Deliverable Number D5.3.2 Protocol for diversification and labeling for more sustainable aquaculture practices

Project acronym: ARGOS
Project ID number: 10255153
Project title: ShARed GOvernance of Sustainable fisheries and aquaculture activities as leverage to protect marine resources in the Adriatic Sea
Priority Axis: Environment and cultural heritage
Specific Objective: 3.2 - Contribute to protect and restore biodiversity
Work Package Number: WP5
Work Package Title: Sectorial know-how development and pilot project implementation
Activity Number: 5.3
Activity Title: WP5 "Improvement of aquaculture operators' behaviours"
Partner in charge: Marche Region PP3 with the scientific support of DiSVA – UNIVPM and technical support of COOP M.A.R.E. (external expertise)
URL: https://www.italy-croatia.eu/web/ARGOS
Status: Final - version n. 1
Distribution: Public
Date: June 2023

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Background and goals the ARGOS "Protocol for the diversification and labelling for more sustainable aquaculture practices"

Under the "WP5 Sectorial know-how development and pilot project implementation", the ARGOS project foresees the implementation of pilot actions at regional level aimed at improving the sustainable behaviours of fisheries and aquaculture operators. To this goal, the development of cross-border "Protocols" to support the adoption of sustainable materials and methods along the supply chains of these two key sectors for the Adriatic blue economy.

Specifically, the Activity 5.3 focuses on the testing and adoption of common sustainable behaviours to maintain aquaculture activities in a balanced relation with environmental needs and Marche Region (PP3) is responsible to develop a "Protocol for the diversification and labelling for more sustainable aquaculture practices". A first draft of the Protocol has been prepared by Marche Region in close cooperation with the Department of Environmental and Life Sciences of the Polytechnic University of Marche which provides scientific advice and support to PP3 in pilot actions implementation. The document takes its grounds from the consultation process carried out from 2021 to 2022 at regional level on the aquaculture key actors: operators, universities and research centers, policy makers, NGOs. Based on the inputs from the regional consultation, the Protocol have been structured in a set of pilot actions aimed at:

- *A.1 reducing the use of plastic in the farming process by introducing alternative and biodegradable materials;*
- *A.2 improving the sustainability of oysters' farms by means of natural valorisation and sustainable practices application along the supply chain;*
- *A.3 reducing the use of plastic in the molluscs' commercialization;*
- *A.4 promoting the product diversification;*

The implementation of the pilot actions A.1, A.2, A.3 have been carried out in the period February 2023 – May 2023 by the external expertise hired for the purpose (M.A.R.E. soc. Coop) and the A.4 by DiSVA- UNIVPM in cooperation with AMAP – Agency for innovation in the agri-food and fisheries sectors of Marche was conducted in July 2022. The pilot actions results enabled the validation of the Protocol and the delivery of a final version for transnational use.

The Protocol target group

The Protocol targets the following regional and transnational stakeholders:

> Aquaculture operators at regional and transnational level;
> Consumers;
> Buyers;
> Policy makers;
> Students
> Researchers

The mentioned target group was reached during demonstrative events arranged by Marche Region with the technical support of DiSVA – UNIVPM and M.A.R.E. soc. coop during the pilot actions implementation. Namely, the following awareness and knowledge transfer initiatives were organized:

- Demonstrative initiatives targeting other aquaculture operators to share and transfer the novel methodologies and equipment used in mussels farming and oysters' refinement as well as in molluscs packaging (held in Senigallia and Porto San Giorgio in May 2023);

- Business to Business event to share and discuss with potential buyers (restaurants, caterers, retailers) the added value of eco-compatible materials and practices towards consumers;

- Storytelling show-cooking to raise consumers awareness on the environmental contribution of mussels farming (held at “Tipicità in Blu”exhibition in May 2023, Ancona)

- ARGOS project thematic events such as the High Level Conference on aquaculture (Zara, April 2023), the Adriatic Advisory Committee meeting (June 2023) and the project Final Conference (Dubrovnik, June 2023) targeting regional, national and local administrations, scientists and technicians, enterprises and operators’ associations from the Adriatic area.

The overall goal of the pilot action is to provide alternative solutions to the use of plastics in mussel farming

Material and methods

Tubing

On 17 February 2023, as planned, using the same batch of seed, 40 fringed rope and 40 traditional socks were tubing, which were then hung on the same long line. To tube the fringed rope, an image of which is reprinted in Figure 1, a special Spanish-made re-tubing machine of the type shown in Figure 2 and Figure 3 was used.

Figure 1 – Fringed rope ready for tubing

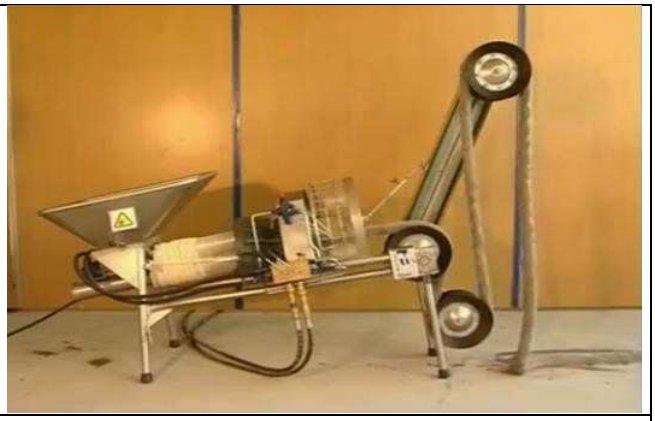


Figure 2 – Re-tubing machine

Figure 3 – Re-tubing machine and mussel-loaded-rope

Figure 4 – Tubing traditional sock

The length and individual weight of socks and ropes were measured, and a sample of the grafted mussels was then taken for biometric measurements. At the end socks and rope were hung on the same section of the long-line.

On 06-03-2023, one socks and one rope was taken on which biometric measurements were carried out. The time between tubing and the sampling date served to 'stabilize' the state of the mussels after the process of de-clumping, sorting and tubing.

Subsequent samples were taken on 17 March, 19 April and 15 May, the latter about three months after being released into the sea. In all cases, five ropes and five socks were also measured and weighed.

In the laboratory, biometric measurements were carried out by dividing the mussels into size classes of 1 mm, and the meat content was measured by cooking 2 kg of cleaned mussels for 5 minutes. Then, for socks and rope, on a population of 40 individuals found at random, the following parameters were measured to define the condition index: shell height; total weight; wet weight of the meat, after dripping on straw paper for 15'; dry weight of the meat, after standing in an oven at 105°C for 24 h; dry weight of the shells, after standing in an oven at 105°C for 24 h.

Mussels de-clumping

The mussel de-clumping trials took place on board a vessel equipped with a processing system consisting of two de-clamping machines arranged in series and a sieve shaker equipped with several grids.

The traditional socks were fed into the ginning machines by inserting sections of about 40-50 cm in length into the first of the two machines, where the plastic net was broken and the product was first degreased. Subsequently, using a conveyor belt, the mussels, still partially aggregated, were put into the second machine, which separated them from each other and from the fouling. By means of conveyor belts, the mussels obtained were sent to the sorter, where they were sorted by size, and where the waste product, consisting of fouling, byssus, broken mussels, etc., was collected.

The separation of the organisms adhering to the ropes was carried out manually, by passing the rope through a metal ring. The product obtained was then placed inside the first de-clumping machine, performing a process similar to that adopted for traditional socks.

