dynamic model, available for demonstration at <https://www.bluefarmenvironment.com/>, includes four modules. This first training focused on the first two modules: "individual" and "population". The individual module allows fish growth estimation, oxygen demands and ammonia excretion rate in relation to feed composition and water temperature. This module can also be used as a stand-alone model for quickly simulation of site-specific growth trajectories, based on water temperature climatology. The second module, "population" upscales the output of the individual model at a population level, in order to simulate the change of the total biomass in a fish cage, as well as the total oxygen consumption, total ammonia and organic particulate (uneaten feed + faeces) emissions. FiCIM as the main inputs requires feed composition and time series of feed quantities provided to the fish and water temperature. The latter parameter was collected within the AAN project by deploying a buoy equipped with dissolved oxygen and water temperature sensors, an example of the use within the models of these monitoring data was provided at the training.



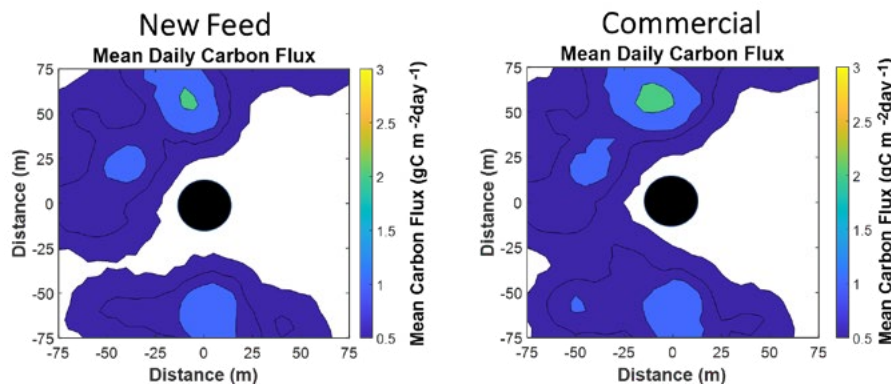
Example of FiCIM online interface and output provided.

FROM HUSBANDRY PRACTICES TO CARRYING CAPACITY: AN INTEGRATED MODELLING APPROACH

Pastres Roberto, Brigolin Daniele, Royer Edouard

Bluefarm s.r.l. subcontractor UNIUD

Assessment of the potential local environmental impact associated to different feed formulations were performed within the AAN project by using FiCIM, Fish Cage Integrated model. This dynamic model, which includes four module, and its application was presented at the training. The “individual” module allows fish growth estimation, oxygen demands and ammonia excretion rate in relation to feed composition and water temperature. The second module, “population” upscales the output of the individual model at a population level, in order to simulate the change of the total biomass in a fish cage, as well as the total oxygen consumption, total ammonia and organic particulate (uneaten feed + faeces) emissions. Time-dependent emissions are taken as input by the “dispersion” module, which allows one to estimate the potential effects on the water column and the deposition of organic carbon particle on surface sediment underneath a fish cage. The fourth module cumulates daily flows of organic carbon and convert them into an increase in the concentration of organic carbon above the background level in surface sediment, considering the dynamics of early diagenesis processes. As a result, FiCIM can provide maps of organic carbon enrichment in a form on which potential damage to the benthic community can be assessed. FiCIM as the main inputs requires feed composition and time series of feed quantities provided to the fish, water temperature, and current. The two latter parameters were collected within the project by deploying a buoy equipped with dissolved oxygen, water temperature sensors and a current meter. Data, collected every 15 minutes, were sent to a cloud and available in real time. The combined use of data and models was described to the end users within the training.



FiCIM model output – sample application at Orada farm, performed within the AAN project.

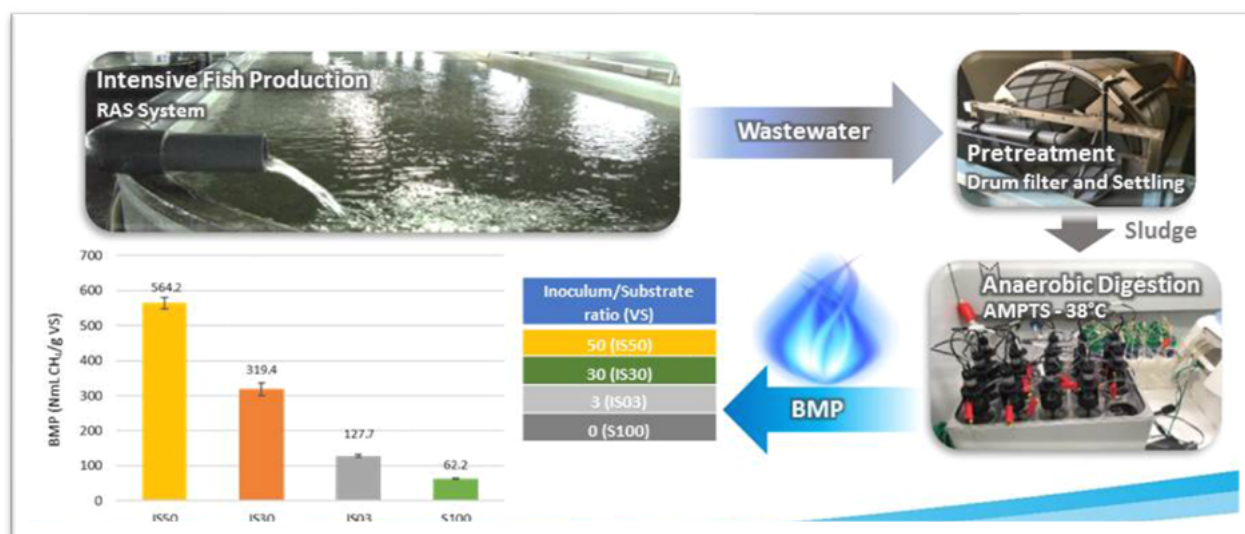
BIOMETHANE POTENTIAL OF SLUDGES FROM BRACKISH WATER FISH FARMS

Da Borso Francesco¹, Chiumenti Alessandro¹, Owono Owono Bartolomè¹, Fait Giulio¹, Novelli Andrea²

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The development of intensive aquaculture is facing the challenge of the sustainable management of effluents. The reproductive sectors (i.e., hatcheries) can use water recirculation systems (RAS), which discharge a portion of wastewater. Anaerobic digestion (AD) could reduce the environmental impact of this waste stream while producing biogas. Our research was focused on the biochemical methane potential (BMP) of brackish fish hatchery sludges. Wastewater was concentrated by microfiltration and sedimentation and thickened sludges were treated in a BMP system with different inoculum/substrate (I/S) volatile solids ratios (from 50:1 to no inoculum). The highest I/S ratio showed the highest BMP (564.2 NmL CH₄/g VS), while different I/S ratios showed a decreasing trend (319.4 and 127.7 NmL CH₄/g VS, for I/S = 30 and I/S = 3). In absence of inoculum BMP resulted of 62.2 NmL CH₄/g VS. AD applied to brackish water sludges can be a promising treatment with interesting methane productions.



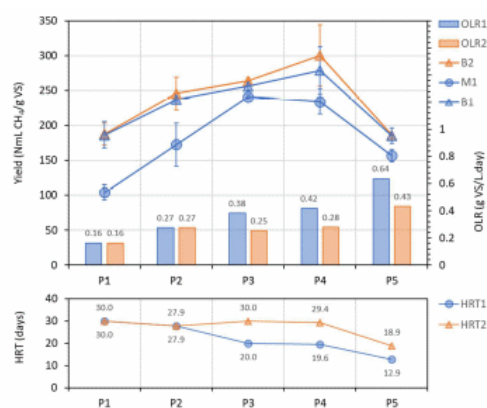
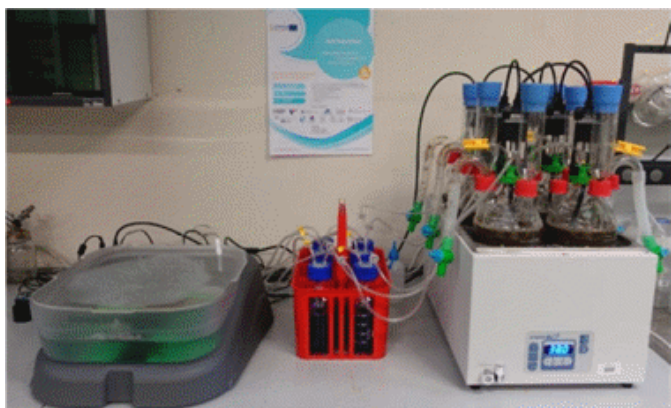
ANAEROBIC DIGESTION OF BRACKISH AND HIGH SALINITY AQUACULTURE SLUDGES

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Anaerobic tests carried out at laboratory scale with an automatic biomethane potential test system (AMPTS, by Bioprocess Control, Sweden) have demonstrated that fish farm effluent have a fairly high biomethane potential (BMP). Further anaerobic digestion tests were conducted using a pilot scale Bioreactor simulator system (BRS, Bioprocess Control, Sweden), which can operate with semi-continuous loading, simulating a full-scale reactor in order to study the optimal process layout and the optimal substrate retention time. The system was initially loaded with inoculum derived from anaerobically treated brackish aquaculture sludges and with digestate originated from a full-scale anaerobic reactor operating with animal manure and corn silage. The incubation period lasted 20 days, and from the 21st day, aquaculture sludges were loaded with organic loading rates (OLR) varying from 0.16 to 0.64 g VS/L day, with hydraulic retention times (HRTs) shortened from 30.0 days to 12.9 days. The anaerobic digestion process resulted proficient in the treatment of effluents from fish production despite the relevant dilution, low organic matter content and salts presence. The highest methane yield was achieved in brackish conditions, corresponding to 300.3 NmL CH₄/g of input VS, with hydraulic retention time of 30 days and low OLR (0.28 g VS/L day). The AD process in marine conditions seemed to have a slower adaptation to salinity, but with an HRT of 20 days the yield raised to 241.2 NmL CH₄/g VS. The application of the retained biomass technology to the AD process could allow to overcome the elevate dilution of this type of wastewater, effectively lowering the HRT and reducing the risk of microbial biomass washout from the digester. Further development of this study is going in this direction.



