

"Piloting of eco-innovative fishery supply-chains to market added-value Adriatic fish products"

Priority Axis: Blue innovation

1.1 - Enhance the framework conditions for innovation in the relevant sectors of the blue economy within the cooperation area

D4.2.4. List of prototyped innovative products and solutions (including processing)

WP4 -INNOVATING TOOLS AND PROCESSES FOR ADDED-VALUE ADRIATIC FISHERY PRODUCTS/ A 4.2. DESIGN, DEVELOPMENT FEASIBILITY OF SPECIALIZED POLYVALENT-MULTIUSE PROCESSING PACKAGING SOLUTIONS

December 2020

Contribution: PP8, PP9 and PP10 Final version Public version



ORDER	FC OMEGA 3, FC ISTRIA, O.P. BIVALVIA		
TYPE OF DOCUMENT	PROJECT PRIZE FISH- Piloting of eco-innovative fishery supply-chains to market added-value Adriatic fish product REPORT D4.2.3.		
DELIVERY PERIOD	M24		
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Document description

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Besides the pilot actions of innovative technologies taken and described in previous D 4.2.3. report, it is important for the producer organizations to detect the possibilities on creation of innovative products with added values, which will be piloted. Due to its own specialties, and the results gathered from the piloting of innovative technologies, each of the producer organization has made a clear insight into what kind of new product is possible to innovate, in order to widen the market possibilities and satisfy consumer needs.

This document is divided into 3 separate cases (OP "Omega 3", OP Istria and O.P. Bivalvia), in order to make it easier for readers to follow each and specific business models which are operating with different sea species.



1. OP "OMEGA 3" CASE

Project innovations are based on the search for opportunities to significantly raise the quality of catch for small pelagic fish and change production processes in the traditional harvesting habits. The current product range of Fisherman's Cooperative Omega 3 is based mainly on the sale of quick-frozen fish (sardines and anchovies). There is a need to search for a possibility to sell fresh / chilled sardines in the fresh fish market. In this context, through the project task, given the very high sensitivity of small blue fish, the possibilities of further raising the quality of the catch were analyzed. Definition was found in possibility to change harvesting traditional habits. Based on all interactions, bases from work packages 3 and 5, existing bases, and pilots from packages 4, we decided on a fresh category of sardines and sardines packed in a plate with various gas mixtures including noble argon gas.

The outcomes of project innovations in Omega 3 relate to a significant change in the production process where a critical point of quality decline has been identified. It refers to the traditional way of harvesting with voliga (hand net to harvest with crane). At the same time, there is a significant squeezing and damages of the fish, which loses the quality as well as the possibility of extending the shelf life.

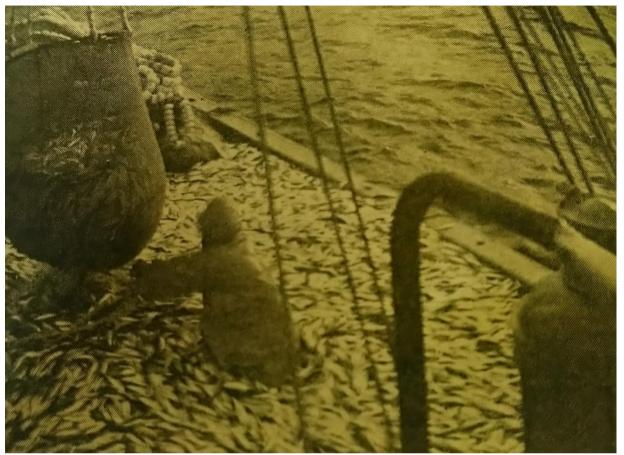
A significant change in the process was caused by a change in the traditional way of fish harvesting in the possibility of harvesting with a pump used in aquaculture. In addition, the process of innovation transfer to a fishing boat was successfully carried out. A number of benefits of such a change of quality, safety, velocity, fish welfare has been identified.

Laboratory analyses defined for the category of fresh sardines caught by the pump and caught in the traditional way:

- Sensory determination of freshness and estimation of shelf life
- Determination of muscle pH
- Determination of water binding capacity
- Determination of total volatile base nitrogen (TVB-N)
- Determination of the degree of proteolysis
- Determination of the degree of fat oxidation (TBARS test)
- Determination of histamine content
- Determination of basic chemical composition, ie nutritional value

A basis has been created for further research in terms of the possible use of selectors on board during harvesting, which would enable the return of live and undamaged fish to the sea to increase the reproductive potential of sardine and anchovy for sustainable approach.





Source: History of pursesenines boats a-A. Viličič

1.1. Introduction

The task of the technical part of the project for the organization of Omega 3 producers in work package 4 was to find innovative process technologies and product definitions to further improve the quality of own catch products and expand the range. Therefore, a study with a technical description of the operational course of the catch was first made, which should show the operational course from the catch to the processing of small blue fish with emphasis on searching and identifying those parts of the operational process and technology which could raise the quality of catches of small blue fish from the Adriatic Sea.



The study looked at today's trends in catching and processing of small blue fish. The trends that bring quality improvement have been clearly pointed out and these trends have been processed from the scientific and background of previous professional experience. Through the same, critical points of the process were presented and a process should be selected that can affect the quality of catch and manipulation in partially controlled conditions on board or the category of catch handling, and whose application in the Adriatic Sea would be innovative. Fishing activities have been developed and the Study has been prepared in accordance with the outcomes from previous assessments and findings carried out through the activities of Work Package 4 of this project. The study provided an overview of the capacity / quality of existing operational procedures in the process from catching to processing and packaging of fishery products, applicable to this case, ie small blue fish.

This overview of existing technological procedures and the possibilities of upgrading / improving the production process showed the possibilities of transforming operational procedures in order to obtain products with added value or raising quality. Based on the analysis, a solution or prototype of the operation or equipment for pilot testing was selected. After the completion of the first unit, guidelines were developed with a technical description of the innovative production solution. The guidelines contain a description of the equipment, procedures and practices in the process of catching small blue fish that may have an impact on raising the quality of the fish caught and improving existing operational procedures.

The technical guidelines described in detail the selected production solution as well as provided information on how to handle new tools and maintenance requirements during the production process from fishing operations to product delivery. Technical schemes are attached and the implementation of the first testing of new technical-technological solutions in handling small blue fish catches is ensured. The solutions were related to the application of aquaculture pumps on a purse seiner boat that took place in Croatia. The third part related to find the possibility of conducting the pilot as well as conducting the pilot testing itself. With regard to the selection of pumps for catching small blue fish, the prototype was included in the existing technical technological processes on board with the conduct of pilot testing.

A producer was selected for which, based on all previous experience, it was assumed that it was able to follow the construction of a pilot unit selected for the purpose of manipulating catch, which has the characteristics of innovation and was ready to apply it in a pilot experiment and confirmed this letter of intent. With this production facility for innovative solutions, a representative ship was found that was ready to conduct a pilot experiment as part of its operations. The entire work included an emphasis on innovation within the Adriatic Basin, and a pictorial explanation of technical and technological procedures. The quality of fish or new products was assessed by standard procedures in cooperation with the reference institution from the project partnership.



The Service Provider precisely documented the entire implementation of the pilot testing and submitted it to the Client in the final documentation of each unit. This was followed by the Distribution of solutions to producers in the program area where the successful distribution of developed innovative production solutions to producers in the program area (Interreg program Italy - Croatia) to ensure the potential benefits of innovative production solutions to the entire Adriatic fisheries sector.

For this purpose, the recording and editing of documentary videos of pilot testing was prepared, educational materials were prepared on which courses and training of interested manufacturers can be conducted, and presentations to interested parties in Croatia and Italy are available in English, Croatian and Italian translation (video). Finally, in case of the need for the final Report on the outcomes of project innovations, this report on all completed objectives has been prepared, which has an insight into the outcomes of project innovations. The report was prepared in accordance with the outcomes generated in Work Package 5 of this project, and related to market research. The report therefore includes an overview of potential innovative products that are a possible result of the innovations tested by this project, and an overview of adequate technical or technological solutions in processing required to create such products.

1.2. Study with a technical description of the operational course of the catch

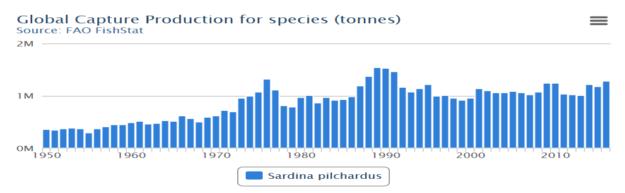
Introduction

Throughout the whole, the basic indicators of the amount of catch and distribution of small blue fish are processed, and the catch of sardines and anchovies in Europe and the Republic of Croatia is presented. An analysis of prices was made, as well as the market of sardines and anchovies in the EU and trends in product development as market preferences. As the next important category, the parameters of catch quality, trends and target species in the catch and catch operations were performed, and an overview of the existing technological and operational procedures in the catch to landing and processing with general recommendations was given. The operational course of fish from landing to processing small fish was given. A narrow circle of identification and selection of an innovative operational solution was made, a general overview of the sustainability and economy of small blue fish catches and an overview of management measures was given.

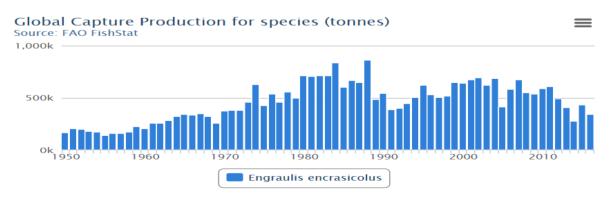


<u>Summary</u>

A brief introductory overview of small blue fish fisheries in Croatia indicates that sardines and anchovies have always been treated as commercial species that were not caught only for their own use and the local market. This was only possible by finding ways to preserve fish from spoilage, which ultimately resulted in new products as well. Sardine and anchovy processing is therefore an integral part of fishing for these species throughout history. These species are also recognized as high quality food and are a constant motive for innovative product improvements in the processes from catch to marketing to end consumers. Pelagic species live in the middle of the water column (up to near the surface) and are very limited in the water column or have no biological interaction with the seabed. The vast majority of small pelagic fishing in the Adriatic targets catches of Sardina pilchardus and Engraulis enrasicolus species, which account for more than 97% of pelagic catches. Sardine pilchardus and Engraulis enrasicolus are the species that form the basis of this study. Sardine pilchardus is an important fishing species in catch areas in zones 34, 37 and 27 while most of the world's anchovy catch is processed for fishmeal and fish oil. Along with Chile, Peru is the largest producer of fishmeal.







Graph 2. Total world catches of Engraulis enrasicolus through the sequence of years 1950-2016.