It should be noted that while the de-clumping process for traditional socks is now well established and has been adopted by farmers for some time, for ropes, on the other hand, there is currently no equipment available to mechanize the detachment of the mussel coat and the manual operation is subject to greater variability in operating methods.

At the end of the operations, data were collected on the weights of the individual components and mussel samples were taken for subsequent laboratory measurements.

Shelf life

Shelf life was measured by observing the mortality trends of two batches, each consisting of 100 mussels, kept in a refrigerator at a temperature of 5°C ± 2 °C, with measurements taken approximately every 12 hours. Mussels with open valves and not reactive to stimuli were considered dead.

All data collected during the surveys were reported on special paper forms and then recorded on computer.

Enterprises involvement

This action took place with the cooperation of the shellfish farm Solomar Ltd, site in Senigallia (AN).

Results and recommendations

Characteristics of mussel ropes and traditional socks

On February 17, 2023, 40 traditional socks, with an average length of 3.01 m and an average weight of 21.16 kg, and 40 fringed rope strands, with an average length of 2.73 cm and an average weight of 9.10 kg were tubed. The traditional socks had a number of about 1021 mussels per meter, while the rope contained an average number of about 466 mussels per meter, equal to about 46% of those present in the traditional socks.

On the following dates, 17 March, 19 April and 15 May, the weight of five units for each type was recorded. The average length and weight values and the relative standard deviations are shown in Table 1, where the average weight per unit of length is also highlighted.

Table 1 – Average length and average weight and relative SD of traditional sock and fringed ropes

Sampling date	Traditional sock					Fringed rope				
	length		weight		Kg/m	length		weight		Kg/m
	average	DS	average	DS		average	DS	average	DS	
02/17/2023	3.01	0.269	21.16	1.94	7.02	2.73	0.218	9.10	0.896	3.33
03/17/2023	3.76	0.321	25.60	1.52	6.81	2.83	0.067	13.20	0.758	4.66
04/19/2023	4.07	0.406	35.00	2.92	8.60	2.92	0.115	18.20	2.049	6.23
05/15/2023	3.90	0.480	41.00	4.47	10.51	2.88	0.08	23.60	1.140	8.19

A significant data is the behaviour of the weight-per-unit-length parameter, which in the case of the traditional sock goes from 7 Kg/m to 10.51 Kg/, an increase of about 50%. Whereas for fringed rope, there is an increase of about 146%, from 3.3 Kg/m to 8.19 Kg/m.

Growth

At the time of the tubing of the traditional socks and fringed ropes, samples of mussels were taken, on which biometric measurements were carried out. Table 2 shows the values for the average size and, in Figure 5, the respective graphical representation. According to the available data, a slightly higher growth is observed for the traditional socks, with differences of 1-2 mm.

Table 2 – Average size of mussels at first sample and in subsequent samplings

	Sampling date - Average size (mm)				
	02/17/2023	06/03/2023	03/17/2023	04/19/2023	05/15/2023
Traditional sock	45.9	43.4	44.4	49.2	51.1
Fringed rope		44.6	43.9	47.0	50.3

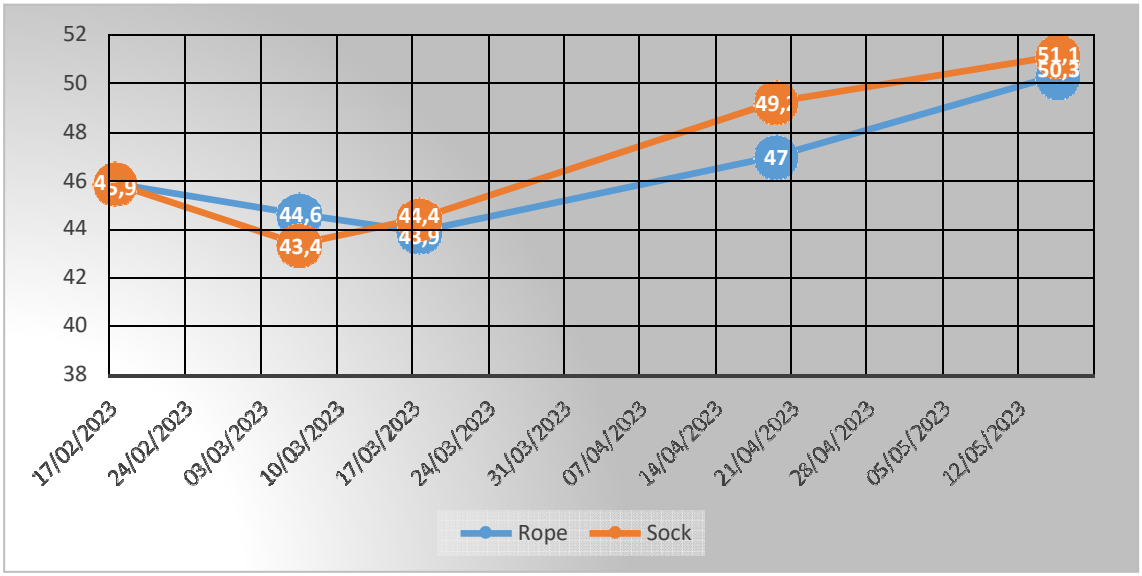


Figure 5 – Trend of the average size of the mussels

The following figures instead show the size distribution of the mussels at the first socked, Figure 6.

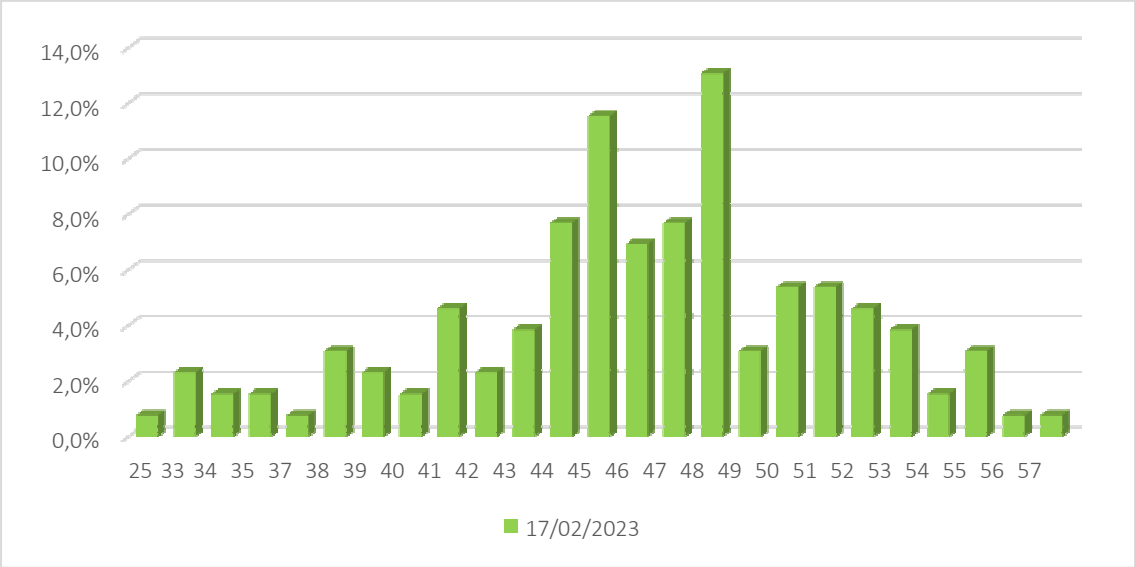


Figure 6 – Size distribution of the mussels at first socked

Meat content

Figure 7 shows the trend in meat content at the sampling dates, where it can be seen that, with the exception of the sample taken on 19 April, the values appear similar.