ELECTRIC PROPULSION ENGINES WITH SOLAR PANEL BATTERY CHARGING STATION FOR SERVICE BOATS. AN ECO-SUSTAINABLE APPROACH TO THE USE OF RENEWABLE ENERGY APPLIED TO CAGE FARMING

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Environmental sustainability is one of the three pillars of global sustainability: Social, Economic, Environmental. The problem of environmental impact and excessive energy consumption is also topical in aquaculture. The use of alternative / renewable sources is well received by all stakeholders (in particular producers, certifiers, control bodies, consumers). Old gasoline-powered outboard engines that are used in small boats for daily operations (cage control, manual feed distribution, transport of divers, etc.) can cause water pollution and, due to their noise, a huge stress to fish farmed in net pens. What «environmental friendly» solution to adopt in an SME to reduce emissions and use clean energy? The AdriAquaNet researchers chose to purchase two electric outboard engines equipped with rechargeable batteries via solar panels. The immediate advantages, personally encountered also by the operators of a small in-shore sea bass and sea bream plant (PP8 Friskina) were: zero environmental impact (silent and non-polluting), the engines were rechargeable via solar panels (no use of fossil fuels) and were not very stressful for the fish, but also for the operators. Furthermore, the model used was light (16 kg of which 6 kg of removable battery against 25/30 kg. of a classic petrol outboard), always ready to use thanks to a small solar panel on board and finally there was no fuel costs. The purchase price was comparable to a classic petrol model of 4 HP, but the power was lower and not suitable in bad weather conditions. Its use is therefore limited to the inspection of off-shore cages, even in larger plants, where they can act as a support to larger boats used for the most important operations (for example, distribution of feed or harvesting of animals for selling to the consumers). In the near future, in addition to a more detailed economic assessment of the costs / benefits of these equipment, thanks to the use of latest generation video cameras or remote monitoring systems (telemetry) it will also be possible to have an objective confirmation of how much they favorably affect the welfare of the animals inside the cages.



PHOTOVOLTAIC (PV) AND HEAT PUMP IN MARINE AQUACULTURE: HIGH EFFICIENCY AND LOW CO² EMISSIONS PROCESSES FOR WATER HEATING

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The European Climate Law, according to the European Green Deal for Europe's economy and society, aims to reduce greenhouse gas emissions at least 55% by 2030, if compared to 1990 levels. Climate neutrality by 2050 means achieving net zero greenhouse gas emissions for EU countries as a whole, mainly by cutting emissions, investing in green technologies and protecting the natural environment. The path to net-zero emission requires the massive deployment of all available clean energy technologies between now and 2030, also in aquaculture sector. Energy efficiency in marine hatcheries is often poor, with no heat insulation of the tanks and low efficiency of heating equipment. AdriAquaNet researchers, in collaboration with Industrial Engineering Department of the University of Padova, have tried to test the efficiency of a latest generation heat pump in heating the water contained in non-insulated fiberglass tanks in comparison with the capacity of a classic system based on heat resistors. Water temperature and power absorption have been continuously monitored. The heat pump tested at different temperature ranges and times, demonstrated a high Coefficient of Performance (COP) much higher than the classic heat resistors (see also the graph below). In the meantime a photovoltaic system has been installed at the partner 9 Fish Farm Caldoli, so the heat pump will subsequently be connected and will supply hot water to some tanks of the hatchery. In this way, the technology transfer between researchers and SME will be fully achieved, with a reduction in electricity consumption (use of alternative sources), greater energy efficiency and a lower environmental impact as it will be possible to gradually replace old water heating systems, like methane gas boilers, with modern energy-powered equipment, thanks to solar energy that is being converted into useable electricity by Photovoltaic panels.



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Workpackage 4 R & I TO IMPROVE HEALTH AND SUSTAINABILITY IN AQUACULTURE

Overview

Workpackage 4 R & I TO IMPROVE HEALTH AND SUSTAINABILITY IN AQUACULTURE

In the last decade *Vibrio harveyi* has been causing increasingly serious losses in Mediterranean and Adriatic aquaculture during the summer months. *Tenacibaculum maritimum* also affects numerous marine fish species around the world. These infections have a significant economic impact on producer companies, and so far there are no commercial vaccines. Researchers from the Croatian Veterinary Institute have now developed two effective vaccines to protect farms from these diseases.

For the control of infectious diseases in aquaculture it is necessary to reduce the use of antibiotics and increase prophylaxis measures by employing new anti-bacterial and immunostimulating molecules, to be incorporated in the feed. Examples include antimicrobial peptides (AMP), components of the innate immune system of almost all living organisms, which can be exploited for their broad spectrum antimicrobial activity. Marine natural products (MNPs) are pure molecules or mixtures thereof that are purified by a range of classes of marine organisms and possess numerous antibacterial, antiviral, immunostimulating, anti-inflammatory and anti-tumoural properties. Researchers from the University of Udine and the CNR have identified new natural molecules of great use in prophylaxis by companies producing farmed fish.

The welfare of farmed fish is of great bioethical, biological and economic importance for fish farms. Measuring the degree of animal welfare is essential for the proper management of the business. Both Operational Welfare Indicators (OWI) and Laboratory Welfare Indicators (LWIs) can be used. Researchers from the Zooprohylactic Experimental Institute of the Venetia and the project partner companies have developed a practical data sheet with 25 indicators based on the external appearance and behaviour of the fish. The monitoring produces a final score in a few hours even on large farms. Researchers from the University of Trieste have developed a new method for analyzing some stress indicators in fish blood micro-samples. The method is accurate and very simple to apply, even in the field.

EXPERIMENTAL VACCINATION AND TESTING THE EFFICACY OF AUTOLOGOUS VACCINES AGAINST *VIBRIO HARVEYI* IN SEA BASS

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Vibrio harveyi is initially described as a cause of mass mortalities in shrimp hatcheries but it was isolated from many disease outbreaks in different fish species reared in subtropical region. In the last decade *V. harveyi* more often causes serious losses in Mediterranean and Adriatic aquaculture during the summer months. The disease is transferred horizontally from fish to fish. First symptoms of the disease are lethargy and loss of appetite, depigmentation, erosions on the skin, and hemorrhages at the fin bases, necrosis and ulcerations, pale gills with bleedings. In pronounced course of the diseases uncoordinated swimming could be noticed together with keratitis, corneal opacity and exophthalmia. On autopsy, hemorrhages and exudate or ascites are present in body cavity, focal congestion and petechial hemorrhages of the liver and serocatarhal enteritis, intestinal necrosis and lumen filled with white to yellowish exudate. The only available management methods are good aquaculture practise (GAP) and antimicrobial treatments.

There is no effective commercial vaccine produced so far and we tested Croatian isolates of *V. harveyi* and we prepared an autologous formalin killed vaccine for experimental vaccination in the laboratory and field condition. Sterility and safety was checked prior laboratory vaccination. Efficacy was tested in triplicate by immersion vaccination and intraperitoneal injection, respectively. Control groups were subjected to mock vaccination. Thirty-six days after vaccination all experimental groups were infected with *V. harveyi* either by immersion or by intraperitoneal injection of high doses of pathogenic bacteria. Mortalities were noted for ten days after challenge and relative percentage of survival (RPS) was calculated. RPS is calculated based on the percentage of dead fish in vaccinated group and percentage of dead fish in control group and in our experiments RPS for immersion vaccinated groups was 63, while in intraperitoneally vaccinated fish was 100. In the field experiment there were no *V. harveyi* outbreak in experimental groups over one year period.



EXPERIMENTAL VACCINATION AND TESTING THE EFFICACY OF AUTOLOGOUS VACCINES AGAINST *TENACIBACULUM MARITIMUM* IN SEA BASS

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Tenacibaculum maritimum is an etiological agent of an ulcerative disease known as tenacibaculosis affecting numerous marine fish species throughout the whole world causing considerable economic impact due to high mortality rates, increased susceptibility to other infections and enormous costs for treatment. It is an opportunistic pathogen that primarily causes extensive skin lesions and gill abrasion, and subsequent systemic infections. Affected fish show loss of appetite, become lethargic and show skin lesions around the eyes and on the head. The lesions are characterized by increased mucus production and the presence of whitish necrotic tissue. Fish with gill infections have increased respiratory rate with visible yellow or brown mats on the pale gills, and extensive areas of severe necrosis. Increased disease prevalence and severity occur at temperatures above 15°C and all age classes may be infected. Younger fish suffer a more severe form but stress and other primary diseases may trigger outbreaks. Disease has several synonyms such as "frayed fins and tail rots", "necrosis of the gills and eyes", "gliding bacterial disease of sea fish" and "eroded mouth syndrome". Good management practices and antimicrobial treatments give some results in disease management. There is no effective commercial vaccine so far and we tested Croatian isolates of *T. maritimum* and prepared an autologous formalin killed vaccine for experimental vaccination in the laboratory and field condition. Sterility and safety was checked prior laboratory vaccination. Efficacy was tested in triplicate by immersion vaccination and intraperitoneal injection, respectively. Control groups were subjected to mock vaccination. Thirty-six days after vaccination all experimental groups were infected with *T. maritimum* either by immersion or by intraperitoneal injection of high doses of pathogenic bacteria. Mortalities were noted for ten days after challenge and relative percentage of survival (RPS) was calculated. RPS is calculated based on the percentage of dead fish in vaccinated group and percentage of dead fish in control group and in our experiments RPS for immersion vaccinated and challenged groups was 100, while in intraperitoneally vaccinated and challenged fish was 67,5. In the field experiment there were no *T. maritimum* outbreaks in experimental groups over one year period.