Geographical distribution of the species Sardina Pilchardus

Sardines (Sardina pilchardus) are widespread in the Northeast Atlantic Ocean and the Mediterranean Sea. In the Atlantic, sardines extend along the continental shelf from the islands of the Celtic Sea and the North Sea to Senegal, with a backward population from the Azores, Madeira, and the Canary Islands (Parrish et al., 1989). The habitat is both the Mediterranean and the Black Sea. Changing environmental conditions affect the distribution of sardines, (Corten and van Kamp, 1996; Binet 1998) Source: ICES stock annex

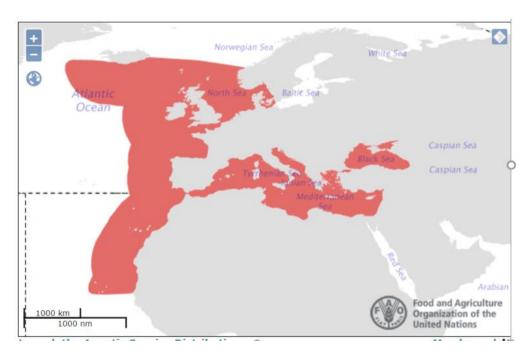


Figure 1. Geographical distribution of the species Sardina pilchardus

Geographical distribution of Engraulis encrasicolus

Anchovies Engraulis encrasicolus are found in the Eastern, North and Central Atlantic (coasts of Europe south of Bergen, Norway, but not the Baltic and rarely in the north; the entire Mediterranean, Black and Azov Seas, with sporadic influences in the Suez Canal and Suez Bay; south along the west coast). Africa to Angola, also recorded from St. Helena).



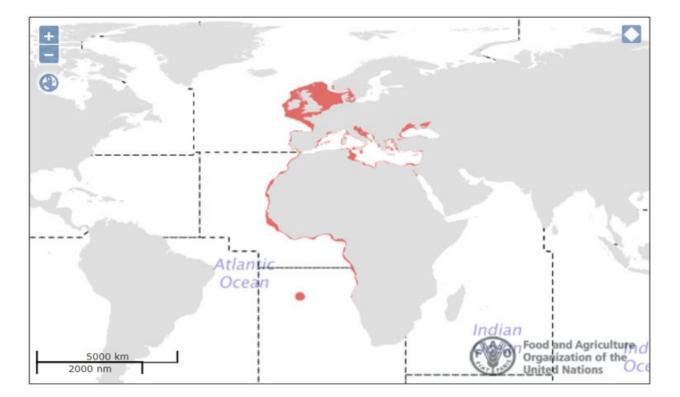


Figure 2. Geographical distribution of Engraulis encrasicolus species

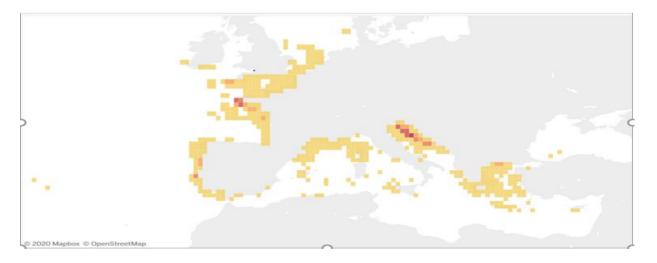


Figure 3. Spatial distribution of sardine catches, Source: <u>https://stecf.jrc.ec.europa.eu/dd/fdi/spatial-land-map</u>



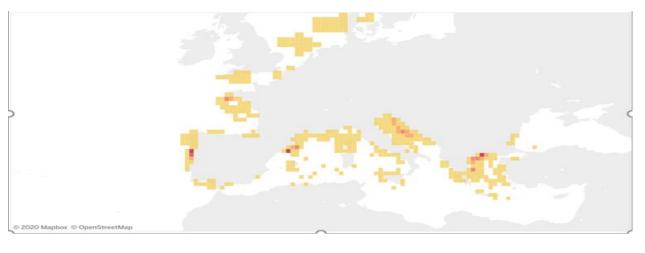


Figure4.Spatialdistributionofanchovycatches,Source:https://stecf.jrc.ec.europa.eu/dd/fdi/spatial-land-map

Although more than 110 different types of fishery products are commercially caught in Croatia, 6 species make up more than 90% of the total weight of the catch. Among them, the largest value has sardines with a catch value of 18.1 million euros and anchovy with a catch value of 10.1 million euros in 2017. Small blue fish are caught by the ourse sainer surrounding nets. The largest part of the fleet are boats from 24 to 40 m (73 active vessels) and from 18 to 24 m (49 active vessels) (SOURCE: STECF - Annual economic report 19-06)

The largest catch of sardines was in 2014, and anchovies in 2015. The price of anchovies is more than twice the price of sardines, and in 2016 the highest average price of 1 EUR per kg was achieved.

Currently, sardine and anchovy fishing is determined by the Ordinance on fishing opportunities in commercial fishing at sea surrounding the sardine purse sainer net (OG 18/2019, 101/2019, 16/2020). Article 4.

(1) **The total catches of small blue fish** by all vessels authorized to fish for sardines may not exceed the following quantities:

a. in 2019 the total catch of small blue fish must not exceed the total catch of small blue fish realized in 2014 reduced by 5% = 64,376.62t * 0.95 = 61,157.78t (data from E-newspaper 01/2015)

b. in 2020, the total catch of small blue fish may not exceed the quantity referred to in paragraph 1, item a) reduced by 5% = 58,099.89t

c. in 2021, the total catch of small blue fish shall not exceed the quantity referred to in paragraph 2, item b) less 5%. = 55,194.90t



The market for small blue fish catches consists of:

• The traditional canning processing market which mainly includes sardines

• Renewed salting processing market, which mostly includes anchovies, and a smaller part of the market is looking for sardines

- Newer market for anchovy processing by marinating
- Freezing processing market
- Fresh produce market
- Market of fresh product intended for animal nutrition (tuna farming)

The market of fresh-frozen products should certainly be included in the above categories with the development of IQF freezing technologies. Some buyers in the market have the opportunity to process the fish in several ways, as well as ship it to tuna farms. For this reason, it is not possible, according to customers, to clearly distinguish the purpose of the purchased fish, or to determine the market of the caught product according to the final product.

The EU has a well-developed small blue fish processing industry, despite high production costs. The total turnover of the fish processing industry in the EU is around 28 billion euros in 2016. The leading countries in this industry are Spain, France, the United Kingdom, Poland and Denmark. Source: Facts and figures on the common fisheries policy Basic statistical data 2018 EDITION; EC

France, Italy and Spain are also the largest importers of fish from other member states. In total, over one million tonnes of small blue fish are imported into the EU. 48 percent of imported fish quantities are frozen products, and only 27 percent are fresh fish. The connection with quality and high price category products is becoming more and more pronounced. About 200-250,000 tonnes of sardines are caught annually in the EU (249,503 2014; 197,618 2015). Around one million tons are fished in the world, most of which is fished by Morocco (70% of the world's catch) with over 800,000 t in 2014. Mediterranean countries (Italy, Spain and Croatia) have maintained a good position in sardine trade, although they are also threatened by declining production due to livestock protection. Atlantic fishing countries have significantly reduced their exports (France from 4,800 tonnes in 2008 to 800 tonnes in 2015 and Portugal from 19,300 tonnes to 3,500 tonnes), meaning that each country consumes almost all of its production.

Given the state of stocks, the EU processing industry will in the future largely depend on imports of frozen sardines, and the fresh sardine market will depend on catches with a tendency for the price of fresh sardines to rise. Most likely, for this reason, an increasing share of sardine catches from the EU will be consumed fresh, due to the highest price. The only sardine product certified under the EU quality scheme is "Cornish Sardines". Some other products have quality certificates, especially in France where premium products carry the "Label Rouge" label. Two EU fleets fishing for sardines are MSC certified, but neither is in the Mediterranean.



These are:

- "South Brittany purse-seine sardine" in France, about 20,000 t per year

- "Cornish Sardines" in the UK, about 1,000 tons per year

The EU fleet catches around 100-132,000 tonnes of European anchovy (Engraulis encrasicolus) per year (132,000 2015; 128,000 2017). Around 7-8 million tons of various species of anchovies are fished in the world (Peru has the largest catch,> 4,000,000 tons), of which 432,000 tons are European anchovies (Engraulis encrasicolus). European anchovies are mostly fished in the EU in Spain (40-50,000 tons) and Italy (30-40,000 tons). The largest producer of European anchovies is Turkey, which catches about 45% of the world's catch in the Black Sea, about 190,000 tons (subspecies Engraulis encrasicolus ponticus). Most countries recorded a decline in catches compared to 2006, except for Spain, which is the only country with a catch increase. EU countries together account for about 30% of world production. Trade in anchovies in EU countries is specific in terms of large imports and exports in certain countries (Spain, Italy and France). Countries that import and export at the same time (for example, Spain also imports and exports frozen products).

Imports of anchovies from non-EU countries amounted to about 30,000 tons. Most of that amount (21,000 t) has already been prepared or processed in some way. The main supplier is Morocco with 14,000 t, followed by Peru (with a long type of anchovy) with 6,000 t. Morocco supplies a semiprocessed product (dried, salted or smoked) which is further processed in Spanish, Italian and French canneries. Spain is the largest processor in the EU, and thus the largest exporter of processed products. In general, the most traded is already processed anchovies. Spain and Italy import salted anchovies from Argentina, Peru and Morocco, and partly from Croatia and Albania. Fresh anchovies are traded mostly within the EU, and Spain is the largest importer with 13,270 t in 2016. It imports mostly from Portugal, Italy and France. Italy is also a major importer of fresh anchovies (3,500 t), and Germany and France import less than 800 t.



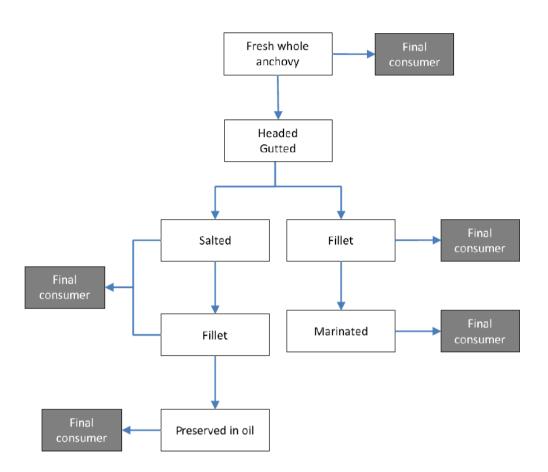


Figure 5. Stages of processing and sales phase of anchovies (source: Case study: Processed anchovy in Italy, EUMOFA 2018)

The scheme in the picture shows that the trade in anchovies is complex because the semi-processed product is traded in retail, but also among individual countries.

There are two geographical indications of anchovy in the EU:

- "Acciughe sotto sale del Mar Ligure" (Italy, 2008),
- "Anchois de Collioure" (France, 2004).