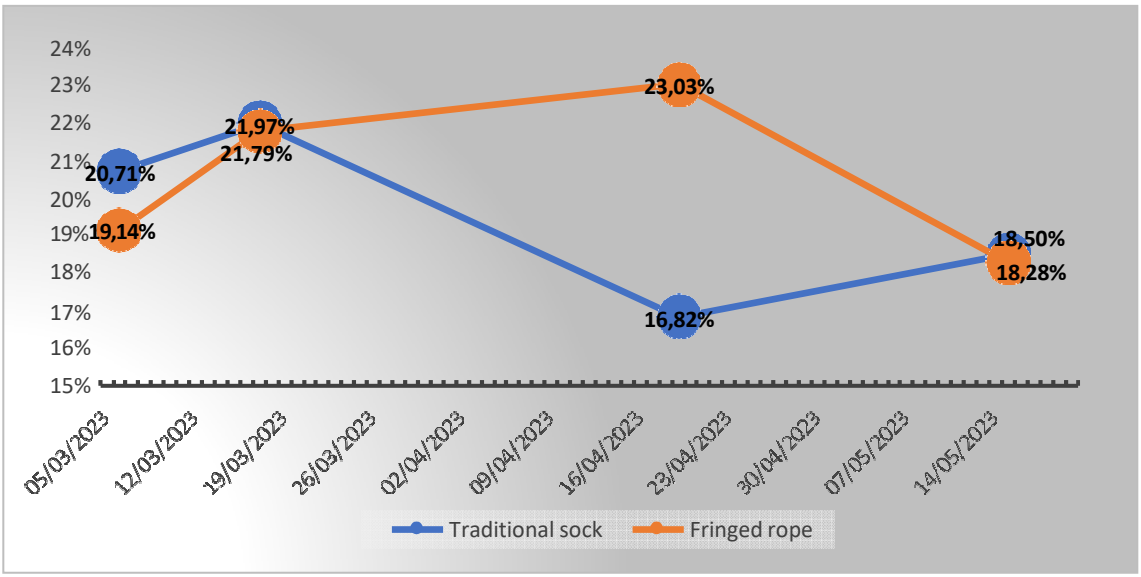


Figure 7 – Trend of mussel meat content

Condition index

Figure 8 shows the trend of the condition index of mussels in traditional sock and fringed rope up to 15 May 2023, where in the last period there is a decrease for both types, more accentuated for mussels from the fringed rope.

The April date, however, does not confirm the lower meat content of mussels taken from traditional sock, although these two indices are correlated.

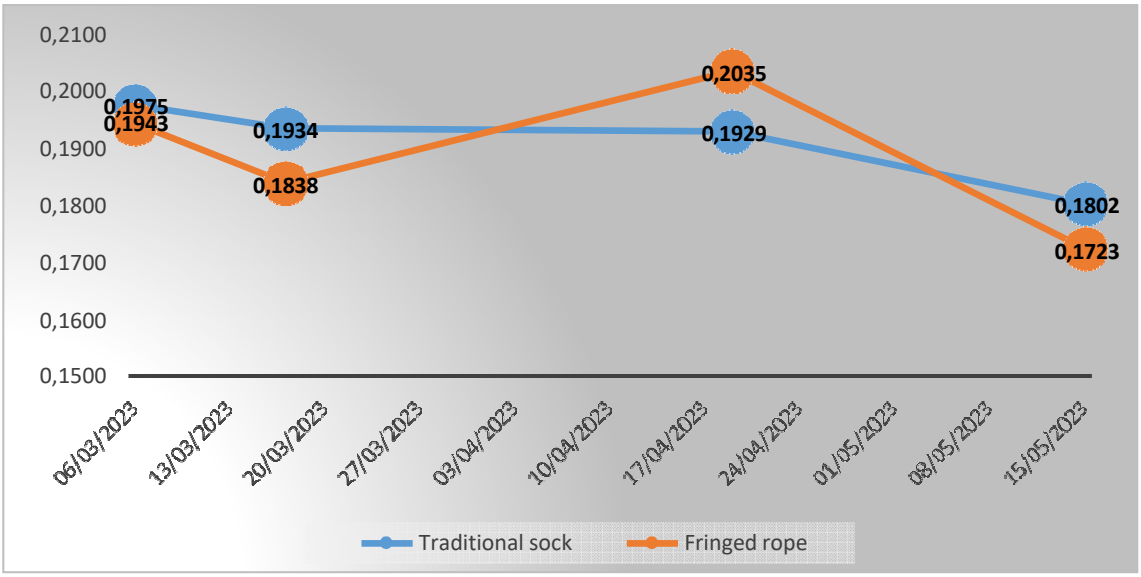


Figure 8 – Graphical representation of the trend of the condition index of mussels in traditional braiding and on continuous rope

Socks in compostable biopolymer

On 6 March 2023, nine socks with a length between 3 m and 3.9 m, average value 3.3 m, and a weight between 13 kg and 19 kg, average value 15.3 kg, were socked with compostable material. On 22 May, after approximately 2.5 months, an average weight of 36 kg was reached on a sample of five socks. At the time of the survey, although in the meantime we had weathered a few storms, all the socks were in perfect condition and showed no signs of net wear.

De-clumping tests

The De-clumping test took place on 22 May 2023 and 29 May 2023, one week apart. Given the approaching end of the project, this was done despite the fact that ropes and socks were not yet in the optimal condition to be subjected to the process.

As can be seen in Table 3 five socks and five rope were used in both tests. On 22 May, the total weight of the traditional socks was 238 kg, and that of the fringed ropes was 113 kg. On 29 May, the total weight of the traditional socks was 253 kg, and that of the fringed ropes was 106 kg.

Table 3 – Characteristics of the remains subjected to de-clumping in the two tests

	05/22/2023				05/29/2023			
	socks		rope		socks		rope	
	m	Kg	m	Kg	m	Kg	m	Kg
	4.68	54	2.84	23	4.52	55	2.68	23
	4.50	46	2.93	19	3.84	47	2.80	21
	4.23	50	2.80	24	4.03	50	2.74	22
	3.70	44	2.90	25	4.12	50	2.72	19
	3.75	44	2.80	22	4.25	51	2.70	21
Total	20.86	238	14.27	113	20.76	253	13.64	106
Average	4.2	47.6	2.9	22.6	4.2	50.7	2.7	21.1

From the de-clumping process on May 22, about 152 kg of mussels were obtained from the socks and about 60 kg from the ropes, equal to 64% and 54% of the total, respectively. On May 29, about 189 kg of mussels were obtained from the socks and about 69 kg from the ropes, equal to 75% and 65% of the overall total, respectively. The remaining was macro fouling, plastic net or rope, mussel juveniles, small fouling, sediment and water.

It can be seen from Table 4 that in both tests the rope has a higher waste rate than the sock, both as fouling and other components. This results in an approximately 10 percent higher yield of the traditional sock than the rope. It is also noted that in the first test, both rope and sock had more than 10 percent more rejection than found in the second test.