THE USE OF NATURAL SUBSTANCES AS THERAPEUTICS IN AQUACULTURE

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Prophylaxis, based on the use of immunostimulant substances and vaccines, is the method of choice for the control of infectious diseases in aquaculture. However, bacterial infections still occur in farms, leading to massive losses and making sometimes necessary the fish treatment with antibiotics. Anyway, their improper use is widely criticized because it leads the development of antibiotic-resistance phenomena among the fish pathogenic bacteria and the potential presence of resistant pathogens in aquaculture food products that may cause the drug resistance gene transfer to human bacteria. Therefore, it is necessary to search for new natural substances with antibacterial and immunomodulatory activity that can be applied in aquaculture as alternative to common antibiotics. Antimicrobial peptides (AMPs) represent a promising class of bioactive compounds that are attracting increasing attention in recent years. They are a group of oligopeptides present in nature as components of the innate immune system of almost all living organisms. The advantages of these molecules are their broad-spectrum antimicrobial activity and the very low possibility of causing resistance phenomena, thanks to the rapid lysis of bacterial cell membranes. Within the framework of AdriAquaNet project, the Veterinary Pathology group of the University of Udine carried out an extensive screening on the antibacterial and immunomodulatory properties of natural compounds according to the protocol described in Figure 1, in order to identify new molecules to be used in European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) farming. In this context, the antibacterial activity of 14 antimicrobial peptides provided by the ICB-CNR of Padua against *Vibrio anguillarum* serotype O1 and *Photobacterium damsela* subsp. piscicida was investigated. For this purpose, serial dilutions of the AMPs were tested in microplates for their ability to inhibit the bacterial growth and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Among the tested AMPs, only the hybrid between the peptides Cecropin A (*Hyalophora cecropiae* Fig. 2) and Melittin (*Apis mellifera* Fig. 3), at two levels of purification, showed an evident action against at least one of the two pathogens. MIC values against *P. damsela* subsp. piscicida were lower than those of oxytetracycline used as the reference antibiotic. Moreover, the in vitro cytotoxicity of these hybrid peptides on the SAF-1 cell line (gilthead sea bream fibroblast-like cells) was evaluated for their possible in vivo application after inclusion in a medicated feed and administration to fish during a bacterial challenge. The purified hybrid peptide was mildly toxic at concentrations comparable to its MIC against *P. damsela* subsp. piscicida. In contrast, the raw peptide showed no toxicity towards SAF-1 at concentrations comparable to its MIC and its IC50 value was found to be higher than that of the purified peptide. The analysis of these results led to the choice of the raw peptide as a candidate for a small-scale in vivo test that will be conducted before the end of the project.

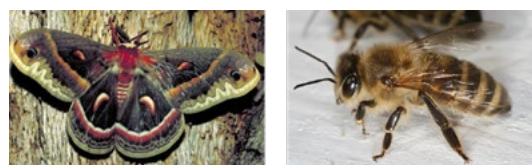
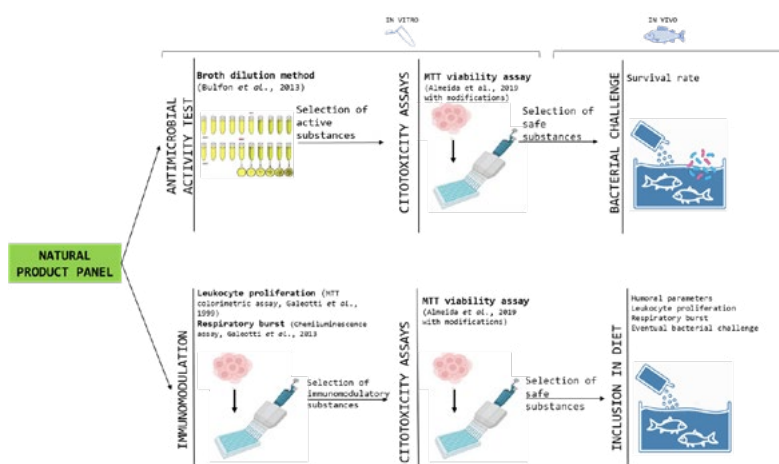


Figure 1. Protocol used for the screening of the antimicrobial and immunomodulatory properties of a panel of natural origin compounds within the framework of AdriAquaNet project. They were tested adopting the broth dilution method to determine their antibacterial activity or they were tested on leukocytes purified from sea bass head kidney in order to evaluate their immunomodulatory properties (effects on cell proliferation and respiratory burst activity); subsequently, they were submitted to the MTT viability assay which allows to evaluate their in vitro cytotoxicity. Then, the substances not showing cytotoxic effects were selected and addressed to a small scale in vivo trial, in order to determine if they are effective as therapeutics when included in a medicated feed and administered to fish after a bacterial challenge or as dietary immunostimulants for enhancing fish immune response and preventing infections.

Figure 2. *Hyalophora cecropiae*

Figure 3. *Apis mellifera*

NATURAL SUBSTANCES FOR SUSTAINABLE AQUACULTURE: STATE OF THE ART AND POSSIBLE APPLICATIONS IN THE CONTROL OF INFECTIOUS DISEASES

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In aquaculture there is a constant demand for new strategies to be used in the control of infectious diseases, in order to reduce the use of antibiotics and provide more safe and eco-sustainable products to the consumers. One of the most promising approaches is the search for natural compounds with antimicrobial or immunostimulatory activity, which can be incorporated into the aquafeeds and easily administered to fish without being toxic or having long-term side effects. In particular, the dietary administration of immunostimulants was found to be effective in promoting the growth, non-specific (and to a lesser extent specific) immune responses and resistance to infections of various farmed fish species, resulting a valuable alternative or complementary treatment to vaccination.

Several categories of substances of natural origin have been studied in fish species for their immunostimulatory properties (Fig. 1), but only a few number of experiments have been carried out in European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) during the last twenty years. β -glucans obtained from yeast, bacteria, plants, algae and fungi are the most studied ones and various commercial products are currently available as functional ingredients for fish feed. Similarly, alginic acid and alginates purified from brown macroalgae and bacteria are commonly included in the formulations addressed to fish feeding. Probiotics (*Lactobacillus* sp., *Bacillus* sp., *Enterococcus* sp..) and prebiotics are widely proposed not only for their ability to improve fish health but also to modulate the intestinal microflora, whereas the possible use of mixtures, extracts or essential oils obtained from medicinal plants, marine microalgae and macroalgae, fungi and products of animal origin (eg. propolis) has attracted increasing attention more recently. In any case, the use of new products as food additives for fish is possible only after a specific authorization and in accordance with the indications given in the EC Regulation no. 1831/2003 (list of food additives for animal use) and subsequent amendments.

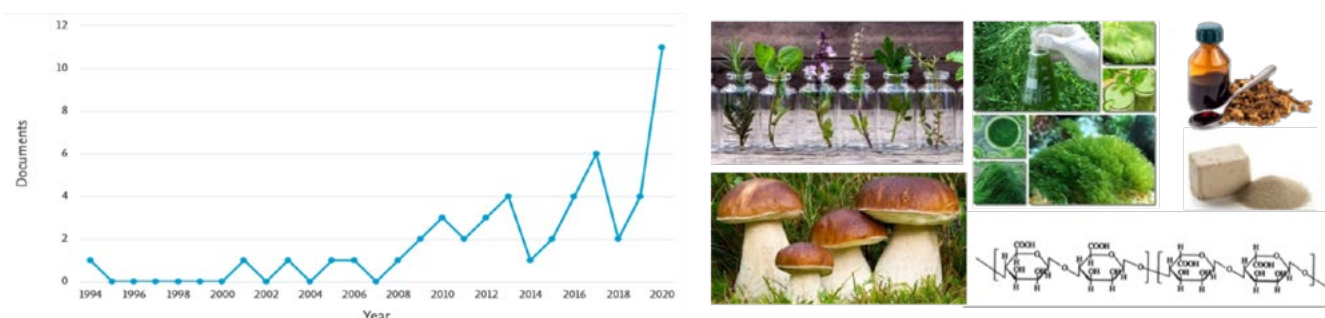


Fig. 1. Examples of substances of natural origin that have been studied in fish for their immunostimulatory properties and result of the bibliographic research on the scientific investigations carried out during the last twenty years.

MARINE NATURAL PRODUCTS: POTENTIAL DRUGS AGAINST *VIBRIO ANGUILLARUM* AND *PHOTOBACTERIUM DAMSELAE PISCICIDA*?

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The promotion and discovery of new natural substances with antibacterial activity to be used for the control of infectious disease in fish farms are strongly requested as alternative to the currently available antibiotics, in order to avoid the selection of antibiotic-resistant bacterial strains among fish pathogens, possible risks for human health or environmental pollution and provide safe and environmentally friendly products to the consumers.

In this context, an intense research aimed at evaluating the antimicrobial and immunostimulatory potential of marine natural products (MNPs) is under progress. The MNPs are compounds (raw extracts, purified fractions, active metabolites) that are purified from different classes of marine organisms and possess numerous biological properties (antibacterial, antiviral, immunostimulant, anti-inflammatory, antitumor ..). To date, thousands of MNPs have been studied for their effective use in human medicine whereas the information regarding their potential application in aquaculture is limited. Within the AdriAquaNet project, the Veterinary Pathology group of the University of Udine investigated the antibacterial activity of more than 140 MNPs provided by the ICB-CNR of Naples against *Vibrio anguillarum* serotype O1 and *Photobacterium damselae* subsp. *piscicida*, in order to identify new substances/molecules to be used in sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*) farming for the treatment of vibriosis and photobacteriosis. For this purpose, serial dilutions of MNPs were assayed in microplates for their ability to inhibit the bacterial growth and the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. Some active metabolites purified from the mollusc *Gastropeteron meckeli* (CBC46B_108/O, CBC46B_108/R, CBC46B_108/Z, CBC46_10H), the crude extract (CBC3A_X) and some enriched fractions (CBC3A_B, CBC3A_C) obtained from the sponge *Crambe crambe* demonstrated an action against at least one of the two pathogens, with MIC values sometimes lower than those of oxytetracycline used as reference antibiotic.