Both labels are used for processed anchovies. The Italian designation has not been used since 2017 because in the Ligurian Sea the size of the anchovy has fallen below the limit size specified in the designation of origin specifications.



Today, the fishing of small blue fish is carried out with the purse seiner's surrounding nets and with the trawl with pelagic trawl (hereinafter - floating trawl). In the Adriatic Sea, fishermen on the eastern Adriatic coast use purse seiner's nets, while Italian fishermen in the 1980s mostly switched to floating hides and turned mainly to catching anchovies. Only a few purse seiners remain on the west coast. Fishermen fishing with purseseiner's nets target the primary or secondary processing market while floating hull fishermen are turning to the fresh produce market. Pulling live fish on deck enables "shock", killing in an ice emulsion of water and ice, which interrupts all catabolic processes, including anaerobic decomposition processes, which significantly affects the life of fresh product in retail. From catch to retail it is necessary to maintain a cold chain. Recently, IQF freezing technology has enabled the primary processing of fish caught in floating trays, which has changed or diversified the market for small blue fish caught in floating trays.

In terms of the quality of the fish caught, sardine fishing differs from floating trawl fishing in the amount of catch in one stock and in the target market. In sardine fishing, the fisherman "grazes" the fish usually once during the fishing day. Italian fishermen practiced multiple stocks (2-3) at night. Croatian fishermen have a tradition of fishing for small blue fish for processing, so the amount of daily catch was related to optimization, which includes the cost of fishing and the price that can be offered by fish processors.

In recent years, Croatian fishermen have turned to the fresh fish market as well as the use of IQF technologies for the placement of frozen fish in distant markets. Here, below, we will pay special attention to the connection of fishing operations in sardine fishing with the quality of small blue fish as a product for the first sale market. It follows from the above that in order to preserve freshness and quality it is necessary:

- 1. Reduce enzyme activity
- 2. Reduce the activity of bacteria that are naturally present in fish

3. Reduce contamination with new bacteria by humans, dirty surfaces, water, ice and anything that comes in contact with fish.

4. Reduce oxidation and hydrolysis by modifying the atmosphere

Biotechnological assessment of fish quality is developing in two basic directions:

- Assessment of fish quality from the aspect of food safety
- Assessment of fish quality from the aspect of organoleptic and nutritional properties

In commercial terms, quality is a term that corresponds to the price that a particular species of fish can achieve in the market and the duration of quality within the framework recognized by end consumers. In areas that are deficient in protein in human nutrition, they are still satisfied with the second and even third quality category. However, in developed countries we strive to stay in the first quality category for as long as possible. In order to achieve this, we work in two basic directions: I. Reducing stress during fishing. Innovative solutions in fishing operations.

II. Slowing of autolytic processes in fish tissues. Cold chain maintenance.

The target species in sardine fishing in the Adriatic Sea are sardines and anchovies. Occasionally long species are also hunted.



Targeting a particular species depends on:

• Long-term fishing experience - the ship's captain's assessment of the expected catch

This assessment comes to the fore at the beginning of the fishing darkness when the fleet is in principle looking for fish. Short-term experience in catching small blue fish. It is based on information on catches in previous days and the position of vessels determined by an inspection of the vessel tracking system

- Estimation of the amount of expected catch of a particular target species
- Estimation of the relationship between fishing costs and the value of expected catches
- Weather conditions. The master estimates the certainty of the catch in the expected weather conditions, so he can choose the fishing area in which he estimates the higher probability of catching
- The cost of fishing trips is proportional to the distance of the fishing area from the port of landing, and the value of the catch depends on the structure of the catch (species, average size), the quality of the fish caught at first sale and the market situation.
- The HACCP system (Hazard Analysis Critical Control Point) serves to control food safety by analyzing risks, recommending design for the safety of space, processes and equipment to achieve optimal hygiene, and setting measurable standards, ie monitoring and control systems.
- The HACCP system is aimed primarily at controlling hygiene, processes and cold chain maintenance and can be applied at all stages of production, from catch to retail.

Some of the critical control points in establishing better quality fish processing are:

- Maintenance of the cold chain during transport and in the processing plant itself, temperature recording
- Fish rinse control
- Removal of fish that are infected with parasites, damaged or of poor quality
- Good hygiene in the plant and reduction of bacterial contamination
- The shortest possible duration of the process of cleaning, filleting and other manipulation of fish

MAP packaging

The next step in raising quality is to control the atmosphere because a reduced amount of oxygen reduces oxidative processes and bacterial growth. In meat products, the influence of vacuum and modified atmosphere on the durability of the product is much greater than in fish, because the spoilage of meat is influenced by gram-negative bacteria such as Pseudomonas. Although mixtures of gases containing about 30% oxygen are used for MAP packaging of white fish, crustaceans and shellfish, mixtures of carbon dioxide and inert nitrogen are used in the packaging of blue and other fatty fish to reduce the oxidation of fatty acids in the tissue. Too high a concentration of carbon dioxide causes acidification which causes water to drain from the fish.



Özogul et al. (2004) experimentally proved that "shelf life" of sardines is 12 days if packaged in a MAP atmosphere with a ratio of 60% CO2: 40% N2 at 4 ° C, and 9 days in a vacuum package, while in a normal atmosphere its duration is only 3 days. They measured several parameters including the amount of histamine, the number of bacteria, the concentration of trimethylamine (TMA) and total volatile nitrogen (TVB-N) (F Özogul, A Polat, Y Özogul,)

Superchilling

Storage at temperatures from 0 to -4 ° C is called supercooling or partial freezing. This method of storage can extend shelf life to as much as 15 to 30 days for some species of white fish, depending on the temperature (source: FAO FISHERIES TECHNICAL PAPER - 348, Quality and quality changes in fresh fish, 1995), which gives resellers additional time to market on the market. The principle of supercooling is that the product is kept at a temperature of 0.5 to 2 degrees below the freezing point. Although pure water freezes at 0 ° C, fluids in fish tissue contain salt, proteins, and other solutes that lower the freezing point of body fluids. Depending on the type of fish, this temperature is different, but in most species about 50% of body fluids are frozen at temperatures from -2 to -2.2 ° C (source: Fresh fish keeps up to two days longer, Eurofishmagazine.com). If the temperature drops to -2.8 ° C, almost all the liquid in the fish is frozen and shelf life is extended to 35 days, but then larger ice crystals are formed and during thawing there is more fluid loss and destruction of fish tissue. Superchilling on fishing boats was first used by Portuguese trawlers who had tanks filled with water and ice in the boat with bulkheads through which pipes with coolant passed. This system maintains a controlled temperature of 0 ° C so that the fish arrives in the port supercooled and is ready for

further processing.

Fishing with a sardine purse seiners involves a series of operations that can affect the quality of the fish. Fishing operations in fishing with sardine purse seiners are:

- Looking for fish
- Attracting fish
- Laying the purse seiner's net in the sea
- Clamping, closing and pulling the net
- Fishing from the net on a fishing vessel
- Killing of caught fish (sudden cooling)
- Preservation of quality from catch to landing
- Landing fish

<u>The search for fish</u> using echolocators has significantly improved sardine fishing. It can be heard among fishermen that individual captains are able to assess biomass and fish species. There is no scientific confirmation for this, so there is a way open for innovations that would increase the probability of a catch that is more in line with the objectives of sustainable fishing.



<u>Attracting fish</u> with light is a traditional technique that currently has no significant impact on the selectivity of fishing or on preserving the quality of the fish caught.

Setting the purse seine net in the sea begins when ultrasonic detection and visual observation determine that the flock is "ready" for the start of the operation. The process of setting the net and surrounding the flock does not have a significant impact on the quality of the fish caught.

Clamping, closing and pulling the net depends on the skill of the ship's crew. In any case, the procedure should be carried out to reduce the stress of the surrounded fish. This is influenced by both sea currents and possible predators in the network. Today, we do not have the ability to monitor the behavior of the besieged flock, so the impact on the quality of fish in this operation is technologically very limited. **However, the last stage of netting can have a significant impact on fish quality.** The speed of pulling the net and squeezing the fish into the bag should be matched with the density (amount of catch) and the speed of emptying the bag (catch). Innovative fishing solutions can significantly contribute to the quality of the fish caught. **The goal is to get the fish out of the bag as quickly as possible with as little damage as possible.**



Figure 6. Catch of fish from a bag on a fishing boat (critical phase determined)

<u>Killing fish after catching (shocking)</u> is done in an emulsion of ice and water. When shocking fish, it is advisable to lower the temperature of the fish to approximately 0 ° C as soon as possible. Combinations are used for the emulsion:

- Freshwater ice sea water
- Sea ice sea water
- Chilled sea ice

Due to the fact that with increasing salinity, the freezing point of water decreases, ie the water temperature decreases while it is in the liquid phase, and ice crystals begin to form in fish at temperatures between -0.5 and -1.1 °C. individual methods.





Figure 7. Subcooling of fish after catching. Source: YouTube; HRT

"Shocking" anchovies that are then placed in cassettes. Then the preservation of the cold chain depends on a sufficient amount of ice with which the cassettes are frozen, the air temperature and the duration of transport until the fish is unloaded at the unloading point. Fish after death do not have the ability to regulate osmotic conditions in body fluids. This means that if they are immersed in a liquid of less osmolarity than the osmolarity of body fluids, water will penetrate the body and the fish will swell. That is, if immersed in a solution of higher osmolarity the fish will lose water and will lose weight. The choice of subcooling method depends on the target market of the first sale and the duration of the trip to the landing of the fish.

<u>Preservation of fish quality from catch to landing</u> site depends on the method of temporary storage. The aim of temporary storage on board is to maintain a cold chain, ie to preserve the temperature of the fish during transport in the range from -0.60 ° C to 0 ° C. They use two methods of temporary storage, traditional in fishing boxes and recently more and more represented transport in thermally insulated containers.

Innovations in the technological process of catching and processing small blue fish - through yesterday today tomorrow - world-European-Adriatic level



Freezing and thawing under high pressure

In conditions of high pressure, the freezing temperature of water is lower, so at an air pressure of 200 MPa, the water remains in a liquid state up to -21 ° C. at the moment when the pressure is abruptly released, due to the isostatic properties of the water, water crystals are instantly formed which are uniformly distributed and of equal size in the fish meat tissue.

This freezing at the time of pressure release is called High Pressure Shift Freezing (HPSF).

The freezing property of water can be used for supercooling or storage at low temperatures without freezing, but also for the opposite defrosting process where the frozen fillet temperature below 0 ° C is placed in a high pressure chamber and due to lowering the freezing point completely thaws (Pressure Shift Thawing).