Table 4 - Composition of the products obtained after de-clumping during the two tests

	05/22/2023				05/29/2023			
	Sock		Rope		Sock		Rope	
	Kg	%	Kg	5	Kg	%	Kg	%
Kg mussels	151.7	63.7%	60.4	53.5%	189.0	74.6%	68.6	65.0%
Kg macro fouling	8.2	3.5%	5.8	5.2%	15.7	6.2%	7.7	7.3%
Kg sheath/rope	0.4	0.2%	1.9	1.7%	0.4	0.1%	1.8	1.7%
Kg other waste*	77.7	32.6%	44.9	39.7%	48.3	19.1%	27.5	26.0%
Total	238.0	100.0%	113.0	100.0%	253.4	100.0%	105.6	100.0%

* Juvenile mussels, fouling, sediment, water

The values obtained show a higher productivity of the traditional socks, a predictable result since the fringed rope initially contained about 46% of the mussels present in the traditional sock.

Table 5 – Production of mussels per meter of traditional sock and fringed rope

	05/22/2023			05/29/2023		
	Kg	m	Kg/m	Kg	m	Kg/m
Traditional sock	151.7	20.76	7.3	189.0	20.86	9.1
Fringed rope	60.4	13.64	4.4	68.6	14.27	4.8

The number of damaged individuals per kg of intact mussels obtained is quite similar in the two tests and between the two different farming systems, resulting in a few decimal places of difference.

Table 6 – Damaged mussels: total number and number per kg produced

	Sock		Rope	
Date	number	N/Kg	number	N/Kg
05/22/2023	254	1.7	83	1.4
05/29/2023	573	3.0	190	2.8

Shelf life

The verification of the survival of the mussels following the de-clumping process relating to the first test lasted for around 14 days, from 22 May to 5 June, with measurements taken about every 12 hours, although generally after a week after capture the chilled mussels are no longer marketed. As can be seen in Figure 9, up to about five days there is a cumulative mortality of 2% with both rearing systems, from day six there is a greater increase in the rope-reared mussels, and then similar values.

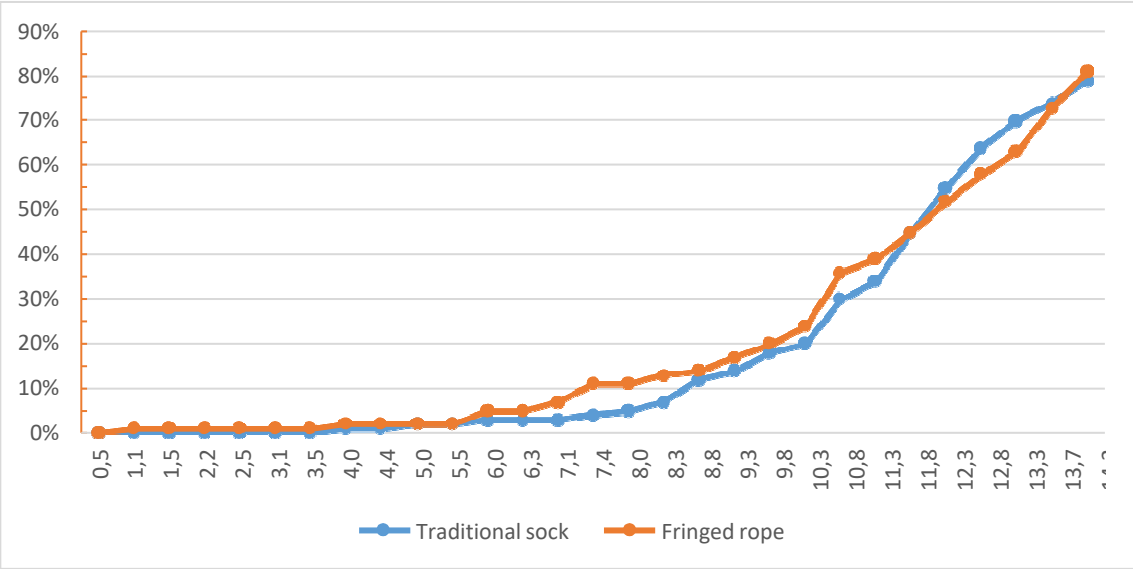


Figure 9 – The mortality trend during the first trial

The second test lasted for about 10 days, from 29 May to 9 June, with measurements taken about every 12 hours. As can be seen in Figure 10, mussels reared on ropes showed a higher mortality already from the third day, with a differential that became more pronounced up to the seventh day, from which point the mortality of mussels reared on traditional socks increased significantly, with a differential of around 10%.

The process of detaching the mussels from the rope was carried out manually, and this may have led to greater variability in the results obtained, as in these processes mortality is generally determined by the traction exerted on the byssus filaments, which, if too vigorous, can result in injury to the soft part of the mussels.

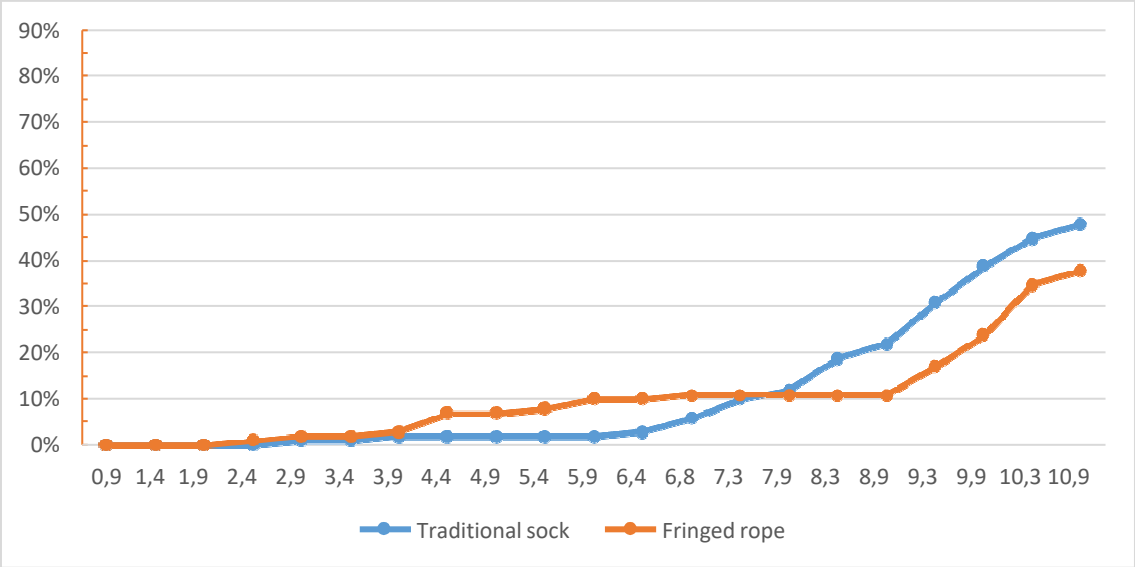


Figure 10 – The mortality trend during the second trial

The overall goal of the pilot action is to improve sustainability and diversification of the activities related to the oyster farming, specifically focusing on Flat Oyster

The aim of this action is therefore to provide indications on the characteristics of the caught product, on the improvement of quality through the refinement of oysters in collaboration with shellfish farms and on the possible use of sustainable equipment.