These findings are promising, however further investigations will have to be carried out in order to verify whether these MNPs are in vivo equally effective in treating the infections caused by *V. anguillarum* and *P. damselae* subsp. *piscicida* and stimulating the fish immune responses. Furthermore, the possibility of their real use as natural antibacterial substances in mariculture cannot ignore the search for new and innovative techniques for the purification/production of these compounds in sufficient quantities and at low costs (Fig. 1).

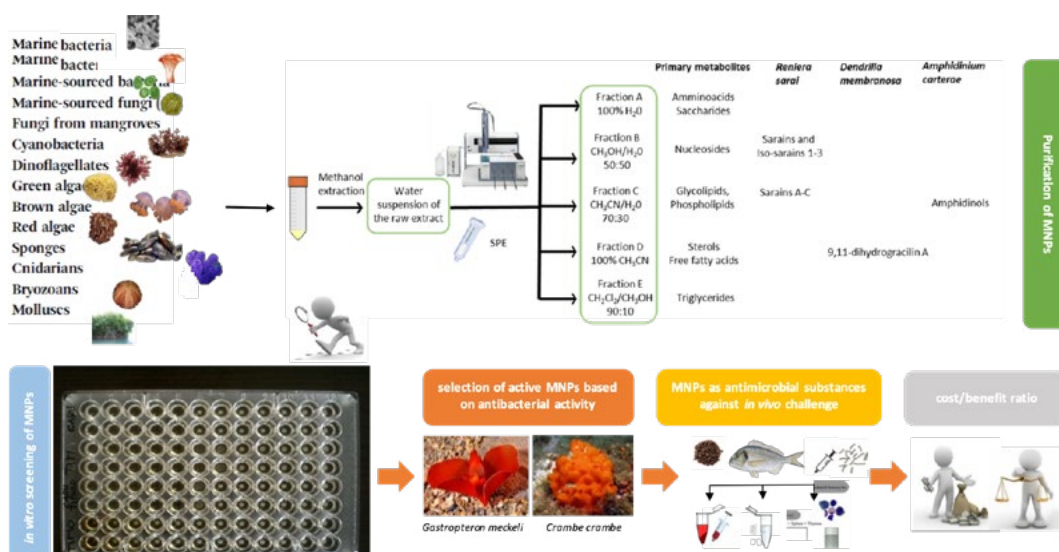


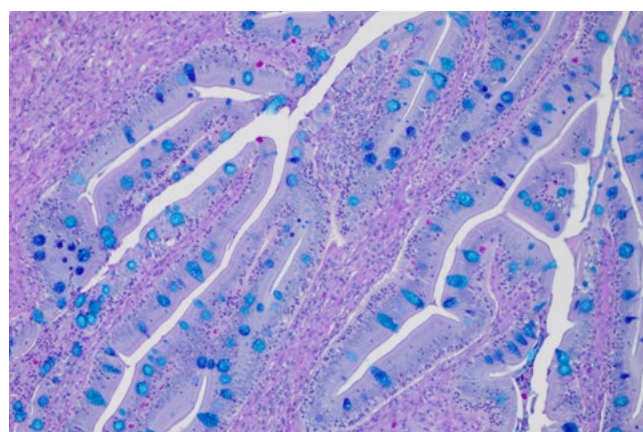
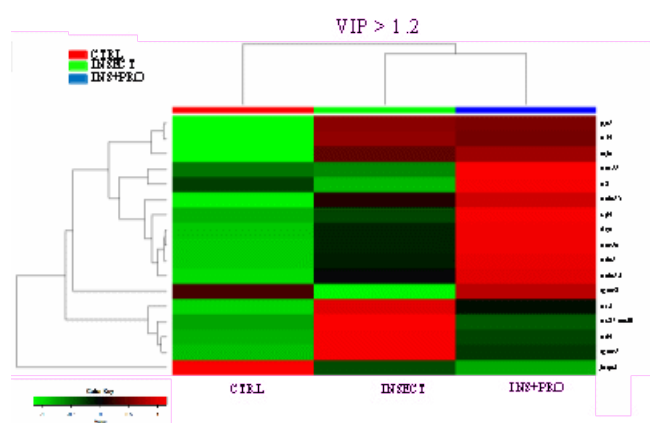
Fig. 1. Screening of MNPs for a potential use in mariculture as natural antibacterial substances.

AUTOCHTHONOUS INTESTINAL *BACILLUS* SPP. AS A PROBIOTIC FOR NEW GENERATION OF FEEDS IN CULTURED SEA BREAM

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In an era of rising antimicrobial resistance, aquaculture strives to find cost-effective, and more importantly, environmentally friendly alternatives to the use of chemotherapeutics in fighting and controlling disease outbreaks, such as probiotics. Within the AdriAquaNet project, we have isolated autochthonous bacteria from farmed sea bream to potentially be used as a novel probiotic(s) in sea bream aquaculture. The selected *Bacillus* spp. isolate has been incorporated in the fish feed containing 10% insect protein from black soldier fly (*Hermetia illucens*), i. e. the feed designed and tested within the WP3. The fish was fed with the probiotic-supplemented diet, insect protein-supplemented diet and commercial diet for six months. Intestinal epithelium of fish fed probiotic-supplemented diet showed no degenerative or inflammatory changes and an elevated number of goblet cells, suggesting the beneficial effect of probiotic supplementation in forming and maintaining the protective mucous barrier. Main changes in microbial community composition occurred already within the first month of the feeding trial. Most importantly, positive changes were recorded for the group of lactic acid bacteria. Many of the epithelial integrity markers were up-regulated already with the insect diet, and probiotic supplementation augmented further this up-regulation. Probiotic supplementation also reversed the up-regulation of inflammatory/immune-regulatory genes that occurred with the insect diet. In conclusion, probiotic supplementation emerges as beneficial for the preservation of gut homeostasis and innate immunity in fish fed alternative protein source.



DEVELOPMENT OF A PRACTICAL METHOD FOR ASSESSING THE WELFARE OF FARMED FISH

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Animals are assumed to have good welfare when they have their needs fulfilled. Welfare needs include: basic needs, which are necessary for immediate survival and good health (including respiration and nutrition) and behavioural needs, which are necessary for long terms success (including social contact).

Welfare indicators (WIs) are observations or measurements that provide information about the extent to which the animal's welfare needs are met. Indicators that can be used in an on-farm welfare assessment are termed Operational Welfare Indicators (OWIs) and must: provide a valid reflection of fish welfare, be easy to use on the farm, be reliable, be repeatable, be comparable, be appropriate and fit for purpose indicators for specific rearing systems or husbandry routines.

Welfare Indicators can be Environment based observations (e.g. oxygen, temperature, salinity) and Animal based (group and individual) observations, made on or from the animal, like abnormal swimming, mortality, fin damage, deformities, etc. At the moment no indicators are available for sea bass and bream (some works and projects are in progress) so the AdriAquaNet researchers, in agreement with fish farmers involved in the project, developed a practical data sheet with 25 indicators and an evaluation grid. Based on the score obtained, the farmer can easily identify some critical issues and immediately prepare corrective actions to improve the welfare of his fish.

Although it is a qualitative-quantitative system, it is possible to carry out an evaluation in a few hours even of large plants and, in case of need, requires the help of a veterinarian or a laboratory. New methods for evaluating some stress indicators in fish blood in the laboratory (Laboratory Welfare Indicators – LWIs) are now available or in the process of being standardized and may also be used by individual farmers in the next future.



Environment based: 5

Animal based (group): 8

Animal based (individual): 12

TOTAL OWIs: 25

SCORE (number of NC)	CONCLUSIONS
0 ≤ NC ≤ 5	Very good welfare
6 ≤ NC ≤ 10	Good welfare
11 ≤ NC ≤ 15	Poor welfare
16 ≤ NC ≤ 25	Very bad welfare

BILIRUBIN ANALYSIS IN BIOLOGICAL FLUIDS

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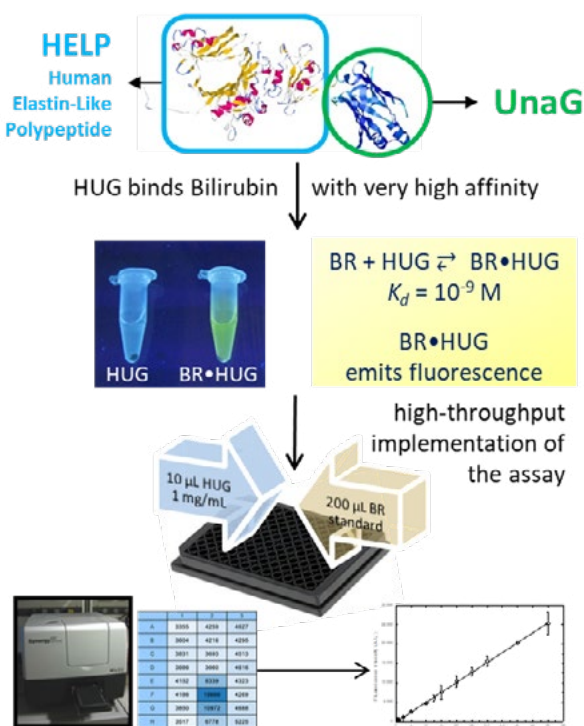
In 1960, Mather stated that “bilirubin determinations are perhaps the most unreliable of any in clinical chemistry”, and despite tremendous analytical progress, this consideration is still partially relevant. Indeed, bilirubin is one of the most complicated analytes to quantify accurately in clinical chemistry. In addition to the importance of its quantification in the diagnosis of jaundice and its use as a biomarker for liver and blood diseases, it has now gained interest for its potential

biological activity at low concentrations as an antioxidant. It is therefore of paramount importance to find an analytical method for bilirubinemia that allows accurate estimation when fluctuations are slightly above physiological levels. Moreover, the need for repeated monitoring requires a sampling technique that is non-invasive and easy to perform.