High pressure processing

High pressure processing (HPP) or pascalization is a technological process for sterilization and preservation of food which achieves the deactivation of microorganisms and enzymes in food by means of very high hydrostatic pressure. Unlike pasteurization, which uses heat to sterilize food and causes changes in the taste and other characteristics of food, HPP preserves the sensory and nutritional properties of food. This technology was proved by B. H. Hite as early as 1899 in the first experiments, and was named after, the French scientist Pascal who studied the influence of pressure on liquids. HPP is also called "cold pasteurization". The process consists of placing the products in their final packaging or in bulk in a hyperbaric chamber filled with water and raising the pressure to 300–600 MPa / 43,500-87,000 psi at a temperature of + 4oC to 10oC. High pressure inactivates microorganisms such as Listeria, E. coli, Salmonella, Vibrio and some enzymes. However, as part of the enzyme remains active, the food has an extended shelf life, but it needs to be kept cool.

Pulsed electric field

Research into the application of short pulses of electric fields (PEF) lasting several microseconds or even milliseconds at an intensity of 10-80 kV / cm to the inactivation of microorganisms began in the 1960s. Electric fields of voltage up to 25 kV were found to inactivate the following microorganisms: Escherichia coli, Staphylococcus aureus, Micrococcus spp., Sarcina lutea, Bacillus subtillus, B. cereus, Clostridium welchii, Saccharomyces cerevisiae and Candida utilis. The mechanism of action is that the charge that is created on the cell membrane causes it to burst, which leads to the destruction of bacteria.

Packing machine

Since electricity is needed as a guide, this technology is mostly used for liquid products such as juices, milk, yogurt, liquid eggs, soups, as well as for fruit and vegetable treatments used for further processing.



<u>Ultrasound</u>

Ultrasound is sound waves, or pressure waves, mostly above the frequency that people can hear, starting at 20 kHz (20,000 cycles / second). The energy of ultrasound is inversely proportional to the square of the frequency, so that the "power" of ultrasound used for food processing is 15-40 kHz, while frequencies higher than 100 kHz are used for imaging, medical diagnostics and other applications. People can hear frequencies up to 15 kHz, and some up to 20 kHz, so workers will need hearing and sound-absorbing enclosures in these bands. Ultrasound creates an electrical device called a generator, which can be adjusted to generate vibrations at the desired frequency. These vibrations, of relatively low amplitude, are transmitted and amplitude increased through another device, called a coupler. Finally, the vibrations are delivered to a third device, called a horn or tool, which actually comes in contact with the material being treated. Sound waves are quickly attenuated in the air, ie they lose energy quickly, so the horn must be in physical contact with the material being treated, which can be solid or liquid. In addition to direct application, the product can be immersed in the so-called. ultrasonic bath.

Intense pulsed light

Pulsed light includes wavelengths from 200 to 1,100 nm and includes UV (200-400 nm), visible (VIS - 400-700 nm) and edge infrared light (IR - 700-1,100 nm). Pulsed light is used to sterilize external surfaces, and has an advantage over UV sterilization due to its shorter duration of action.

Ohm heating

Ohm heating is a process in which electricity is converted into heat due to the resistance of the conductor through which it passes.

Microwaves

Microwaves are electromagnetic waves with a frequency of 300 MHz to 300 GHz. Microwaves are used for heating, drying, blanching, baking and defrosting.

Innovative packaging

Classic packaging for the transport of fishery products must have many layers in order to protect the product and maintain a cold chain, and at the same time prevent squeezing from the packaging - the possibilities are:

Active packaging

Innovative active packaging creates special conditions within the packaging to extend the shelf life of the product.



Active packages can have:

- System for absorbing unwanted substances such as oxygen, carbon dioxide, ethylene, excess water, paint or other substances
- System for adding desirable substances such as carbon dioxide, antioxidants and preservatives
- Additional systems such as cooling

Intelligent packaging

Intelligent packaging is one that can provide information about the condition of packaged food and its quality during transport and storage.

Today, there are various indicators on the market that can collect and provide various information on the principle of mechanical, chemical or enzymatic processes. Indicators that measure time and temperature are important for maintaining a cold chain. These are usually external indicators.

Packaging with antimicrobial properties

Some of the systems for reducing the number of microorganisms in packaging are:

- Addition of organic acids benzoates, parabens, sorbates and propionates
- Addition of enzymes lysosin, nisin, glucose oxidase
- Fungicides and bacteriocins
- Polymers chitosan and nylon
- Natural spices mint, oregano, cumin, clove
- Other silver, zeolite, antibiotics

Selection of an innovative operational process with the possibility of transformations -pilot

To raise the quality of blue fish, it is important to handle the catch and sub cool the catch. Blue fish are particularly vulnerable to ill-treatment and storage, but with good production practice, spoilage at all stages of production and processing can be assessed and minimized. The small blue fish has a delicate structure and has a thin skin. Therefore, it is easily damaged during handling, and since it has a relatively larger surface area than body volume and is thus more exposed to bacteria, it is more susceptible to bacterial spoilage. Blue fish in the tissue contains large amounts of fat which soon after the fish is taken out of the sea begin to oxidize and significantly change the organoleptic properties and quality of fish meat.



Cooling - coverage in operations

FAO general recommendations:

The handling of the catch should be such as to ensure the maximum quality of the raw fish. Excessive squeezing of the fish should be avoided to avoid increased stress and consequent spoilage of the meat. It is also necessary to respect animal welfare and ensure a quick "painless-unconscious" death. It is necessary to avoid surface damage and crushing by a different and more efficient way of fish transfer. In addition, landing procedures should be such as to facilitate the work of the ship's crew and reduce the physical effort of the fishermen. The use of human strength when lifting heavy loads, as well as unsafe and unsuitable positions for workers, should be avoided.

Recommendations for raising the quality of today's catch system are:

- Choose the smallest possible net pull volume floating hulls possible category efficiency issue
- Reduce the duration of the pull-floating skin-possible category
- Pull fish out of the net with a small filling of sake to prevent squeezing due to high weight the category delays the collection of fish unfavorable category

• Use pumps when pulling fish out of the net, instead of lifting sake - possible category - recommendation for conducting pilot testing in the surrounding nets of swimmers on the Adriatic Pumps for lifting fish from the net to the boat have an underwater suction and it is necessary to control the ratio of water and fish during pumping and it is necessary to separate the water from the fish as much as possible to maintain the temperature in the cooling tanks.

Given that the transfer of fish from the closed network through the bag to the boat or tanks is recognized as the riskiest category that can significantly affect the quality of fish, for further continuation of the project will be selected to pilot the use of pumps. Pumps used in aquaculture transfer live fish vertically and horizontally without any problems, so it is imperative not to damage the fish. For the purposes of this project, a model of an aquaculture pump with a special adaptation for water discharge or separation and overcoming height differences will be proposed. It is also planned to use pumps without a vacuum tank to ensure smaller dimensions on board and facilitate handling. Since such a pump model is not used in the work with the catch, the same system will represent a certain innovation because it will need to be adapted to the fishing vessel.



1.3. Project Innovation Outcome Report

Project innovations are based on the search for opportunities to significantly raise the quality and change production processes in the catch and processing of small blue fish. The current product range of RZ Omega 3 is based mainly on the sale of quick-frozen fish (sardines and anchovies). There is a need to seek and be able to sell fresh sardines in the fresh fish market. In this context, the project task, given the very high sensitivity of small blue fish, sought opportunities to further improve the quality of catches through the process part and later the definition of products and promotion of fresh fish. Based on all interactions and bases from work packages 3 and 5 and existing bases and pilots from packages 4, it was decided to use a fresh category of sardines and sardines packed in a folder with various gas mixtures including noble argon gas.

The outcomes of project innovations in Omega 3 relate to a significant change in the production process where a critical point of decline in quality has been identified, and refers to the very catch of fish that today on all ships on the Adriatic takes place in the traditional way through the use of voligas. At the same time, there is a significant squeezing and kneading of the fish, which loses the quality as well as the possibility of extending the shelf life. A significant change in the process was caused by a change in the traditional way of fishing in the possibility of fishing with a pump used in aquaculture. And the process of innovation transfer to a fishing boat was successfully carried out. A number of benefits of such a change have been identified.

Laboratory analyzes defined for the category of fresh sardines caught by pump and caught in the traditional way:

- Sensory determination of freshness and estimation of shelf life
- Determination of muscle pH
- Determination of water binding capacity
- Determination of total volatile base nitrogen (TVB-N)
- Determination of the degree of proteolysis
- Determination of the degree of fat oxidation (TBARS test)
- Determination of histamine content
- Determination of basic chemical composition, ie nutritional value



With the additional product definition, it was decided to try to extend the shelf life of fresh / chilled sardines through packaging in MAP in the category of Argon noble gas additive.

For this category of innovative product, 6 theses have been identified that are recommended to be tested:

- TQ: Product washed with drinking water and packed in air drawers
- TQ O3: The product is washed with ozonated drinking water and packed in air drawers
- Thesis 1: Product washed with drinking water, packed in drawers with a modified atmosphere Mix1 (60% nitrogen 40% carbon dioxide)
- Thesis 2: Product washed with ozonated drinking water, packed in drawers with modified atmosphere Mix1 (60% nitrogen 40% carbon dioxide)

• Thesis 3: Product washed with drinking water, packed in trays with a modified atmosphere Mix2 (60% argon - 40% carbon dioxide)

• Thesis 4: Product washed with ozonated drinking water, packed in drawers with modified Mix2 atmosphere (60% argon - 40% carbon dioxide)

1.4. Overview of potential innovative products

The main goal of all the above achievements is to propose the piloting of potential innovative products that will provide added market value to all sectoral producers of interest. The possibilities of creating an innovative product of the target type of sardine are divided into two categories; frozen and fresh / chilled.

PROPOSAL FOR EXTENSION OF POTENTIAL FROZEN PRODUCTS BY IQF TECHNOLOGY

FC Omega 3 classically uses the IQF system for freezing fish (individually quick-frozen / set method of freezing) the set capacity is 5 tons per hour. In this way, the product freezes individually, where it reaches a temperature of - 18 degrees next to the bone. After freezing, the product takes on a thin layer of glaze to protect the product from drying out, retaining all its natural properties. The whole freezing process from the entry of the raw material into the line to the packaging takes 15 minutes, which is extremely important to avoid the formation of larger ice crystals inside the tissue of the product that significantly affect the quality. It is recommended to expand the range to a more significant framework of cleaned fish and work on packaging in terms of the target market.