Material and methods

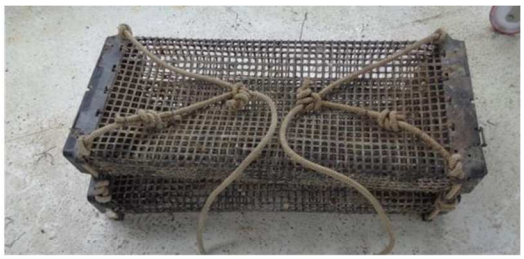
For this action were collected three batches of oysters, each weighing approximately 100 kg, one from a natural oyster bed north of Ancona and two from beds North and South of San Benedetto del Tronto, the location of which was determined using AIS data provided by the company KosmosAmbiente.

The oysters in each batch were sorted by size, eliminating those that had damaged, dead shells, were too clumped together or had an appearance that did not meet the marketing parameters.

The suitable oysters were distributed among the different containers used: lantern net; baskets made from biodegradable materials using a 3D printer; poches - plastic bags mainly used in oyster farming in France.

Baskets

Lantern net



Poches

The following measurements were taken to characterize the oyster beds and during the trial:

- Degree of Polydora infestation by the presence of blisters and by counting Polydora individuals after placing the shells in a solution containing phenol;
- Wet weight of the meat;
- Condition index;
- Amount of sediment or mud inside the valves.

Measurements were then repeated fortnightly for sediment and monthly for all other parameters.

In addition, a classification of *Polydora* individuals is carried out to determine the species.

Sensory tests were carried out comparing refined oysters and those from natural beds.

Enterprises involvement

This action took place with the cooperation of the shellfish farm Civitacozza Soc. Coop. a r.l., with farm off the coast of Porto San Giorgio.

Results and recommendations

Initial sorting and grading

The eliminated part consisting in empty shells, oysters clumped together or with inappropriate shape were 8.8% of individuals and 8,6% of caught biomass. Proportion of undersize oyster were respectively 23,0% in batch 1, 29,1% in batch 2 and 24,9% in batch 3.

Inter valves sediment

Table 1 shows that until April, T1.5, the trend of this index is unstable, while after this date it steadily decreases to values significantly lower than the initial ones Figure 1.

Table 1 – Amount of inter valves sediment in oyster batches

		Batch 1 - Ancona		Batch 2 - SBT 1	Batch 3 - SBT 3	**Average**
		Basket	**Lantern nets**			
Time	Date	**g/ 1.000 g**				
t.0	21/02/2023	1,79		2,62	1,31	1,91
t.0,5	08/03/2023	1,10	-	0,98	0,84	0,97
t.1,0	25/03/2023	1,57	-	1,30	1,21	1,36
t.1,5	07/04/2023	1,66	1,27	0,90	0,88	1,18
t.2,0	20/04/2023	0,73	0,68	0,80	0,48	0,67
t.2,5	07/05/2023	0,36	0,45	0,37	0,55	0,43
t.3,0	29/05/2023	0,30	0,17	0,34	0,23	0,26

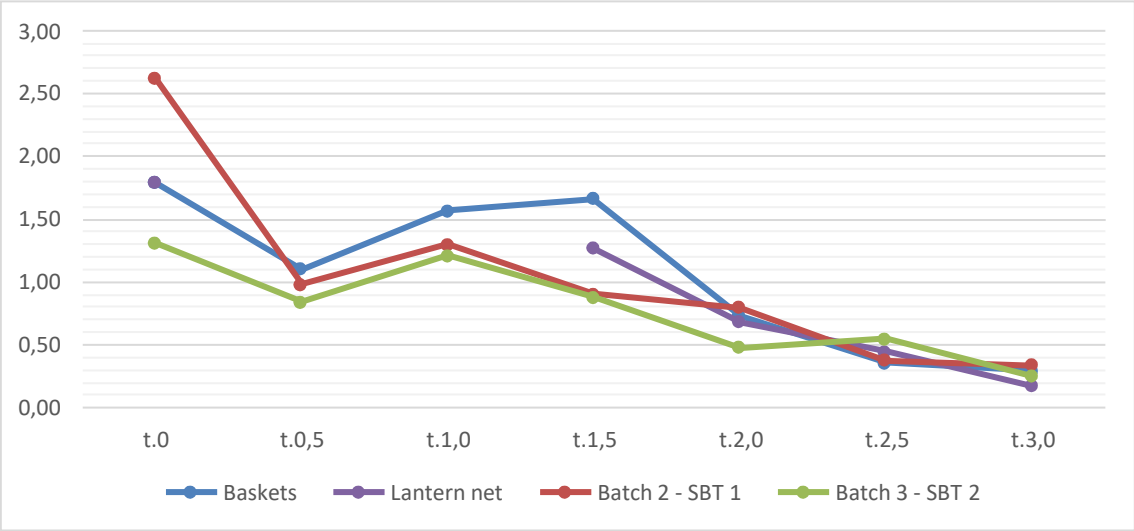


Figure 1 – Trend of inter valves sediment in oyster batches

Wet meat content

Table 2 shows the values of the percentage of meat content measured at the sampling dates and Figure 5 shows the trend over time.

The values shown in the table show a constant slight decrease for batch 1, batch 3 after an increase at time t1.5 also shows a slight decrease, while batch 2 at the last sampling date shows a slight increase. The decrease shown in the last sampling dates is due to the presence in the batches of oysters in an advanced state of gamete release.

Table 2 – Percentage of average meat content over total measured individuals

		Batch 1 - Ancona		Batch 2 - SBT 1	Batch 3 - SBT 3
Time	Date	**Basket**	**Lantern nets**		
t.0	21/02/2023	15,5%		14,5%	13,9%
t.0,5	08/03/2023				
t.1,0	25/03/2023				
t.1,5	07/04/2023	15,3%	14,2%	14,5%	15,7%
t.2,0	20/04/2023				
t.2,5	07/05/2023	14,3%	13,0%	12,9%	13,9%
t.3,0	22/05/2023	12,4%	12,7%	13,7%	12,7%

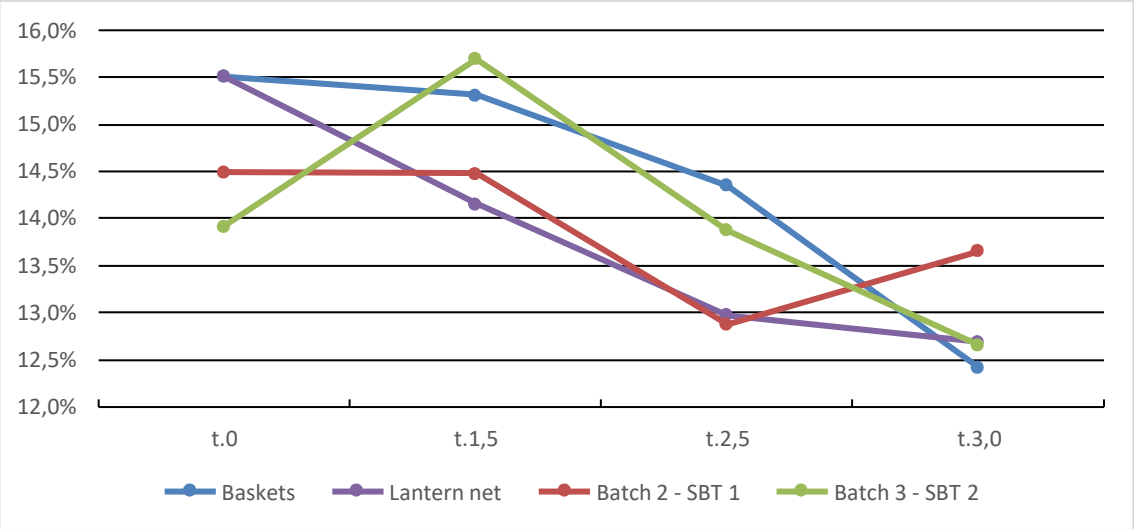


Figure 2 – Trend in average meat content over the total number of individuals measured

Condition index

Table 3 shows the condition index values of the oysters belonging to the three distinct batches and, as far as batch 1 is concerned, distinguished between those contained in biodegradable baskets and in lanterns, whose respective trend over time is highlighted in graphical form in Figure 8. The trend of the condition index follows that of the meat content and shows a gradual decrease in values, less regular for batch 3, and also in this case the influence of the gamete release is evident.