Our research group has developed a fluorometric method based on the highly specific binding of bilirubin to the recombinant bifunctional protein HUG. This fusion protein, which can be easily purified by exploiting the thermoreactive properties of the elastin-like domain, binds bilirubin via the UnaG functional tract. The extremely stable complex formed corresponds to the emission of an intense fluorescent signal.

The evaluation of bilirubin concentration in biological fluids requires careful calibration of the method with suitable standard solutions. The poor solubility of bilirubin, its photodegradation and its easy oxidation are all parameters that we took into account when optimizing the analytical protocol. The goal of obtaining stable and reproducible standard solutions was achieved by using a solvent consisting of bovine serum albumin in phosphate buffer.

The assay proved to be robust and applicable over wide ranges of variability in analytical conditions (pH, temperature, albumin concentration).



BILIRUBIN ANALYSIS IN WHOLE BLOOD

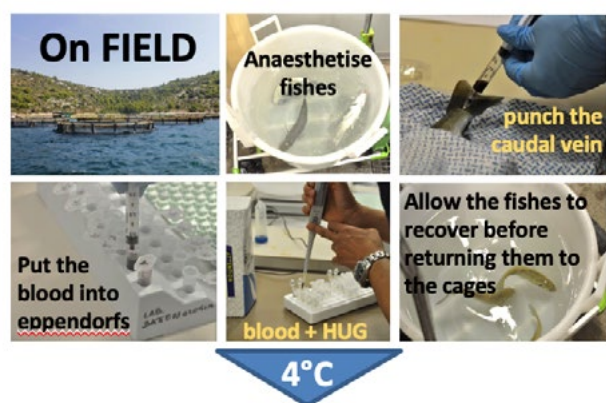
Paola Sist¹, Antonella Bandiera¹, Federica Tramer¹, Chiara Bulfon², Donatella Volpatti², Jerko Hrabar³, Ivona Mladineo⁴, Emilio Tibaldi², Sabina Passamonti¹

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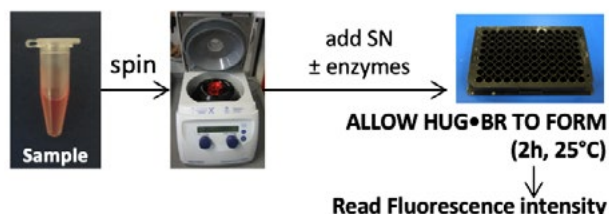
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Like all vertebrates, fish produce biliverdin and bilirubin, two molecules that give blood plasma its yellow or green color. These molecules can be analyzed in serum and have diagnostic value. Toxic substances or pathogenic microbes can cause hemolysis or liver damage resulting in elevated serum bilirubin (hyperbilirubinemia). Available analytical methods do not fully exploit the diagnostic significance of these two bile pigments, so called because one of them, bilirubin, is excreted with bile. Automated methods, such as those used in human diagnostics, require such large sample volumes that the animal must be sacrificed. Other powerful methods require sophisticated instrumentation, sample preparation with solvents and filters, and produce environmentally harmful laboratory waste.

To overcome these technological limitations, we have developed a new high-performance fluorimetry method that requires a small drop of blood that is diluted and analyzed directly to calculate blood levels of bilirubin and biliverdin. Blood collection is not painful or unduly distressing to the animal, and the animal returns to the water immediately after collection. The method ensures that blood samples can be easily stored and transported to the analytical laboratory. In addition, the analytical equipment is relatively simple and can be installed in peripheral laboratories. Minimal training is

In the LAB



required to perform the test. Finally, there is no hazardous waste that would require expensive disposal. Because of these characteristics, the method can be used widely and allows monitoring of the health of farmed fish and their response to environmental situations. By collecting data sets on a regular basis, it will be possible to learn more about aspects of farmed fish pathophysiology and use this knowledge to improve farm management. For this work, it is useful for companies, research centers, and universities to collaborate and share biological samples (companies) and laboratories and research personnel (universities).



AdriAquaNet

Enhancing Innovation
and Sustainability in
Adriatic Aquaculture



Workpackage 5 IMPROVING THE QUALITY AND MARKETING OF FRESH AND PROCESSED FISH

Overview

Workpackage 5 IMPROVING THE QUALITY AND MARKETING OF FRESH AND PROCESSED FISH

The creation of new long-life fish-based products is very important to promote fish consumption and reduce food waste. Researchers from the University of Udine, in collaboration with the company Friultrota Srl, a project partner, have created a new product based on smoked sea bass fillet, preserved in a protected atmosphere using biological methods. This new product may meet the needs of consumers to buy a ready for use fish product.

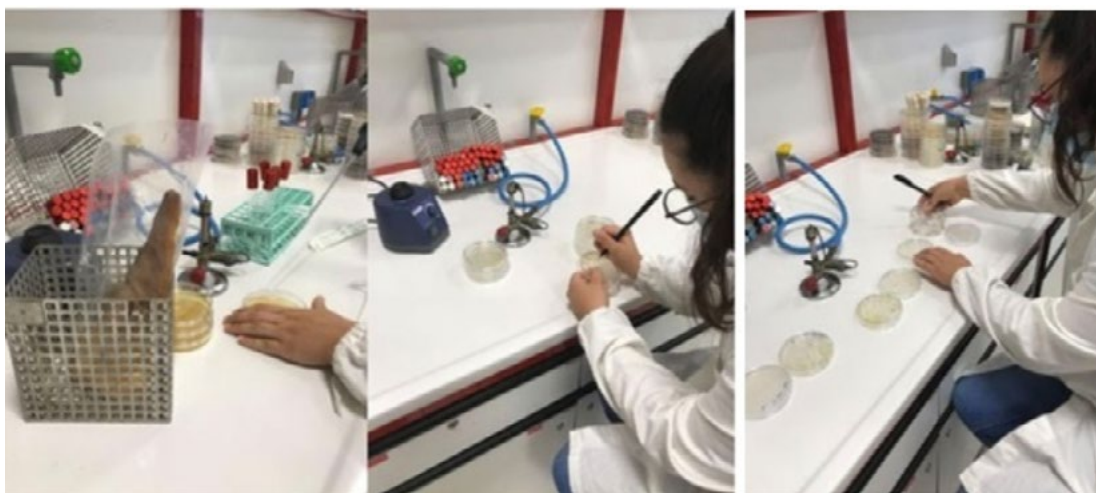
Researchers from the Croatian Veterinary Institute analyzed the meat of sea bass and sea bream raised in two locations on the Adriatic Sea. The percentage of total fat varied considerably over the seasons, while the lipid quality indices were in line with those recommended by international health organizations and rose with the use of the experimental feed developed by researchers at the University of Udine. This said, data from a survey conducted by researchers from the Faculty of Tourism and Hospitality Management of the Rijeka University show that there are many negative prejudices with respect to farmed fish and are proposing a new market strategy, based on consumer information on the nutritional quality of products from reared fish.

ANALYSIS OF THE BIOPROTECTIVE POTENTIAL OF LACTIC ACID BACTERIA TO IMPROVE THE SHELF-LIFE OF FRESH SEA BASS AND SEA BREAM PACKAGED UNDER VACUUM AND STORED AT 6 ± 2 °C

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Fish meat is highly perishable due to indigenous and microbial enzymes that determine spoilage and shelf life. The spoilage processes that lead to a significant, sequential and progressive change in the original state of freshness are rapid and depend on the conditions of rearing, harvesting, slaughter, handling and storage. Normally, the shelf life of gutted fish stored at 4 ± 2 °C under vacuum packaging (VP) and modified atmosphere (MAP) is about 9 days. Our objective was to improve the shelf life and maintain the microbiological and sensory quality of cultured farmed gutted sea bass and sea bream by using different methods, including VP, MAP and a bioprotective culture containing *Latilactobacillus sakei*, up to 12-14 days. Our analyses showed that the VP samples were more acceptable than the MAP fish, although the shelf life of the VP and MAP fish was similar and was about 12 days when stored at 6 ± 2 °C, which is the normal temperature in supermarkets or consumer refrigerators. Finally, we succeeded in extending the shelf life of the VP fish to 14 days at 6 ± 2 °C by washing the gutted sea bass and sea bream in a suspension of bioprotective starter cultures. The bioprotective culture reduced the growth of spoilage microorganisms. Non-professional and untrained evaluators confirmed the acceptability of the inoculated samples by sensory analysis. Read more in: L. Iacumin, A.S. Jayasinghe, M. Pellegrini, G. Comi, Evaluation of Different Techniques, including Modified Atmosphere, under Vacuum Packaging, Washing, and *Latilactobacillus sakei* as a Bioprotective Agent, to Increase the Shelf-Life of Fresh Gutted Sea Bass (*Dicentrarchus labrax*) and Sea Bream (*Sparus aurata*), *Biology* (Basel). 11 (2022) 217.



COLD-SMOKED SEA BASS, A NEW PRODUCT PACKAGED UNDER VACUUM AND STORED AT 6 ± 2°

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Cold smoked sea bass is a new product, not yet marketed, developed in the production facility. Cold smoked fish is often contaminated with *Listeria monocytogenes*, which can grow in these products when stored at relatively low temperatures, due to favorable conditions such as low acidity and high humidity. To prevent this contamination by *L. monocytogenes*, which poses a risk to human health, cold-smoked sea bass can be inoculated with strains of lactic acid bacteria. We tested the efficacy of two commercial lactic acid bacterial strains and a newly selected bacterial strain isolated from spoiled beer (LAK-23) in preventing the growth of *L. monocytogenes* intentionally inoculated onto cold-smoked sea bass, vacuum-packed, and stored at 7 °C for 60 days. The addition of this new starter prevented the growth of *L. monocytogenes* at a level higher than that of the inoculum. Although a drop in pH was observed in these samples, they were considered acceptable by 20 non-professionally trained evaluators who found no sensory difference between them in a triangle test. Therefore, cold-smoked sea bass can be included in category 1.3 (Reg. EC 2073/05) of products that do not support the growth of *L. monocytogenes*.