<u>1. Frozen cleaned headless sardines</u> 2. Frozen sardine fillets



PROPOSAL FOR POTENTIAL INNOVATIVE REFRIGERATED FRESH SARDIN PRODUCTS PACKED BY MAP TECHNOLOGY IN COMBINATION WITH PRECIOUS GAS

The next step in raising the quality and marketing of fresh fish is to pack the sardines in a controlled atmosphere. The ideal gas mixture for a particular product reduces oxidative processes and stops bacterial growth, but the combination of gases or the mixture in the packaging is extremely important. In meat products, the impact of vacuum and modified atmosphere on the durability of the product is much greater than in fish. Although mixtures of gases containing about 30% oxygen are used for MAP packaging of white fish, crustaceans and shellfish, mixtures of carbon dioxide and inert nitrogen are used in the packaging of blue and other fatty fish to reduce the oxidation of fatty acids in the tissue. Too high a concentration of carbon dioxide causes acidification that causes water to drain from the fish. The proposed categories of new innovative products are:

- 1. Chilled and cleaned sardines packed in a mixture of gases
- 2. Chilled headless sardines packed in a mixture of gases

3. Chilled sardine fillets packed in a mixture of gases

1.5. Conclusion and recommendations for further development

- A change in techniques and technologies for catching small blue fish on board was defined and successfully applied through the innovative use of aquaculture pumps, which significantly increased the quality of the catch and the possibility of extending the lifespan through process changes.
- Creation and testing of an innovative sardine fillet product in MAP in an attempt to extend the life of the product, which allows it to reach distant markets and creates the possibility of expanding the range of tests in the category of noble gas atmosphere
- Created a basis for further research in terms of the possible use of selectors on board during fishing, which would allow the return of live and undamaged fish to the sea to increase the reproductive potential of sardines and anchovies



2. OP ISTRIA CASE

The preparation of raw materials is followed by the preparation of recipes and shaping of the burger into the final mixture and its thermal or cold processing and packaging for final use. For this process, in addition to the definition of the mixture ingredients, its mixing, weighing, there is a significant set of the steps. Some ingredients need to be chopped separately (vegetables and spices, fish, shrimp or mussels). When making burgers from musky octopuses, they need to be blanched before grinding. The ingredients are then mixed and, if necessary, ground again to the desired texture. The ingredients are pressed into the mold of special machines to form the burgers. The production line includes immersion in the mixture, breading, squeezing and packaging, and eventual freezing in relation to the target market. The shelf life of a well-prepared product stored below -18oC is 24 months. Burgers can be shaped using a VER (Forming machine) machine can make different shapes using an interchangeable mold. The pilot will continue in Italy in specialized development laboratories where mechanically separated meat from shrimp and mullet will be sent. The final processing of the semifinished product obtained in Istria goes to the category of complete product, ie the final formation of the product.



Figure 1. Preparation of semi-finished minced shrimp meat and mullet fillets for sending to Italy



A food preparation laboratory will be hired for this purpose. The aim of the pre-industrial study is to verify the shelf life of fish burgers from sent material subjected to HPP treatment- (high pressure). A small amount of lemon fiber can be added to the final product formulation to increase the water retention capacity of the final products. Frozen meat (about 10 kg for each species) will be prepared and sent to a test laboratory, stored at -18 ° C, processed and packaged.

Preparation of samples in possible formulations or adapted after the first tests;

Thesis 1: Burger formulated with shrimp and mullet meat (e.g., 60 - 40%), skin packaging;

Thesis 2: Burger formulated with shrimp and mullet meat (e.g., 60 - 40%) and lemon fiber, skin pack; Thesis 3: Burger formulated with shrimp and mullet meat (e.g., 60 - 40%), skinned and treated with hydrostatic pressure (HPP);

Thesis 4: Burger formulated with shrimp and mullet meat (e.g., 60 - 40%) and lemon fiber, skin packed and treated with hydrostatic pressure (HPP).

For each sample, 20 packs of about 100-150 g will be prepared for a total amount of about 80 packs. For each sample, 10 additional packages will be prepared for any sensory assessment performed by the client company.

Analytical measurements

To assess the shelf life of the obtained products, for microbiological and chemical substances (at least in three copies) will be carried out before and after HPP treatment and during storage:

- total mesophilic microbiological load (TMC);
- Enterobacteria;
- Total coliform load;
- Total pseudoomoni;
- Paint with tristimulus colorimeter;
- pH;
- Texture with texture analyzer, using Kramer test.

The number and typology of analyzes may have some variation as a result of the sampling time duration. The analysis will be performed after day "zero" (before and after HE treatment), 7, 14, 21 and 28 days in a refrigerator (2 - 1°C).



2.1. Burger placement options

The burgers will be intended for sale in supermarkets and the HORECA sector. The products in supermarkets are meant to be prepared at home and the packaging will include two burgers. As the product is of high value, packaging with the longest possible shelf life, vacuumed either in a MAP atmosphere, or frozen is recommended. Because the aspect of healthy food and a clean environment is emphasized, ecological solutions such as packaging in aluminum trays or packaging on a cardboard tray covered with film are recommended for packaging. In this type of packaging, the amount of plastic is reduced by up to 80%, and all material can be separated and recycled.

Figure 2. Fillet packaging in foil and burger offer



For the HORECA sector, burgers will be frozen separately and packed in larger packages, and separated by foil so that they do not stick. In the HORECA sector, burgers are especially suitable for "street food" facilities. "Street food" has been on the rise in Croatia in recent years because it offers fast, but also imaginative meals at affordable prices.

Figure 3. Preparation of burgers in the project promotion under Covid 19 measures





People's eating habits have changed by choosing more smaller meals a day, there is a growing demand for non-meat options, organic products and exotic cuisines and spices. Burger bars offer in addition to beef and chicken burgers, vegetarian and fish burger options. There is a weak supply of delicious ready-made fish burgers on the market, so caterers use tuna steaks in fish burgers.

Figure 4. Burger bar



Figure 5. Innovative forms of packaging





Figure 15. Preparation of pilot burgets after making the mixture - professional staff of chefs



Figure 16. Preparation and tasting of pilot burgers



2.2. Determining a possible approach in declaring

Before a complete product is formed, it is difficult to make an assessment of the declaration, but the basic guidelines should be the following:

Species: Parapenaeus longirostris (Adriatic shrimp)

Product properties: Frozen minced shrimp and mixed fish meat (packed in skin packaging, with welded polyethylene foil) are produced from fresh shrimp caught in the Adriatic and white sea fish mullet, high nutritional quality,

Delivery: From the warehouse - refrigerators of the department for fish processing through wholesale and retail, Horeca



Packaging: The frozen product is packaged in skin packaging. The weight is fixed (200 g / pack). Depending on the distribution channel

Storage and distribution: Products packed in Skin packaging are stored in a warehouse - refrigerators at a temperature of up to + 2 ° C, and at the same temperature is distributed.

How to use: The product is prepared for direct consumption after opening the PE packaging. Each box indicates the shelf life of the product and storage conditions.

Potential beneficiaries: Local population, tourists, customers in the countries of export of products (EU).

It is recommended that the product is in the form of a frozen form so that its long-term storage is possible.

2.3. Recommendations for further system development, food safety, implementation and dissemination

Technical implementation depends on the possibility of making high quality mixes for industrial production of burgers. The use of machining is unquestionable in this case and the innovative application can satisfy the fast processing or speeding, but also filleting. The same means quickly disposing of high seasonal catches. A pilot innovation in the use of a shrimp meat separation machine and its adaptation indicated possible significant improvements in quality improvement, the creation of new products to speed up the process, and easier and less strenuous work. In the sale, the additional cost is represented by plastic trays and their fixing with PE foil or any other packaging. A special segment in the work of the cooperative will be the packaging and design of new products, which can significantly affect the cost price. The requirements of potential customers are becoming more complex and demanding in terms of seeking smaller packages (which increases the cost of the process and multiplies the cost of packaging) but also the application of new technologies for making goods.

Preliminary agreements with packaging suppliers will be constantly upgraded in order to create special products for FC Istria (by pressing the logo of special design and / or veterinary control number) on the packaging itself, which will reduce handling costs and ultimately make products more acceptable and more competitive on the market. In accordance with more innovative packaging solutions, the icing of products will be adjusted by introducing new methods of thermal preservation of a particularly fresh range. In the context of the above and the HACCP system, special attention will be paid to packaging that must be exclusively certified for food packaging, but also the application of new technologies, the most important of which is the so-called controlled atmosphere especially for fresh and dried products.



In order for a product to have a long life on the market and be constantly recognizable, it is necessary to pursue food safety management policies. The food safety management policy is to operate in a given period with the application of modern technology with an organization and staff that will be able to ensure a quality and safe product and meet customer needs, which should result in continuous production growth and control of production costs. It is necessary to ensure the procurement of high quality raw materials from own catches and by selecting quality suppliers from which raw materials will be selected and purchased only according to quality criteria. Business policy is continuous automation and modernization by investing in new production processes and improving existing ones.

Food safety management policy is the timely delivery of quality and healthy products, and achieving satisfaction not only with our own customers, but also other stakeholders: owners, employees, suppliers and the community in which we operate to achieve satisfaction not only of our customers but also other stakeholders : owners, employees, suppliers and the community in which we operate. The products that are produced are recognized as a dedicated manufacturer of safe and healthy food. The food safety management policy is that all employees have an obligation: to meet the requirements and needs of customers and all other users for safe and quality food, to comply with laws and regulations, to act in accordance with assigned responsibilities, to constantly monitor and improve the effectiveness of their procedures and business processes.

The main goals are:

- Preservation of the Adriatic within the framework of sustainable and responsible commercial fishing, as a guarantee of the quality of the breeding process

- Minimal use of chemical agents to treat catches
- High quality input raw materials from verified suppliers for catches from other vessels

- Educating employees in terms of developing awareness of the importance of producing high quality products

- Developing awareness of the need to implement hygienic measures in order to obtain healthy products

- Develop products with maximum use of natural ingredients and minimum use of preservatives

- Constantly work on the development of new products, especially those in the group of delicacies

- Presenting the product to customers in a way that is acceptable to them, while presenting all the positive features

- Use new technologies and knowledge that can increase the quality of products
- Accept customer comments and incorporate positive ones into the business system
- Conduct continuous education of all employees



2.4. Demonstrations of innovative packaging

Packaging is a barrier between the product and its environment. Packaging prevents contact of the product with bacteria and other contaminants, with oxygen, can protect against moisture, heat or mechanical damage. It also prevents liquids, odors, grease and other substances from escaping from the packaging. The first packaging materials were paper, glass and metal, but today plastic materials are mostly used due to low cost, flexibility, mechanical resistance, light weight, water resistance and various possibilities of use. Certain types of plastic materials are resistant to freezing or high temperatures, so they can also be used in microwave ovens. Recently, more and more attention is being paid to packaging that is environmentally friendly, made of natural materials, biodegradable or recyclable. When calculating environmental acceptability, a number of factors should be taken into account, for example raw material, its origin and distance, biodegradability, recyclability, CO2 footprint in packaging production, packaging weight in case of product distribution over long distances. When choosing packaging, it is necessary to determine which type of protection is most needed - protection from moisture, heat, oxygen, light and so on.