Table 3 – Oyster condition index referring to the various batches

		Batch 1 - Ancona		Batch 2 - SBT 1	Batch 3 - SBT 2
Time	Date	**Basket**	**Lantern net**		
t.0	21/02/2023	0,057		0,048	0,041
t.0,5	08/03/2023				
t.1,0	25/03/2023				
t.1,5	07/04/2023	0,051	0,044	0,044	0,054
t.2,0	20/04/2023				
t.2,5	07/05/2023	0,042	0,045	0,041	0,050
t.3,0	29/05/2023	0,033	0,033	0,036	0,034

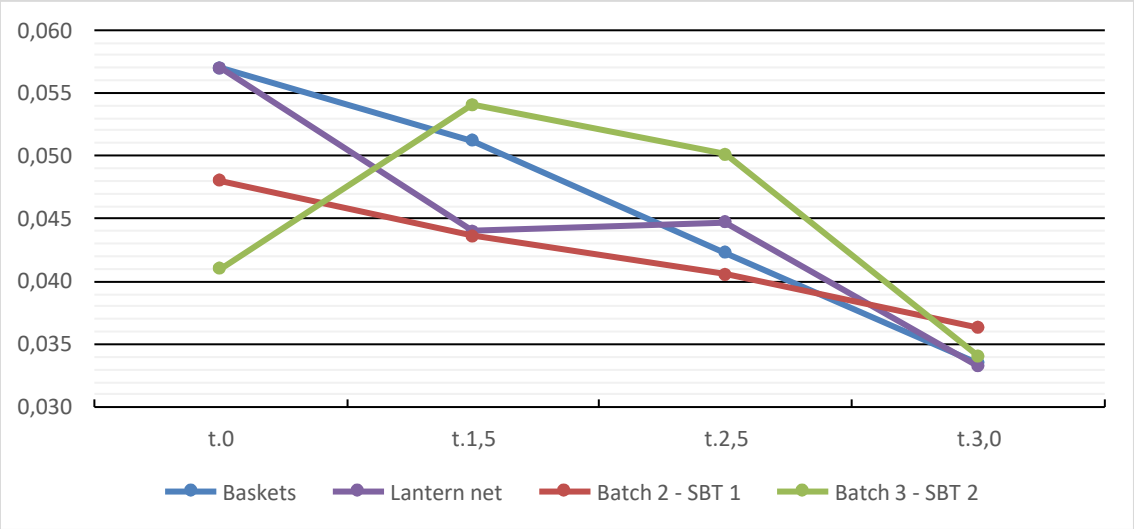


Figure 3 – Trend in oyster condition index referring to batches

Polydora infestation

With regard to infestation by Polydora, the data in Table 4 show high variability both in the index, based on observations of the inner part of the shells, and in the number of individuals found following immersion in vermifuge. Batch 1 and batch 2, with 24 and 31 individuals respectively, show the greatest infestation, although this is not adequately reflected in their index values. Both parameters appear to be independent of the period of suspension.

Table 4 – Polydora infestation index and number of individuals found in batches

		Batch 1- Basket		Batch 1 – Lantern nets		Batch 2 - SBT 1		Batch 3 - SBT 3	
Time	Date	Index	Oyster	Index	Oyster	Index	Oyster	Index	Oyster
t.0	21/02/2023	11,7%*	9*	11,7%*	9*	12,5%	31	8,75%	13
t.1,5	07/04/2023	20,4%	5	23,3%	24	22,1%	17	34,5%	10
t.2,5	07/05/2023	16,3%	12	28,8%	14	26,7%	14	18,3%	15
t.3,0	29/05/2023	15,8%	14	15,8%	14	16,3%	23	33,8%	10

* The values for batch 1 correspond to the overall sample

Final survival

Depending on batch, survival rates were respectively 93,1% for batch 1, 90,4% for batch 2 and 91,3% for batch 3. Mortalities occurs mainly in the 2 weeks after sorting and grading. Survival rate of undersize oysters was 95,8%.

Sensory test

On 14 April, a sensory test was held at the AMAP headquarters in Osimo in which 12 panelists participated. The test was of the Triangular type, in which two batches of flat oysters were compared, one consisting of oysters fished from natural beds two days before the test date and the other of flat oysters fished from natural beds but left to refine for the duration of 51 days: from 21 February to 13 April 2023.

Both batches of oysters came from natural beds located in the waters off the southern coast of the Marche region.

To this end, two rounds of tastings were carried out in which each participant was presented with a plate containing three oysters, identified by a three-digit alphanumeric code, one belonging to one lot and two belonging to the other lot. The taster's task was to identify the oyster that was different from the other two. Without making quantitative assessments. In the first round the oyster to be identified as different was the one from natural beds, in the second round it was the one from the refining process.

The codes were assigned by the technician responsible for opening the oysters and kept secret until the end of the tests. Both batches had previously undergone a purification process of approximately 12 hours at a plant located in San Benedetto del Tronto. The opening of the oysters took place using a special tool and was carried out by only one person. After opening, each oyster was lightly washed with running water to remove shell residues and traces of mud.

Due to the presence of oysters in the reproductive phase, even at an advanced stage, it was necessary to discard all individuals showing gonads in the gamete emission phase or, in some cases, the presence of larvae in various stages of development. This resulted in the discarding of approximately 30/40% of the available oysters. As for the batch coming directly from natural beds, individuals with an excessive abundance of mud inside the shells were also discarded.

The test results are shown in

Table 5, where the column corresponding to the oyster to be identified is highlighted in yellow.

As can be seen from the analysis of the table, in both comparisons, only 5 tasters out of 12, equivalent to about 42% of the total, identified the oyster as different, and only two tasters, number 7 and number 11, responded correctly to both tests.

This result shows that from a statistical point of view, the thesis that oysters subjected to refining treatment can be identified as different from those taken directly from natural beds cannot be supported.

Table 5 – Sensory evaluation test result

	First test			Second test		
	Refined	Natural bed	Refined	Natural Bed	Refined	Natural bed
Num.	A3X	T2C	E6B	A1J	R2K	P4Z
1	1				1	
2		1		1		
3			1		1	
4		1				1
5	1					1
6		1		1		
7		1			1	
8	1				1	
9			1			1
10			1			1
11		1			1	
12	1			1		
Total	**4**	**5**	**3**	**3**	**5**	**4**

Shelf-life test

A shelf-life test was carried out at the end of the experiment with oysters packed in wooden traditional boxes used for cupped oysters and oysters packed in net bags. After 13 days about 25% of the oysters were still closed.