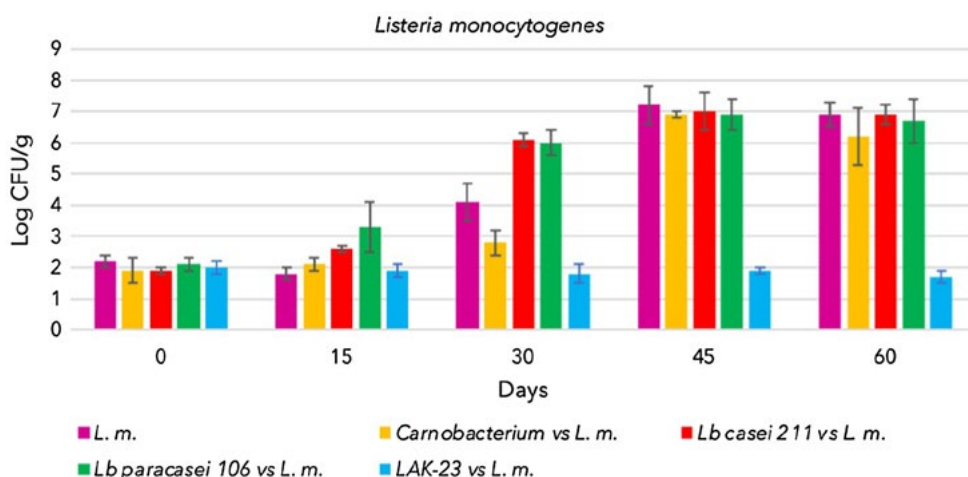


Figure. Evolution of *Listeria monocytogenes* intentionally inoculated in cold-smoked sea bass with or without bioprotective starter added, stored at 6 ± 2°C. Source: L. Iacumin, G. Cappellari, M. Pellegrini, M. Basso, G. Comi, Analysis of the Bioprotective Potential of Different Lactic Acid Bacteria Against *Listeria monocytogenes* in Cold-Smoked Sea Bass, a New Product Packaged Under Vacuum and Stored at 6±2° C, Front. Microbiol. 12 (2021)

NUTRITIONAL QUALITY OF SEA BASS AND SEA BREAM FARMED IN THE ADRIATIC AREA

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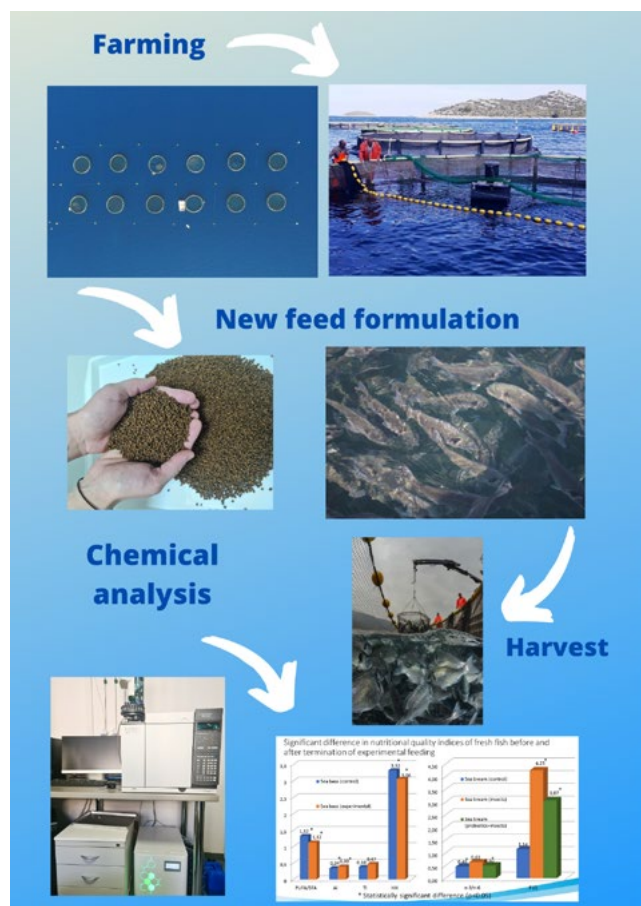


Fish meat has a balanced nutritional composition, since it contains proteins, fats, and important micronutrients in the amounts beneficial to human health. During this project, nutritional quality of sea bass and sea bream as the farmed fish species most important from the commercial standpoint, was investigated. Basic nutrient composition (water, proteins, carbohydrates, ash, fats, and fatty acid profile), as well as mineral (K, P, Na, Ca, Mg, Fe, Zn and Cu) and vitamin (A and E) content were determined in fresh fish samples farmed at two locations in the Adriatic Sea over four seasons during a two-year period using validated analytical methods. The levels of saturated (SFA) and unsaturated (MUFA and PUFA) fatty acids and their mutual ratios, as well as lipid quality indices, including the atherogenic (AI) and the thrombogenic (TI) index, the flesh lipid quality index (FLQ), and the ratio of hypocholesterolaemic over hypercholesterolaemic fatty acids (HH), were established, too. Sea bass and sea bream farmed at both Adriatic Sea locations were found to be of a high nutritional value, with the proportion of total fat varying considerably across seasons, whereas the lipid quality indices were in line with those recommended by international health organizations during the entire study period.

THE INFLUENCE OF NEW FEED FORMULATIONS USED IN MARICULTURE ON THE QUALITY OF FARMED FISH

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Nutritional composition of fish is influenced by many factors, including species, age and sex, environmental parameters, such as seasonal changes in temperature and sea salinity, and the composition of feed used during fish farming. Numerous studies have highlighted the differences in farmed fish quality, especially their fat content, i.e., the composition of saturated (SFA) and unsaturated (MUFA and PUFA) fatty acids, seen even in simultaneously caught fish of the same species, age, and sex. Fish nutrition directly affects its growth rate and the quality of meat, especially the composition of fats and fatty acid profile. However, intense development of aquaculture and steady growth of farmed fish production have fuelled consumer prejudices about the poorer quality of farmed as compared to readily caught fish. Therefore, an extensive research has focused on the development of sustainable nutrition that can provide for farmed fish of high nutritional quality up to consumers' expectations. In this project, basic chemical composition, fatty acid profile, and proximate fatty acid shares, as well as mineral and vitamin content, were investigated in sea bass and sea bream before and after experimental feeding with feed supplemented with insects and probiotics. Based on the levels of fatty acids determined in fresh fish fed on various experimental feeds detailed above, feeding on experimental feed supplemented with insects, in which the highest levels of polyunsaturated fatty acids (PUFA) were determined, resulted in the highest PUFA fish meat content, especially when it comes to the sea bream muscle, suggesting the high influence of diet on fatty acid composition.

FISH CONSUMPTION: HEALTH EFFECTS AND FACTORS INFLUENCING CONSUMER CHOICE

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Fish is low in fat and contains high quality proteins and numerous micronutrients such as vitamins, minerals and especially omega-3 fatty acids, which are beneficial in many diseases. The positive effects of fish consumption on health are mainly related to the anti-inflammatory effect of omega-3 fatty acids. Due to the anti-inflammatory properties, regular fish consumption of at least 240 g per week is associated with a lower incidence of cardiovascular disease, obesity and metabolic syndrome, cancer, better cognitive function and mental health. However, fish consumption worldwide is below recommendations for a variety of reasons. As experts continue to emphasize the need to increase fish consumption, the only way to ensure an adequate supply of fish is to promote the consumption of fish from aquaculture. Aquaculture is currently the fastest growing food production industry in the world, with an average annual growth of 5.3% since the 2000s. With the goal of increasing fish consumption, it is very important to identify the drivers and barriers to fish consumption in different populations. The main drivers of fish consumption are sensory preference for fish, perceived health benefits, and habits of eating fish, while the main barriers are sensory aversion to fish, concern about health risks, perception of high price, lack of convenience, lack of availability of preferred fish products, and lack of knowledge in selecting and preparing fish. In addition, consumers also have prejudices against farmed fish. They believe that wild-caught fish tastes better, is safer, healthier, and more nutritious than its farmed counterpart.

Market research could provide valuable information on fish consumption habits, attitudes, and beliefs of different consumer groups, which could serve as a background for marketing activities.

The results of the market research conducted in Croatia confirmed that Croatian consumers like to eat fish, but they are price sensitive and have prejudices against farmed fish. They are relatively interested in fish from sustainable aquaculture and therefore should be informed and educated about the quality and availability of this type of fish. Italian consumers enjoy eating fish, both at home and in restaurants. More than half of consumers have prejudices against farmed fish. A large number of consumers are willing to buy sea bass fillets that are cold smoked, sustainably farmed, and have a longer shelf life due to the packaging method, if available. The attitudes of Italian and Croatian consumers towards farmed fish are summarized in Figures 1 and 2.

When planning marketing activities, small and medium enterprises (fish farms and processors) should consider the needs and desires of the target markets, i.e., consumers. The proposed marketing activities should be related to sustainable aquaculture products. For the purposes of the proposed marketing activities, a sustainable aquaculture product should be defined as a nutritious, healthy, and high-quality food product with a limited environmental footprint.

Effective collaboration between universities and small and medium enterprises should be established with the aim of knowledge transfer in the development of effective marketing strategies, thus making a valuable contribution to increasing the competitiveness of producers or processors in the market.

