



Integrated Multi-Trophic Aquaculture: A Laboratory and Hands-on Experimental Activity to Promote Environmental Sustainability Awareness and Value of Aquaculture Products

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Aquaculture is among the industries growing at the fastest rate in the world. This industry has been recognized to play a critical role in food production for a continuously expanding world population. However, despite various technological innovations and improvements in production techniques, this sector is still associated with misperceptions and negative opinions hampering its implementation and wide consumption of its products. The integrated multi-trophic aquaculture (IMTA) concept was developed as a way to increase the sustainability of intensive aquaculture systems, using an ecosystem-based approach. In this study, following this sustainable aquaculture concept, a closed recirculation IMTA system, at laboratorial scale, was developed and tested with the simultaneous production of fish, sea urchin and seaweed for 70 days. Based on this proof of concept, a hands-on experimental activity was developed to teach and communicate recent scientific advances in environmental sustainability and value of aquaculture products to young students and the general public. This experimental activity was tested and evaluated with students ($n = 60$) of basic and high-school (secondary) learning cycles. A quantitative assessment was carried out through a short questionnaire provided to the students before and after the experimental activity. After the experimental activity, a qualitative assessment was also performed through questions expressed without preconceived categories or hypotheses. Results indicated that the overall frequency of students who consider the ocean to be “very important” and “extremely important” increased from 68 to 81% after performing the experimental activity. Moreover, the percentages of correct answers to the questions related to IMTA concepts also increased significantly after the experimental activity. In the discussion of the experimental activity results, the students stated that they appreciated the opportunity to develop a hands-on experimental activity, which allowed them to increase their knowledge and obtain information on aquaculture and the quality of its products.

Keywords: acceptability of aquaculture products, public image of aquaculture, IMTA, recirculation systems, water quality

INTRODUCTION

Aquaculture is an old practice thought to have begun over two thousand years ago in China (Rabanal, 1988). Crafted fish farming is known for hundreds of years throughout the world (Freeman et al., 2012). However, it has only been in the last four to five decades that commercial production became widespread, moving from an annual output of 4.7 million tons in 1980 to 80 million tons in 2016 (Bacher, 2015; Food and Agriculture Organization [FAO], 2018). Owing to an increasing food demand, combined with fish stock depletion, it is now recognized that fisheries will not be able to meet the projected global needs in high-quality protein (Food and Agriculture Organization [FAO], 2018). In face of this development need, aquaculture is currently the food production sector showing the fastest growth in the world, with an average rate of 5.8% in the last 15 years (Food and Agriculture Organization [FAO], 2018) and is a priority of the Blue Growth strategy adopted by the European Union (EU). This sector is expected to supply 109 million tons of fish by 2030, with a growth of 37% in 2016 (Food and Agriculture Organization [FAO], 2018).

Aquaculture growth took place over several decades in a context of increasing environmental awareness and public preoccupation about the quality of its products (Tacon and Metian, 2013). In particular, topics such as food safety and quality, their potential health impacts, environmental sustainability of the sector and animal welfare came up as of utmost public concern (Aarset et al., 2004; Gormaz et al., 2014; Olsen and Osmundsen, 2017). Among these are the issues related to: (i) the presence of parasites in aquaculture fish, (ii) the use of antibiotics to promote growth and prevent diseases, (iii) the origin of the aquafeed supplied to the farmed fishes, (iv) the excessive release of nutrients to natural water bodies, (v) the accumulation of aquaculture wastes in the seabed, and (vi) the impacts of aquaculture on wild populations and on the introduction of non-indigenous species (i.e., aquaculture as introduction vector/pathway) (Van Osch et al., 2017; Mangano et al., 2019a). This insecurity regarding environmental, social and safety features of aquaculture products, together with the increasing dependence of developing countries on farmed seafood, has also attracted considerable negative media attention to aquaculture (Whitmarsh and Palmieri, 2011; Bacher, 2015).