The overall goal of the pilot action is to provide alternatives to the use of plastics in the molluscs commercialization by using different materials in packaging

This action is about packaging certain product lines such as mussels and oysters using innovative biodegradable and compostable packaging to replace the traditional plastic net packaging currently used in the packaging industry.

Material and methods

The market research to identify alternative packaging materials to plastic was carried out both by directly contacting companies producing and/or distributing packaging that can be used in the seafood sector and by means of a web search. The search criteria favoured national and/or local companies already operating in the food sector, in order to test materials already available on the market, leaving out experimental packaging still in the industrialisation phase and lacking certification for contact with food (Table 7).

Table 7 - List of suppliers and alternative materials to plastic packaging tested

Name Material	Supplier	Type	Notes
Bioplastic - Novamont patent	ROM Plastica Srl	Biodegradable and compostable biopolymer	New patent
Greenpack	M.T. RETI Srl	Cellulose net	Used in the citrus sector
Hemp Canapa	Kuku International	Hemp net	Used in the fruit and vegetable sector, various netting formats tested
Cellulose polycoupled	Grafostil snc	Tray	Cellulose tray and bioplastic protective film
Bioplastic	Ekoe' soc coop	(PLA- PBAT) Bioplastic	Vacuum bag

Materials used in tests: bioplastic, cellulose, hemp, bioplastic vacuum bag

The tests were conducted under the real operating conditions of preparing fresh product for sale.

The materials were tested in the 2 formats most commonly used for packaging: 1kg and 5kg packs. The seafood products used were: mussels, oysters and a test was also carried out on clam packs. A total of 70 packs were made with the new materials suitable for sale (for mussels: 35 1kg packs, 15 5kg packs and for oysters 20 individual packs). The tests were divided into:

- bagging test;
- storage test;
- transport test;
- public acceptance test.

Enterprises involvement

The project was entrusted to Blu Marine Service Soc. Coop. and the packaging tests were carried out at the Space Fish S Ltd plant, both based in San Benedetto del Tronto.

Results and recommendations

Results of the physical tests

The tests to which the various materials were subjected were as follows: bagging, storage, transport, public acceptance. The tests were organized in a consecutive manner so as to continue the tests only if the previous test had been successful. Packaging that did not prove to be suitable was discarded for the next stages. Packaging test results are showed in Table 8.

Table 8 - Packaging test results

Material	Packaging type	Rating	Public acceptance
Cellulose	Pack 1 Kg	Suitable	8
	Pack 5 Kg	Not suitable	
Hemp – 1	Pack 1Kg	Not suitable	
	Pack 5 Kg	Not suitable	
Hemp – 2	Pack 1 Kg	Suitable	7
	Pack 5 Kg	Not suitable	
Hemp – 3	Pack 1 Kg	Not suitable	
	Pack 5 Kg	Not suitable	
Bioplastic	Pack 1 Kg	Not suitable	
	Pack 5 Kg	Suitable	6
Cellulose tray and bioplastic film	Pack 10 oysters	Suitable	ND
Vacuum-packed bag	Pack singol oyster	Suitable	ND

Consumer acceptance

All the innovative packaging shown to the public had a positive approval rating, being substantially similar to the plastic net, but made from renewable resources (e.g. hemp, cellulose). Only the material made of bioplastic was rated lower than the other types, mainly due to its similarity to classic plastic.

Mussels shelf life results

The checks carried out showed that all the packaging had a shelf life similar to the normal plastic packaging used. Verifications were carried out with daily checks during the verification period by taking samples from the packs to check the vitality of the animal. The bags were kept tightly sealed at all times in order to simulate operational storage conditions. Considering the results of the assessment of the inspection analysis at T0-T6, and taking into account the acceptability criteria set by the company, it is considered appropriate to establish a shelf-life in the net bag of a maximum of 5 days, in compliance with the storage temperature of 6 ± 3 °C in order to be cautious in defining the commercial life of the product, guaranteeing a product with high freshness standards.

Oysters shelf life results

The vacuum sealing machine used did not have to undergo any changes in the operating parameters as the vacuum was performed correctly on all packs without any problems. None of the bioplastic wrappings suffered cracks or holes during the vacuum process, due the pointed ends of the animal's shell. The vacuum-packed product presents itself well aesthetically as well, and being transparent allows the product contained in it to be seen. The data show a good resistance of the animal, the retailer found the data acquired on the vacuum shelf life very interesting considering it also a different packaging method that could have market opportunities.

A.4 Pilot action towards "Diversification"

The overall goal of the pilot action is to provide alternative solutions to the use of plastics in mussel farming

Material and methods

The pilot action A.4 of the Protocol is about product innovation focusing on molluscs. The overall goal is proposing a new product to the market, testing the enrichment of oysters and mussels with Spirulina. In the following action, the preliminary activity consisted in the collection of the material necessary for the experimentation and in the enrichment test to define the experimental procedure. In the realization of the pilot action the COPEMO company was involved for a better development of the action. Specifically, significant quantities of oysters, mussels and Spirulina were acquired, and all the material was therefore transferred and housed at the DiSVA aquarium infrastructure.

Different percentages of enrichment of molluscs were initially tested by exposing them for several hours to different percentages of Spirulina dissolved in water subjected to vigorous aeration. At the end of this first test, an enrichment protocol was drawn up which provided, for both the oyster and the mussel, to expose the molluscs to increasing concentrations of spirulina dissolved in water (0,1, 2, 5% of spirulina compared to the biomass to be enriched) for 4 hours.
To evaluate the potential appreciation of the enriched products, thanks to the support of AMAP-Marche Agriculture and Fisheries Agency, two specific evaluation forms have been drawn up (Tab. 1 and 2) to be submitted, as a second step, to the tasters. The panel test took place in July 2022 at the AMAP panel test room under the supervision of n.3 senior panelists that also contributed in the cards preparation and the results elaboration. Below are the cards used for the panel test which have been extensively discussed by the two parties.

SCHEDA_COZZE_REV_0_13/07/2022	MARCHEAgricolturaPesca Agenzia per l'innovazione nel settore agroalimentare e della pesca Progetto ARGOS

Nome Cognome Assaggiatore _____

Identificazione campione _____

Attributo	Intensità								
	1	2	3	4	5	6	7	8	9
Prodotto crudo (tal quale)									
Odore complessivo cozza									
Odori estranei (specificare)									
Prodotto cotto									
Odore complessivo cozza									
Odori estranei (specificare)									
Salato									
Amaro									
Sapori estranei (specificare)									
Consistenza alla masticazione									
Persistenza									
Rispondenza a odore/sapore tipico cozza									

Colore	Bianco	Giallo/giallo chiaro	Arancione	Verdognolo

Data _____

Firma _____

AGENZIA PER L'INNOVAZIONE NEL SETTORE AGROALIMENTARE E DELLA PESCA - L.R. 12/05/2022 n.11
60027 OSIMO (AN) – Via dell'Industria, 1 – Tel. 071 8081 – Fax 071 85979 – P.I. e C.F. 01491360424
PEC: marcheagricolturapesca.pec@emarche.it

Tab. 1 Evaluation table for mussels

SCHEDA_COZZE_REV_0_13/07/2022	MARCHE Agricoltura Pesca Agenzia per l'innovazione nel settore agroalimentare e della pesca Progetto ARGOS

Nome Cognome Assaggiatore _____

Identificazione campione _____

Attributo	Intensità								
	1	2	3	4	5	6	7	8	9
Prodotto crudo (tal quale)									
Odore complessivo cozza									
Odori estranei (specificare)									
Prodotto cotto									
Odore complessivo cozza									
Odori estranei (specificare)									
Salato									
Amaro									
Sapori estranei (specificare)									
Consistenza alla masticazione									
Persistenza									
Rispondenza a odore/sapore tipico cozza									

Colore	Bianco	Giallo/giallo chiaro	Arancione	Verdognolo

Data _____

Firma _____

AGENZIA PER L'INNOVAZIONE NEL SETTORE AGROALIMENTARE E DELLA PESCA - L.R. 12/05/2022 n.11
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Tab. 2 Evaluation table for oyster.