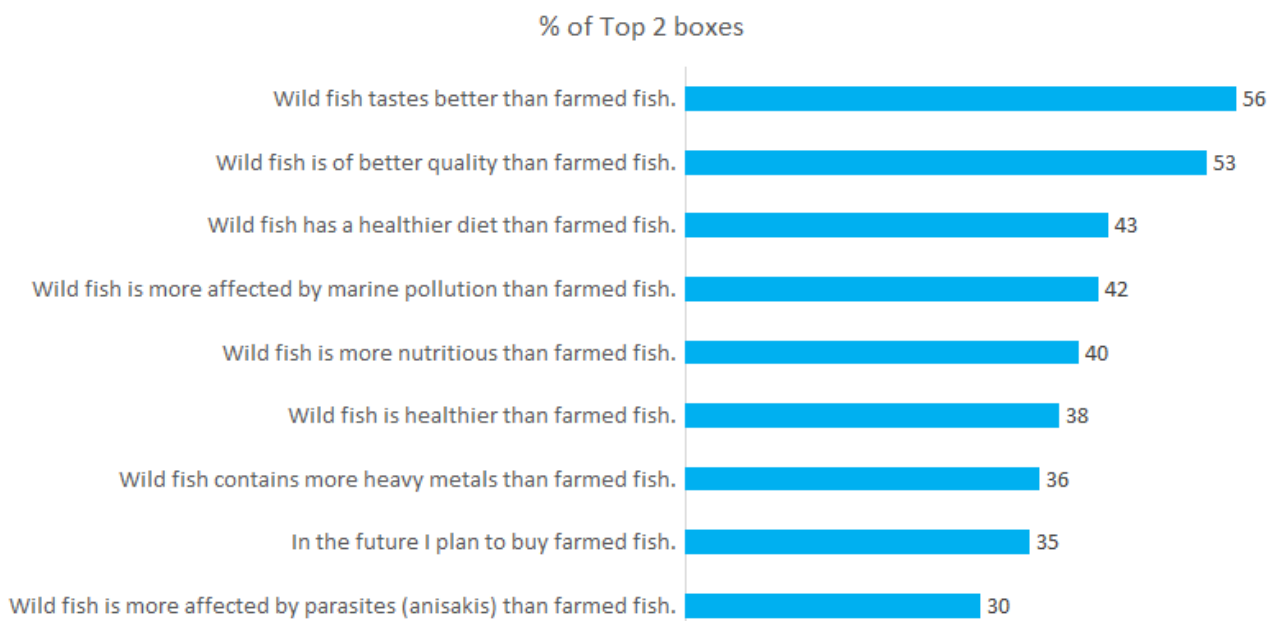


Figure 1. Attitudes towards farmed fish among Italian consumers

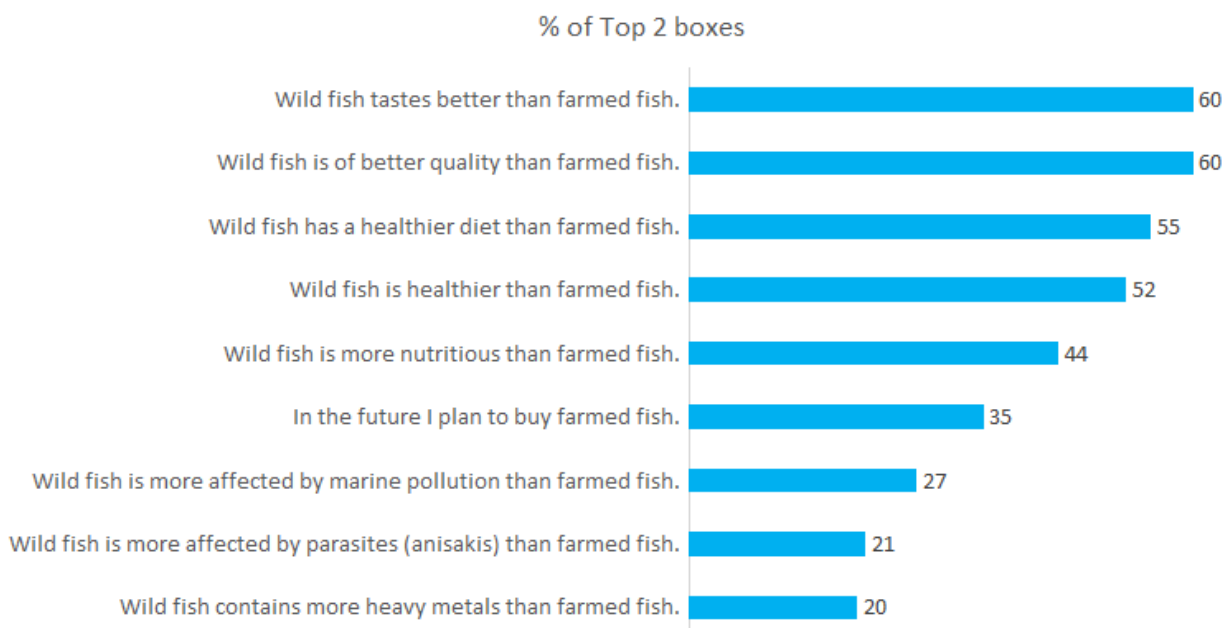


Figure 2. Attitudes towards farmed fish among Croatian consumers

PROMOTION OF SUSTAINABLE AQUACULTURE PRODUCTS

Dina Lončarić, Elena Dujmić, Greta Krešić

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Promotional activities are carried out by suppliers to inform, persuade, and remind consumers about a company, its offerings, and its activities. Promotion refers to marketing communications, the means by which companies attempt to appeal to and engage people in order to get them to buy and experience the market offerings they are promoting. Unfortunately, small and medium enterprises (SMEs) often lack the knowledge or capacity to design and execute promotional activities.

Although the objectives of promotional activities can vary, the most important are to provide information, differentiate the product, increase product demand, stabilise sales, and highlight product value.

Developing effective marketing communications requires performing the following steps: identifying the target audience, defining the communication objectives, designing the communication, selecting the communication channels, determining the budget, deciding on the promotion mix, measuring the results and managing the integrated marketing communication. Target audience refers to the audience for which the message is intended. This is a part of the market that the SME has decided to target.

The communication objectives can be different, such as informing consumers, changing consumer attitudes, differentiating the product from the competition, highlighting the product's value, etc. However, to achieve this goal, it is necessary to inform and educate consumers about the nutritional value of sustainably farmed fish and to reduce prejudice against farmed fish.



Figure 1. Mark
 "Sustainable from Adriatic"

Once the target audience is identified and the communication objectives are set, the communication needs to be designed and the communication messages created. The message should attract attention, generate interest, create desire, and motivate action. To promote the sustainable aquaculture products produced by the project, a mark (logo) and slogan were developed that can be used for product identification, differentiation, and promotion. A mark is a symbol, image, design, distinctive lettering, colour, or combination thereof. A slogan is the verbal or written part of an advertising message that summarises the main idea in a few memorable words (Figure 1).

A communication channel is the way a company communicates its offering and value to its audience. Communication channels are media through which SMEs can send a message to their target audience.

Most Italian and Croatian consumers obtain information about fishery products through the Internet and television (Figures 2 and 3).

Companies use different approaches to determine the budget for promotion. This is one of the most difficult decisions to make. Decisions must be made about how much to spend on the promotion and how to allocate the budget among the elements of the promotional mix.

A promotional mix is a set of promotional activities such as advertising, personal selling, sales promotion, public relations, and publicity. Each element of the promotional mix has unique characteristics that, taken together, inform, persuade, and remind consumers about the available offers SMEs need to monitor the results of their marketing campaigns. Different performance indicators can be used for this purpose. Some of them aim to monitor sales growth, while others aim to determine brand awareness among consumers.

The ultimate goal for SMEs is to increase sales of farmed fish. Despite the specifics of sustainable aquaculture products and the goals to be achieved, in a collaboration with academic it is possible to develop effective marketing communication.

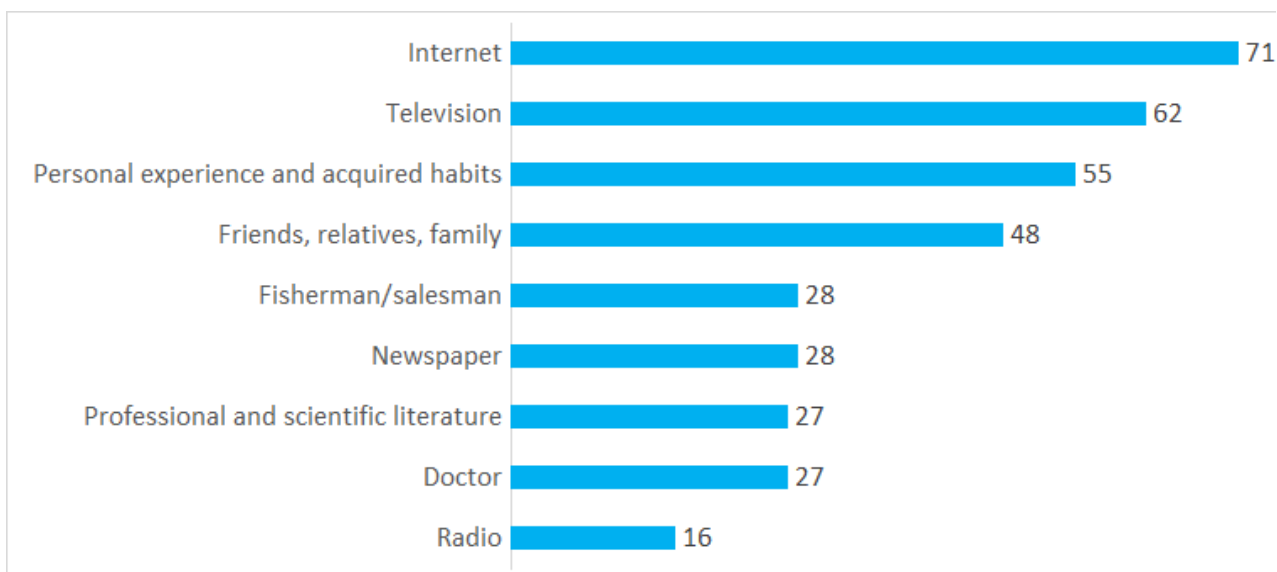


Figure 2. Source of information about fishery products among Italian consumers (%)