Gas barrier

When using a modified atmosphere in the packaging, a barrier that is resistant to the passage of carbon dioxide, oxygen and nitrogen is used. Such barriers often consist of multilayer laminates containing ethylene / vinyl alcohol (EVAL). Polyvinyl alcohol (PVAL) is also used as a barrier to oxygen and odors.

Water barrier

Water tightness is important for fish packaging because the quality of the fish is significantly reduced by dehydration, in addition, the liquid from the fish can drain from the packaging, and condensation in the refrigerator can also wet the packaging. Various plastics are good barriers to moisture passage, such as PVDC, polypropylene (PP-O) and high density polyethylene (PE-HD). Other materials (cardboard, paper, styrofoam) can be laminated with plastic to make them waterproof.

Thermal deterioration

Many types of plastic materials have the property of dissolving when exposed to heat so that the edges of the package can be joined by welding and closed so that the inside of the package does not come into contact with the surrounding air or contamination.



Mechanical protection

Fresh fish and fish products are soft in structure and can be easily damaged or crushed. Therefore, hard trays and polystyrene or high density polyethylene substrates are often used in packaging. The trays are either deep enough that the foil covering them does not touch the product or the foil is made of a material that is resistant to mechanical damage (polyamide).

Transparency

It is important for fresh products to be visible to customers, so at least part of the packaging must be transparent. PET and PP films have excellent optical clarity. Anti-fogging agents are sometimes added to prevent condensation from forming on the foil.

Liquid absorbers

Fresh fish packed in a modified atmosphere is drained of a liquid in which bacteria can multiply. For this reason, cellulose pads are placed in the packaging, which can also be integrated into PS-E trays.

Thermal insulation

Styrofoam boxes and trays also serve as thermal insulation during the transport of packaged products.

Light insulation

Products stored frozen in supermarkets are exposed to light in refrigerators, and light stimulates and catalyzes oxidation. For this reason, pigment is added to plastic films.

Vacuum packaging

Materials with very low gas permeability (<15 cm3 / m2 per day) are used for vacuum packaging.



MAP packaging

The next step in raising quality is to control the atmosphere because a reduced amount of oxygen reduces oxidative processes and bacterial growth. In meat products, the influence of vacuum and modified atmosphere on the durability of the product is much greater than in fish, because the spoilage of meat is influenced by gram-negative bacteria such as Pseudomonas.

Although mixtures of gases containing about 30% oxygen are used for MAP packaging of white fish, crustaceans and shellfish, mixtures of carbon dioxide and inert nitrogen are used in the packaging of blue and other fatty fish to reduce the oxidation of fatty acids in the tissue. Too high concentration of carbon dioxide causes acidification which causes water to drain from the fish.

Özogul et al. (2004) experimentally proved that "shelf life" of sardines is 12 days if it is packaged in a MAP atmosphere with a ratio of 60% CO2: 40% N2 at 2 ° C, and 9 days in a vacuum package, while in a normal atmosphere its duration is only 3 days. They measured several parameters including the amount of histamine, the number of bacteria, the concentration of trimethylamine (TMA) and total volatile nitrogen (TVB-N)

Active packaging

Innovative active packaging creates special conditions within the packaging to extend the shelf life of the product.

Active packages can have:

• System for absorbing unwanted substances such as oxygen, carbon dioxide, ethylene, excess water, paint or other substances

- System for adding desirable substances such as carbon dioxide, antioxidants and preservatives
- Additional systems such as cooling



Type of		Application	
substance			
Decreasing	Chemical compounds	Fresh and dried fish,	
oxygen	(iron oxide powder, iron	sausages, dried fish	
substances	carbonate, iron sulfite,		
	ascorbic acid, etc.)		
	Enzyme systems (glucose		
	oxidase, alcohol oxidase)		
CO2	Ascorbic acid, iron	Fresh fish and clams	
emitting	carbonate and metal		
systems	halides		
Moisture	Silica gel, propylene	Fresh and dried fish	
absorption	glycol, polyvinyl alcohol,		
systems	diatomaceous soil		
Ethanol	Ethanol in capsules	Freash and semi-dried fish	
emissions		products	
Release of	Sorbates, benzoates,	Fish and other processed	
antibacterial	propionates, ethanol,	products	
substances	ozone, peroxide, sulfur		
	dioxide, antibiotics, silver		
	zeolite, ammonia salts		

Mohan and asociates have found that the addition of an oxygen scavenger (O2 scavenger) extended the shelf life of fresh blue fish from 12 to 20 days and reduced the production of histamine, putrecin and cadaverine (source: Mohan, CO & Ravishankar, C. & Gopal, T. & Kumar, Ashok & Lalitha, K ... (2009). Biogenic amines formation in seer fish (Scomberomorus commerson) steaks packed with O2 scavenger during chilled storage. Food Research International. 42. 411-416.) There are foils on the market made of oxygen-impermeable materials and are used for packaging instead of metal foils that were once in use. They have the added advantage of being transparent and safe to use in the microwave.

Inteligent packaging

Intelligent packaging is one that can provide information about the condition of packaged food and its quality during transport and storage. Today, there are various indicators on the market that can collect and provide various information on the principle of mechanical, chemical or enzymatic processes. Indicators that measure time and temperature are important for maintaining a cold chain. These are usually external indicators.



TTI (Time Temperature Integrator) remembers and records the total time when the temperature was higher than a certain number of degrees. Thus, indicators for 3 or 5oC in the form of labels can be purchased. If the product has been at a temperature lower than specified at all times, there is no colored indicator. If the specified temperature was higher for a period of 2 hours, staining occurs in the first mark, and after 4 hours in the next. The indicator is accurate and irreversible and allows the customer to assess the risk of the product. Indicators indicating the amount of oxygen or carbon dioxide in the package are located inside the package and may indicate a possible rupture of the package by changing its color. Microbial indicators work on the principle of changing pH or reacting with certain metabolites. Indicators for pathogens, e.g., E. coli can react on the principle of immunochemical methods.

Packaging with antimicrobial properties

Some of the systems for reducing the number of microorganisms in packaging are:

- Addition of organic acids benzoates, parabens, sorbates and propionates
- Addition of enzymes lysosin, nisin, glucose oxidase
- Fungicides and bacteriocins
- Polymers chitosan and nylon
- Natural spices mint, oregano, cumin, clove
- Other silver, zeolite, antibiotics

Wang and associates have tested an edible surface coating of collagen and lysosin on salmon fillets and found that it significantly improved the durability and quality of fresh fillets without unwanted color or pH changes compared to regular fillets (source: Wang, Zhe & Hu, Shuaifeng & Gao, Yupeng & Ye, Chen & Wang, Huaiyu. (2017). Effect of collagen-lysozyme coating on fresh-salmon fillets preservation. LWT - Food Science and Technology. 75.). Collagen is a protein that builds connective tissue in animals, while lysosin is an enzyme with antimicrobial properties that has a particular effect on gram-positive bacteria. The authors investigated the effect of different combinations of concentrations of these two substances on the durability of salmon fillets. Given the above wide range of products, it is necessary to follow those that already have defined packaging on the example of the meat industry.



3.OP BIVALVIA CASE

3.1. Prototypes of new fishing gears used in piloting

Four fishing vessels were involved in this piloting action, all part of the Clam Management Consortium (Co.Ge.Vo.) of Venice, on which was tested the fishing gear and the sorting sieves.

Table: List of involved fishing vessels and used fishing gears and sorting sieves in the piloting action.

Name	Identification number	Fishing gear type	Sorting sieve type
Nani Gata	3VE946	Standard	Standard
Nicoletta	3VE780	Experimental	Experimental
Giove	3VE777	Experimental	Experimental
Freccia Azzurra III	3VE955	Experimental	Experimental



Figures: The standard fishing gear on board the vessel Nani Gata.



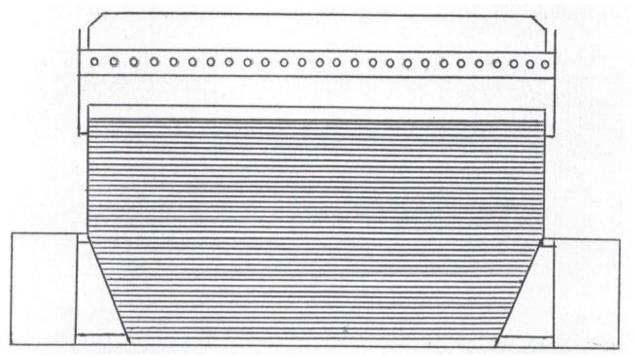


Figure: Fishing gear N. 1 on fishing vessel Nicoletta



Figures: Detail of the new fishing gear on board the fishing vessel Nicoletta.



The second fishing gear is installed on board the fishing vessels Giove (3VE777). This gear measures $2,50 \text{ m} \times 1,50 \text{ m} \times 0,35 \text{ m}$ and uses steel rods spaced 11 mm. This gear has an alternating configuration of steel rods, both horizontally and vertically, where the vertical sections should improve the escapement of undersized specimen. Moreover, the row of nozzles is put at angle towards inside to reduce the pressure of water jets on the benthic macrofauna.

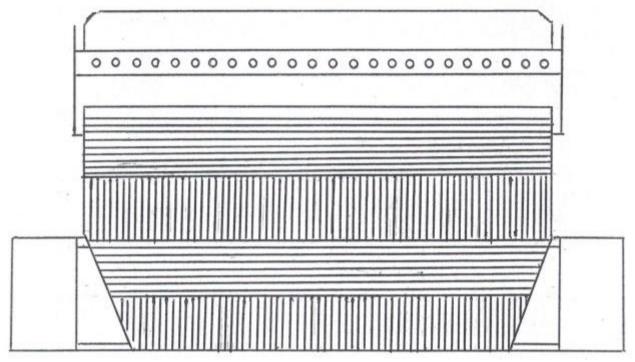


Figure: Fishing gear N. 2 on fishing vessel Giove





Figures: Detail of the new fishing gear N. 2 on board fishing vessel Giove.

The third fishing gear was installed on board the fishing vessel Freccia Azzurra III (3VE955). The fishing gear measures 2,50 m x 1,50 m x 0,35 m and the design is a sieve consisting of horizontal bars spaced 11 mm gives the basic design. The innovation consists in water jet nozzles placed in multiple rows and different angles.



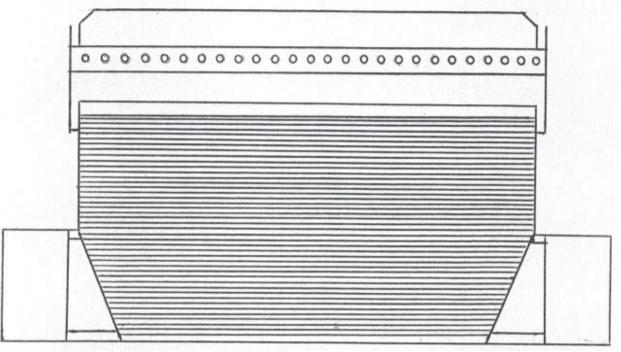


Figure: Fishing gear N. 3 on fishing vessel Freccia Azzura III.