Therefore, once the enrichment protocol was established and the evaluation sheets were perfected, the quantity of mussels and oysters (50 kg each) was prepared for the gustatory test. Oysters and mussels were divided into the different experimental groups (0, 1,2 and 5% enrichment with spirulina for 4 hours-0 indicates the mollusc as it is) and subjected to the phases defined by the enrichment protocol.

Fig.1 Spirulina solution preparation.

At the end of the 4 hours of enrichment, the molluscs were washed with salt water, visually inspected to assess their vitality, suitably packaged and stored in the cold room at + 4 ° C for about 20 hours. During this phase, the shellfish batches were constantly inspected to evaluate their shelf-life. No significant differences emerged between the different shellfish groups and survival was 100%.

The day following the enrichment of the molluscs, a taste test was organized at the MAP-Marche Agriculture and Fishing which involved a number of 10 panelists with different abstractions: expert tasters, academic staff, operators in the mollusc farming sector and simple consumers of molluscs.

Oyster test

Alphanumeric codes were assigned to the 4 groups of oysters (control, enriched with 1,2,3,5% spirulina).

Each panelist initially tasted a control oyster and received the evaluation form in order to discuss it with the specialized staff who organized the panel test.

Subsequently, each panelist received a number of three oysters per treatment served on 4 different dishes, each bearing an alphanumeric code to be reported in the evaluation forms in order to find a correspondence between the mollusc tasted and the treatment.

All oysters were served without any seasoning.

Fig. 2 Example of oysters enriched with spirulina.

Oyster test results

All data was collected on computer media and processed.

- As the concentration of spirulina increases: 1) the overall oyster odor decreases, while extraneous odors and tastes increase; 2) the intensity of the salty decreases, while bitter and acid remain substantially unchanged; 3) Consistency and elasticity do not show particular differences among the various theses, while persistence gradually decreases as the concentration of spirulina increases; 4) The correspondence to oyster smell and taste progressively decreases as the concentration of spirulina increases.
-The sample with the concentration of 5% strangely does not present anomalous odors, although the correspondence is lower than all the others, while it highlights anomalous flavors.
-The color shows greenish shades (Fig. 2) in all the samples treated with spirulina, unlike the control which maintains a creamy white color.

Mussel test

Alphanumeric codes were assigned to the 4 groups of mussels (control, enriched with 1,2,3,5% spirulina). The mussels were cooked without adding oil or other ingredients, at 80 ° C for 8 minutes. Each panelist initially tasted a control mussel and received the evaluation sheet in order to discuss it with the specialized staff who organized the panel test.
Subsequently, each panelist received a number of three mussels per treatment served on 4 different dishes, each bearing an alphanumeric code to be reported in the evaluation forms in order to find a correspondence between the mollusc tasted and the treatment.

Fig. 3 Example of mussels enriched with spirulina.

Mussel test results

All data was collected on computer media and processed.

From the data analysis it emerged that as the concentration of spirulina increases:

1) the overall smell of mussel decreases, while extraneous smells and tastes increase;
2) the intensity of the salty slightly decreases, while the bitterness slightly increases;
3) Consistency and persistence do not show particular differences among the various theses.
4) The correspondence to the smell and taste of mussel progressively decreases as the concentration of spirulina increases, up to 2%.
5) The sample with the 5% concentration is strangely more responsive than the other two concentrations (same compliance as the control), despite the fact that extraneous odors and tastes of greater intensity have been perceived.
6) The color does not show particular differences between the theses (Fig. 3); the differences within the same sample are instead attributable to the sex of the mussel.

Results and recommendations

Microalgae have long been known to be well endowed to enhance the nutritional quality of conventional foods and positively affect human health owing to their high macro- and micronutrients content. Indeed, for thousands of years, edible microalgae Arthrospira spices have been used for food. Being a rich source of promising compounds with biological activity that could be used as functional ingredients, they have been labelled them as super food. Owing to increasing awareness of health promoting nutrients and nutraceuticals necessary to ward off the lifestyle disorders, microalgae are receiving renewed attention in nutritional and food sciences. Spirulina platensis belonging to the family Oscillatoriaceae, is a multicellular and filamentous blue-green microalga (cyanobacteria) that thrives in warm, alkaline fresh-water bodies. This cyanobacterium contains 55–70% protein, 15–25% polysaccharides, 5–6% total lipids, 6–13% nucleic acids and 2.2–4.8% minerals. Among the micronutrients, it contains a complex of vitamins A, B, D, E and K. 4

Spirulina is rich in minerals such as calcium, potassium, iron, nickel, chromium, magnesium, manganese, copper, sodium, zinc, selenium and lead along with carotenoids and essential fatty acids (3,6 γ-linolenic acid, α-linolenic acid, stearidonic acid, eicosapentaenoic acid, docosahexaenoic acid and arachidonic acid) necessary for a holistic balanced diet. Besides, Spirulina is the richest natural source of digestible protein that offers all essential amino acids to the human body.
The higher digestibility of these microalgae is realized because of lack of cellulose in the cell wall of Spirulina, its wall material being composed of 86% digestible polysaccharides. Potential health benefits of Spirulina consumption include immunomodulation, antioxidant, anticancer, antiviral and antibacterial activities, as well as positive effects against malnutrition, hyperlipidemia, diabetes, obesity, inflammatory allergic reactions, heavy metal/chemical-induced toxicity, radiation damage and anemia without any toxicological effects. Owing to these features, Spirulina is widely approved in several countries and has earned GRAS (Generally Recognized as Safe) status. It has been authorized by the FDA (Silver Spring, MD, USA) and The Brazilian Health Regulatory Agency (ANVISA, Brasília, Brazil) for consumption as food or food supplement. Additionally, spirulina belongs to the "low trophic" food products, underlying its low ecological impact during production.

The present action demonstrated that both mussels and oysters are able to uptake spirulina from the culturing water, but the general analysis of the collected data underlined that consumer better accepted mussels respect to oysters. This is mostly related to the fact that both odor and color dramatically changed in the treated oysters, as well as to the fact that oysters were served raw. On the contrary, as regards mussels, one of the main positive aspects is that the color did not change, and this aspect was positively considered by the panelists. As a further perspective of this action it would be interesting to investigate possible ameliorative effects of the addition of spirulina to the mussels by investigating, through laboratory techniques, their nutraceutical characteristics with emphasis on polyunsaturated fatty acids and antioxidant molecules enrichment