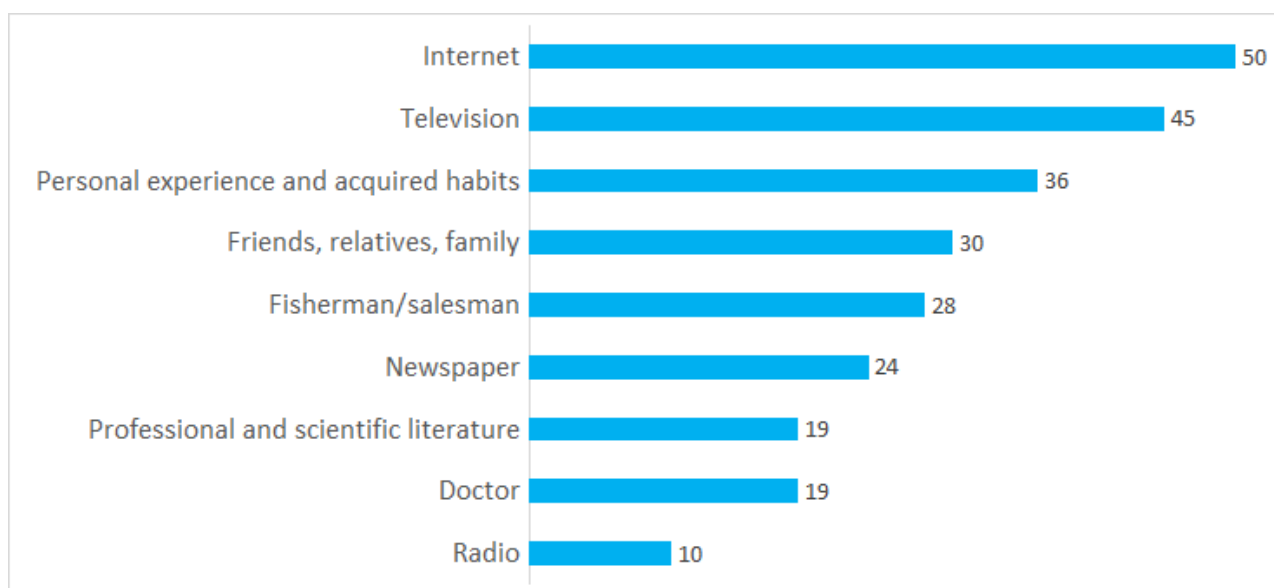


Figure 3. Source of information about fishery products among Croatian consumers (%)

MARKETING OF SUSTAINABLE AQUACULTURE PRODUCTS

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Marketing is the activity, set of institutions, and processes for creating, communicating, delivering, and exchanging offerings that are of value to customers, clients, partners and society at large. SMEs should make a series of decisions related to the so-called marketing mix elements. The marketing mix is also known as the 4 Ps of marketing after the first letters of the elements that make it up (product, price, place and promotion).

A product is anything that is offered on the market with the aim of satisfying the wants and needs of consumers in the exchange process. It is the main element of the marketing mix and the platform on which the other elements of the marketing mix are built. According to the market research conducted, the following three characteristics of the product from aquaculture are the most important for Italian consumers: no additive, acceptable price and from sustainable aquaculture (Figure 1). The most important aquaculture product attributes for Croatian consumers are no additives, easy preparation and pure boneless meat (Figure 2).

Quality is one of the most important product characteristics for consumers. From the marketing point of view, we distinguish between the technological and market quality of food products. Technological quality is based on prescribed standards and refers to the ability of a food product to maintain good health through consumption. Market quality is determined by consumers through their choice of products.

Packaging refers to the process of making packaging and the container used to protect, promote, transport, and/or label a product.

Farmers and small and medium enterprises (SMEs) bring a variety of fishery and aquaculture products to market. The choice offered to the consumer within a given class of goods is called the assortment or product range.

Price is the amount a customer must pay to purchase a product. To the consumer, price is an expression of the value of a product. Unlike other elements of the marketing mix, consumers always recognise differences in the prices of products from different manufacturers.

The term distribution refers to the marketing and transportation of products to consumers. It is also used to describe the extent of market coverage for a particular product. Along with product, price and promotion, distribution - represented by place or placement - is one of the 4 Ps of the marketing mix. The role of distribution is to deliver the product to the consumer. Distribution includes two elements: distribution channels and physical distribution.

Promotional activities are carried out by suppliers to inform, persuade, and remind consumers about a company, its offerings, and its activities.

In order for aquaculture products to be more successfully placed on the market and for SMEs to increase their competitiveness, it is necessary to plan a series of marketing activities. If SMEs do not have sufficient capacity to do this themselves, they are encouraged to connect with the academic community, for gaining knowledge transfer.

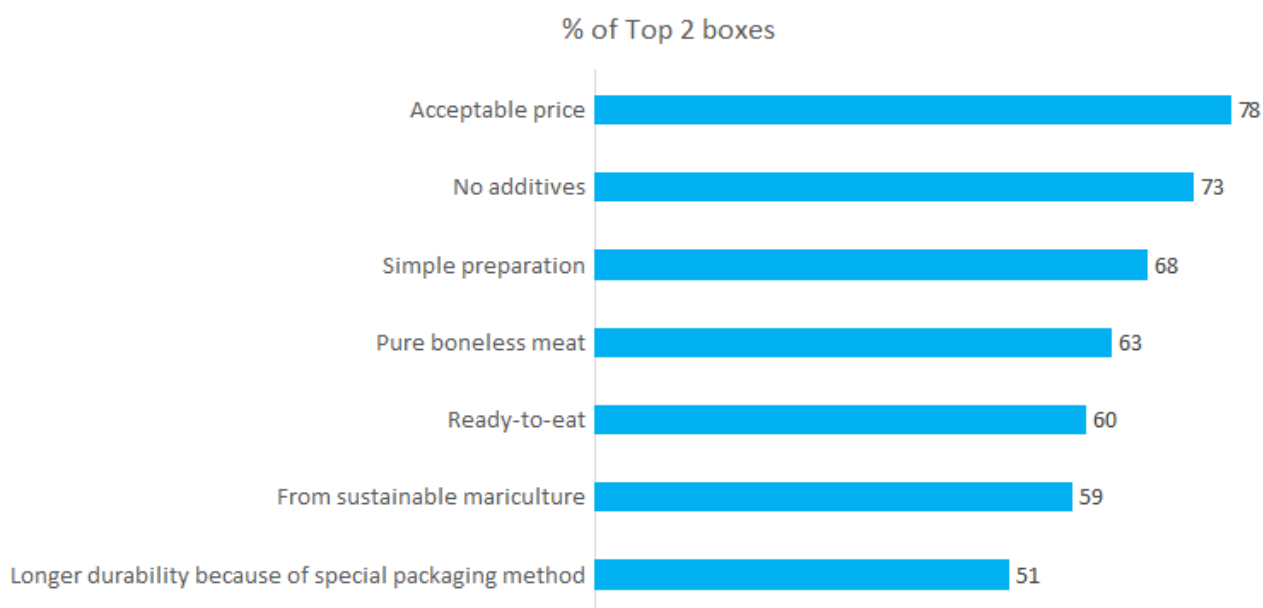


Figure 1. Characteristics of fishery products important for Italian consumers

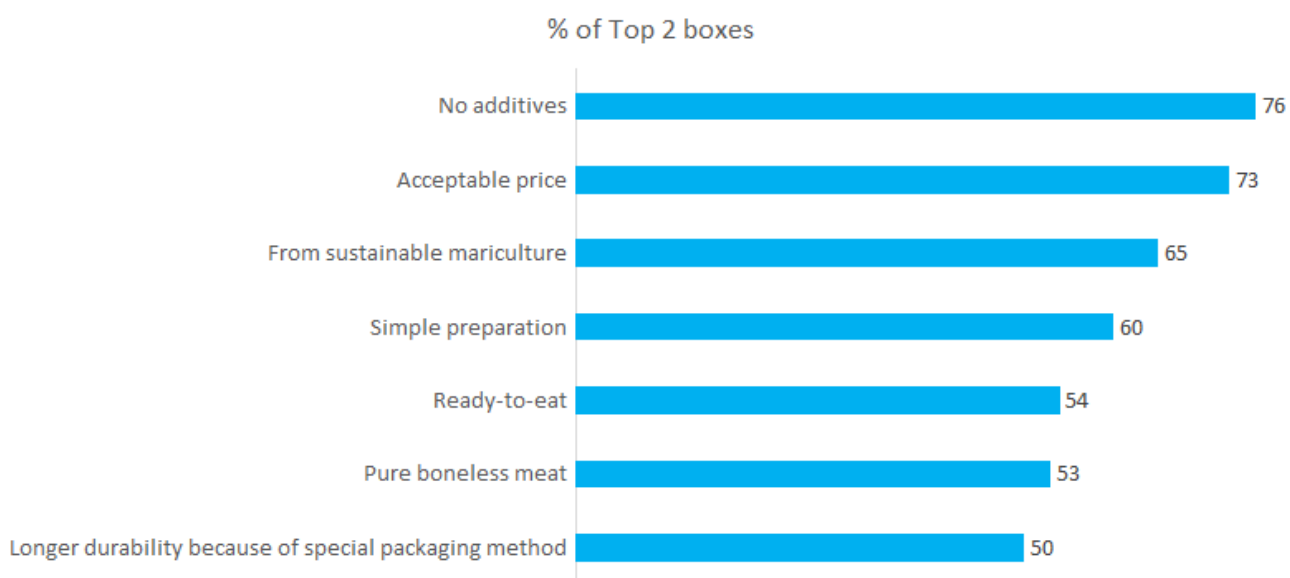


Figure 2. Characteristics of fishery products important for Croatian consumers

D.2.2.4

TRAINING MATERIALS

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