Figures: Detail of the new fishing gear N. 3 on board Freccia Azzura III.



3.2. Trials on sea of the fishing gears

The piloting activities for testing the fishing gears and selecting lines were conducted in three different periods. The primary analysis during on sea trials was the evaluation of the fishing properties of the innovative gears and the selectivity of the target specie. Such trials evaluated the catches of the stripped venus (*Chamelea gallina*) and the length frequency distributions, allowing to evaluate the contribution of undersized and commercial size specimens in the catch. The trials were conducted in natural habitats of the stripped venus and where this specie is the dominant specie in the macrobenthic community, as in the regular fishing grounds. There were 21 identified taxa in the catch samples, mainly molluscs: 10 mollusc bivalves and 6 gastropod molluscs. The analysis of the catch composition during the trials indicates that the target specie, the stripped venus, makes roughly 90% of the overall catches, and the rest of the macro-benthos is composed by other bivalves (5,08%), gasteropods (3,19%) and cruastaceans (2,44%) and sipunculids and polychaetes (Figure 1)

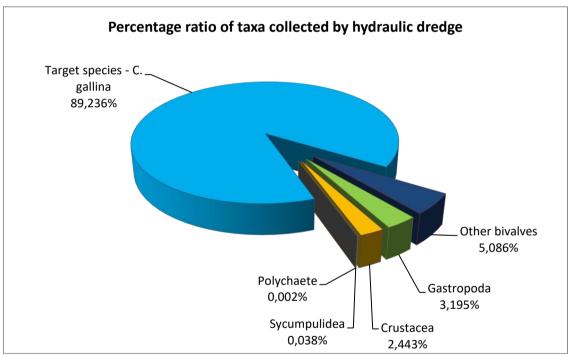


Figure 1: Composition of taxa in the collected samples by hydraulic dredging.



A representation of catch samples based on economic value indicates that the majority of the catches consist of commercial species, where the stripped venus composed 89,2%, other commercial species 5.3% (*Acanthocardia tuberculata, Donax semistriatus, Paphia aurea, Bolinus brandaris, Nassarius mutabilis, Sipunculus nudus*) and 5.5 % by other non-commercial species (Figure 2).

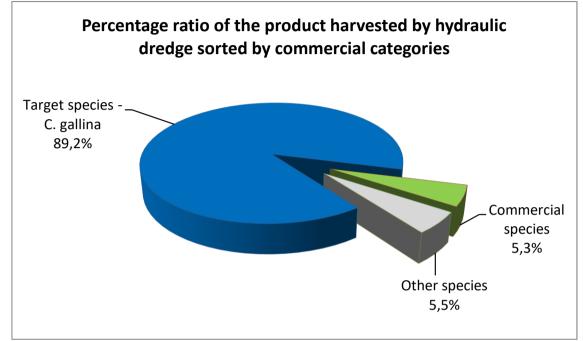


Figure 2: Composition of catches based on economic values.

3.3. Fishing gear

The first evaluation of the catches allowed to observe that caught quantities between the standard fishing gear and the three new ones were comparable (Figure 3). The standard gear used on board the fishing vessel Nani Gata had higher catches in one of the four trials, on the second scheduled test, but in other trials had lower yields than other gears. Among the new fishing gears, the one on board the vessel Giove had in average the highest catches, being the most efficient in 3 out of 4 trials. The second most efficient gear was the one on installed on board the vessel Nicoletta, while the third new gear on board the vessel Freccia Azzura III seems to have the lowest catches among the three new gears. However, it is remarkable that all the new fishing gears were able to catch similar to the old fishing gear, and that could be explained by the ability of the fishermen setting up the fishing gears in a very short time, making them similarly efficient as the old fishing gears. Further trials were also scheduled for improving the setup and the operational protocol for the new fishing gears.



The fishermen had mixed feelings about their new fishing gears. Some are willing to adopt them even after the on-sea piloting and testing, others are more rooted to their traditions and prefer the old standard fishing gear, and there are ones positive to continue the development and setup of the new fishing gear.

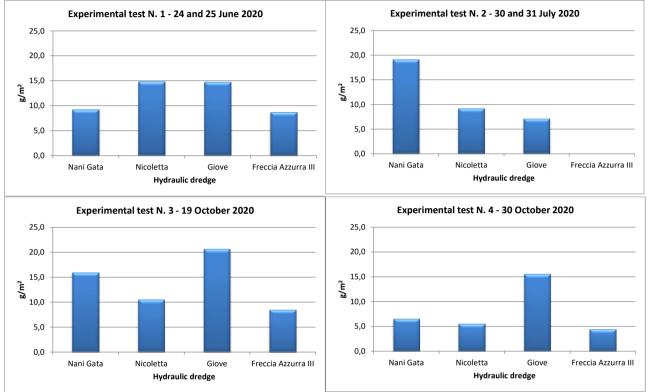


Figure 3.Catches of different fishing vessels and fishing gears observed during on sea trials. The old fishing gear is on board the vessel Nani Gata, while the three new gears are on board vessels Nicoletta, Giove and Freccia Azzura III.

All the fishing gears were evaluated for their selectivity for the target specie (stripped venus, *C. gallina*) and the quantity of by-catch caught. Samples were taken directly from the metal basket in the bow, where the catch of the fishing gear is unloaded and further sent through the sorting line, usually consisting of two sorting machines (one cochlear, and one with vibrating screens). The comparison of the old with the three new fishing gears indicates there a reduction in macrobenthos bycatch from 15,9% to 7,9%-12,6% (Figure 4). On the other hand, the selectivity for commercial sizes of the clams varies between the different gears (Figure 4). The highest selectivity for commercial sized clams is observed for the fishing gear installed on board the fishing vessel Nicoletta, having comparable ratio as the old gear (Figure 4). Larger proportions of undersized specimen are observed in the other two fishing gears on board fishing vessels Giove and Freccia Azzura III (respectively 63,7% and 67,5%), while having the commercial size specimen around 25 % (Figure 4).



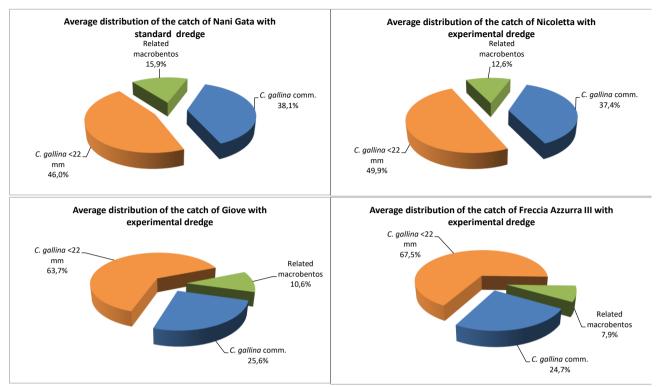


Figure 4. Catch proportions of different stripped venus (*C. gallina*) categories and macrobenthos. The old fishing gear is on board the vessel Nani Gata, while the three new gears are on board vessels Nicoletta, Giove and Freccia Azzura III.

The population structure of the stripped venus caugth with the four fishing gears shows different length contributions (Figure 5). The commercial size (above 22 mm) is most abundant in the catches of the old fishing gear (25,7%) and decreasing in the new fishing gears (from 18,5% to 9,3%). The fishing gear used on board vessel Nicoletta has the most similar ratios as the old fishing gear and the highest proportion of commercial sized specimens (Figure 5). In all the new fishing gears the proportion of specimen under 22 mm is larger than in the old fishing gear, where the gear on board Giove has the smaller specimens and the gear on board Freccia Azzurra III has the largest proportion of specimen 16 and 19 mm (Figure 5).



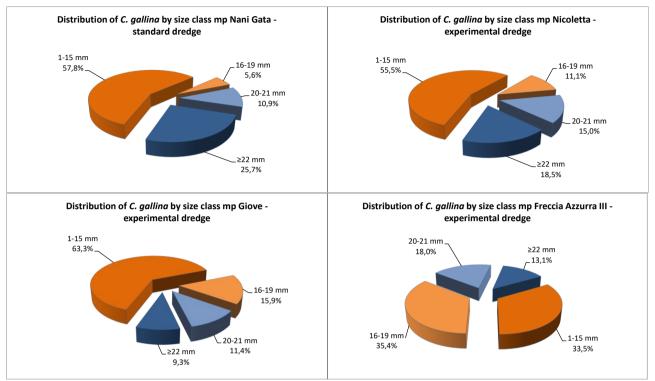


Figure 5. Distribution by sizes of the stripped venus (*C. gallina*). The old fishing gear is on board the vessel Nani Gata, while the three new gears are on board vessels Nicoletta, Giove and Freccia Azzura III.

3.4. Trials on sea of the sorting machine

A selectivity of the traditional standard sorting machine was compared with the new sorting machine on board each of the fishing vessels. During the trials on 30-31 July the fishing vessel Freccia Azzura III was unable to sort the catches due to a malfunction of the sorting machine causes by a defect during its construction. The sorting machine was later replaced with a working one.

The comparison on board the vessel Nicoletta indicates that the new sorting sieves ha lower selectivity for commercial sized specimen (89,9 %) than the traditional sieves (96,0%) (Figure 6). On the other hand, the new sorting machine on board fishing vessel Giove had a comparable selectivity as the traditional sorting sieves (Figure 7).



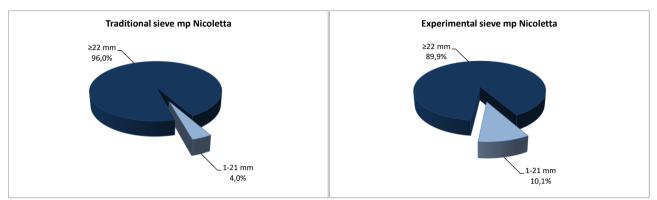


Figure 6. Proportion of specimens retained after sorting on the traditional sieve (left) and the new sieve (right) on board vessel Nicoletta.

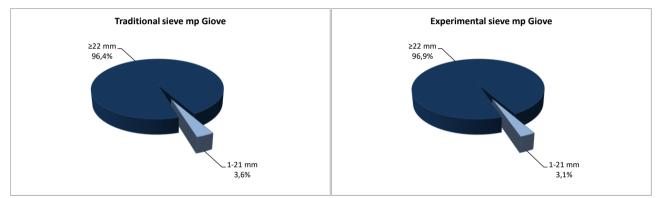


Figure 7. Proportion of specimens retained after sorting on the traditional sieve (left) and the new sieve (right) on board vessel Giove.

3.5. Evaluation of turbidity during fishing operations

The impact of the hydraulic dredging with the new fishing gears on the benthic environment was evaluated visually using a mini underwater remotely operated vehicle (ROV; FIFISH V6 by QYSEA) and the physical-chemical properties using an oceanographic multi-parameter probe (Hydrolab HL7; temperature, salinity, PH, dissolved O2, chlorophyll-a and turbidity). In particular, the impact of the resuspended sediments on the environment, created by fishing gear due to the high-pressure water jets evaluated by measuring the turbidity. The physical-chemical properties of the water column were measured before any activity, as a control situation. The turbidity was measured before, during and after the fishing operations, at sampling intervals between 30 seconds and 5 minutes.



During the monitoring on 24th June 2020 the weather situation on sea was not suitable since the wind influenced the turbidity in the water column. The control value of turbidity measured 150 NTU. The different hydraulic dredges had different maximum levels, being the highest for the fishing vessel Giove (400 NTU, Figure 8), followed by Nani Gata (300 NTU, Figure 9) and Nicoletta (180 NTU, Figure 8). The residual turbidity 5 minutes after the fishing operation was evaluated for the fishing vessel Nani Gata, showing turbidity getting back to normal values (Figure 9).

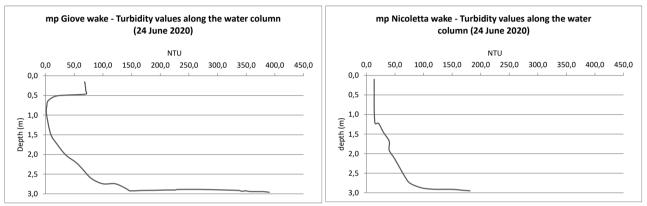


Figure 8. Turbidity wake created during fishing activities of fishing vessels Giove (left) and Nicoletta (right).

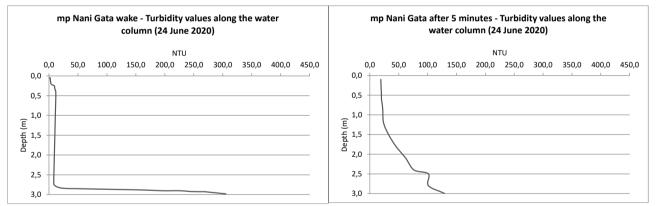
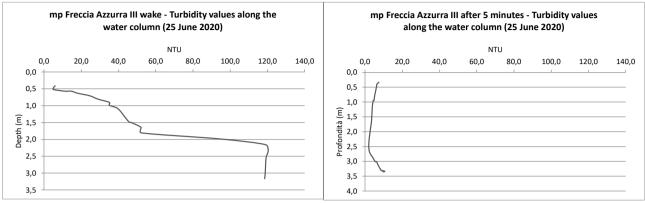
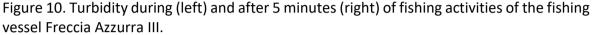


Figure 9. Turbidity during (left) and after 5 minutes (right) of fishing activities of the fishing vessel Nani Gata.

On the 25th June 2020 the evaluation of habitat perturbation was measured for the fishing vessel Freccia Azzurra III, with low values of initial turbidity (20 NTU). During the fishing operations the turbidity was 120 NTU and after 5 minutes the turbidity was again at its normal values (Figure 10).







On 30th June 2020 was carried the testing of the fishing gear on board the vessel Giove, and there was very low turbidity in the water (0,1 - 5,5 NTU). During fishing activities, the turbidity rose to 230 NTU and within 2 minutes the sediments settled bringing back the turbidity to normal values (Figure 11).

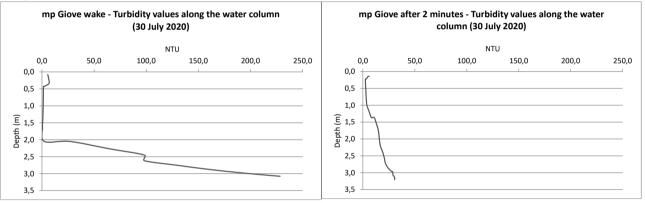


Figure 11. Turbidity during (left) and after 2 minutes (right) of fishing activities of the fishing vessel Giove.

The turbidity trend in time, without and during the fishing activity, highlights the turbidity created by the fishing gear settled within 30-40 seconds (Figure 12)



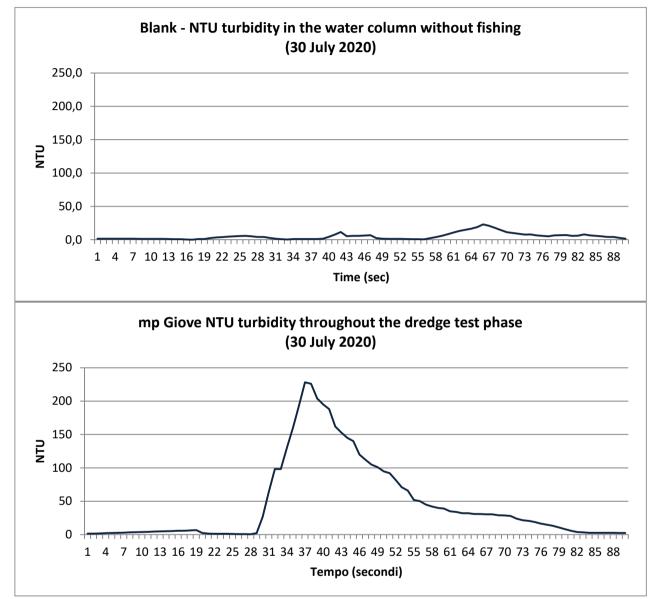


Figure 12. Turbidity of the water measured in time, without (top) and with (bottom) fishing activity of the fishing vessel Giove.



On 19th October 2020 was conducted the testing of three new fishing gears, and turbidity levels were measured during the fishing activities and 30 seconds after. The maximum turbidity of the water measured 2,7 NTU.

The fishing activity of the fishing vessel Freccia azzurra III increased the turbidity to 112 NTU and the cloud was 1,5 meters higher than the seabed (Figure 13). The suspended matter settled quickly and after 30 seconds it measured 4 NTU (Figure 14). The fishing gear on board the fishing vessel Giove produced a lower turbidity (42,2 NTU, Figure 15), that settled 30 seconds after (Figure 16).

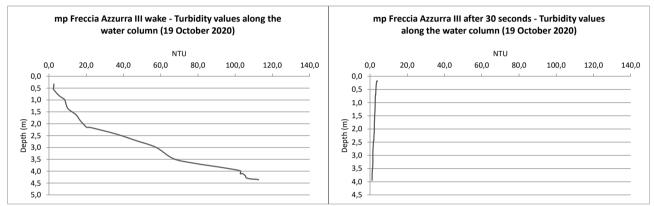
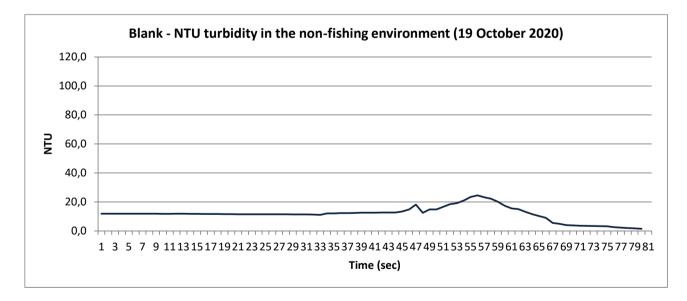
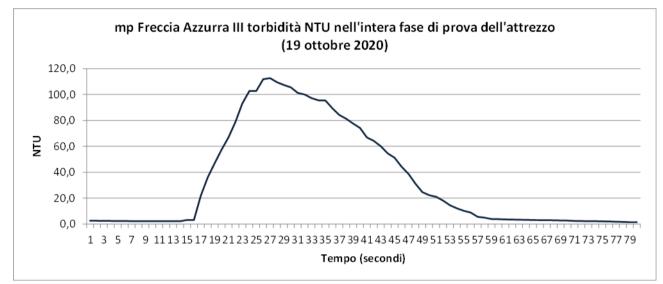
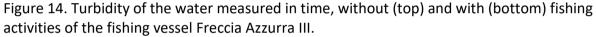


Figure 13. Turbidity during (left) and after 30 seconds (right) of fishing activity of the fishing vessel Freccia Azzurra III.









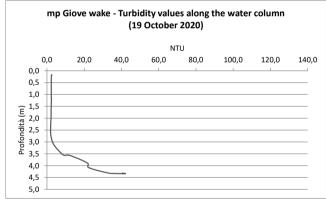


Figure 15. Turbidity during fishing activities of the fishing vessel Giove.



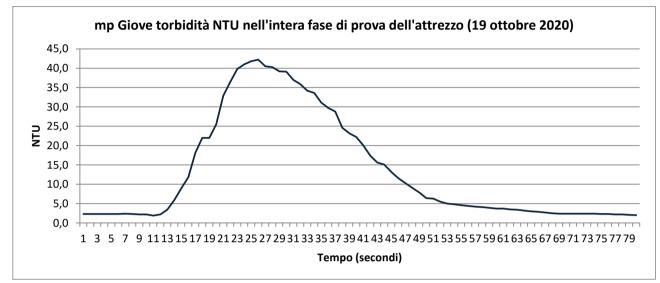


Figure 16. Turbidity of the water measured in time during the fishing activity of the fishing vessel Giove.

3.6. Visual inspection of the seabed before and after fishing

A visual inspection of the seabed using an ROV was carries other that observations made with scientific instruments to assess the status before and after fishing. The activity of dredging left few clams unburrowed (Figure 17), while the specimen of stripped venus discarded to the sea seemed have regular vitality and the ability to burrow again and resume the benthic life (Figure 18).





Figure 17. Visual inspection of the seabed after the dredging for clams.







3.7. Evaluation of physiological responses to different fishing gears

The influence of the fishing gear and the induced stress level on the clams was assessed by measuring physiological responses in laboratory. Visual and smell inspections were carried by preserving the clams in the tray inside a fridge on the temperature of 6°C. The visual inspection indicated that the colour of the clams was regular in the first 4 days for all fishing gear, and later showing signs of alteration. For all fishing gears, the smell resulted regular for the first 3 days, at the fourth day showed first signs of alteration and on the sixth day had a smell of decaying flesh. The analysis of psycrophyll bacteria, indicators of the vitality level of the organisms, suggest that the fishing gear of Freccia Azzurra III had the lowest bacteria count in the first three days, and later having higher values as the gear from Nicoletta (Figure 19). The traditional fishing gear on board Nani Gata and the new fishing gear on board the vessel Giove had the lowest weight loss, i.e., the integrity of the organism, the new fishing gear on board the vessel Giove had the lowest weight loss (Figure 20). Among the other three gears, the traditional gear on board Nani Gata seems to have caused less weigth loss than the new fishing gears on board Nicoletta and Freccia Azzurra III (Figure 20).