In recent years, however, a huge effort has been done to enhance aquaculture practices to support both stakeholders and policy makers during siting activities and in building more sustainable systems (Mangano et al., 2019b). Various technological innovations have been developed to improve the efficiency of water use and decrease the environmental impact of aquaculture (Girard and Payrat, 2017). Among the actions taken are, for example, the restrictions imposed by several countries to the use of chemotherapeutic agents or ground innovation reducing the use of fish meal and oil in feeds (Gatlin et al., 2007; Moutinho et al., 2017), investigation and use of natural feed additives to improve growth and feed efficiency (Magalhães et al., 2016), power the immune system and enhance the resistance to disease (Azeredo et al., 2017). Despite this effort, intensive aquaculture production still releases high amounts of

nutrients and organic wastes into the environment that can cause eutrophication of coastal areas and other aquatic systems (Sarà et al., 2018a). This is because only about 20–40% of the nitrogen (Oliva-Teles et al., 2020) and less than 50% of the energy intake (Bureau et al., 2002; Peres and Oliva-Teles, 2005) are retained by the species produced. The recognition of the significant environmental and social impact of intensive production increased the interest in alternative sustainable practices, such as integrated multi-trophic aquaculture (IMTA) (Alexander et al., 2016a; Sarà et al., 2018b).

Integrated multi-trophic aquaculture aims at the integrated production of aquaculture species of different trophic levels under a circular economy approach, minimizing energy losses and environmental deterioration (Food and Agriculture Organization [FAO], 2009; Chopin et al., 2012; Hughes and Black, 2016; Buck et al., 2018). Under IMTA production, the uneaten feed and wastes of one species are recaptured and converted into feed, fertilizers, and energy to another species. IMTA can promote aquaculture sustainability, with environmental, economic, and social advantages. This can be achieved through nutrient cycling, increased economic resilience arising from increased production efficiency, product diversification, and potential price premiums (Chopin et al., 2012; Van Osch et al., 2019).

There are multiple configurations of IMTA systems, integrating the production of vertebrate and invertebrate species and macroalgae. Cultivated organisms are fed aquatic species, like fish or shrimp, and species extracting the organic and inorganic matter from the water. Species extracting the organic matter may be mussels, oysters, clams, sea urchins or polychetes. These species feed on organic waste, such as uneaten food and feces. Species extracting the inorganic matter, such as macroalgae (e.g., species of the genera *Ulva*, *Gracilaria*, *Saccharina*, *Laminaria*), capture and use the inorganic nutrient wastes. IMTA allows the creation of more sustainable production systems because wastes of fish/shrimp production are valued as a resource rather than considered a burden or pollution. This contributes to environmental sustainability and a more efficient use of resources, while favoring economic diversification (product diversification, bringing company stability through risk reduction), and social acceptability (best management practices).

Integrated multi-trophic aquaculture has additionally been recognized as a contributor to reducing public opposition toward intensive aquaculture (Ridler et al., 2007; Alexander et al., 2016b; Buck et al., 2018). Nevertheless, there is still a pressing need to enhance societal awareness, perception, and acceptability of aquaculture products, and disseminate sound and rigorous information to consumers about the aquaculture industry and its environmental sustainability. Activities fostering understanding about sustainable aquaculture practices, the benefits of its products to consumers, and how they meet end-users needs are also required to implement steady consumption of aquaculture products. In this regard, the IMTA concept, and its advantages over the conventional methods, provides an excellent opportunity to teach students, and inform the general public, about environmental sustainability, increase their ocean literacy,

as well as improve social acceptance of aquaculture products, enhancing its consumption (Shuve et al., 2009). The aim of the present work was, therefore, to develop a laboratory and hands-on experimental activity based on the IMTA concept. The ultimate goal was to provide teachers and other educators with sound resources to work environmental sustainability and value of aquaculture products with their students.

MATERIALS AND METHODS

Experimental IMTA

Two IMTA units were implemented at laboratorial scale (210 L) in the Marine Zoological Station of Porto University. Each of these two indoor systems comprised four tanks, including one for European seabass (*Dicentrarchus labrax*) as marine carnivorous fish, one for sea urchin (*Paracentrotus lividus*), a grazing invertebrate, and one for a seaweed (*Ulva* sp.). This was a closed system with recirculation, in which each tank was supplied by a continuous flow of seawater from the previous tank (the direction of the flow was: fish, sea urchin, seaweed and back to the fish unit). The fish tank was built with the incorporation of a particulate organic matter trap. The trap prevented the sedimentation of organic particles (feces and surplus feed) in the fish tank and ensured their continuous transference to the sea urchin tank. The trial lasted for 70 days. During this period, the water temperature was regulated to $18.0 \pm 0.5^\circ\text{C}$, salinity averaged $34 \pm 1\text{‰}$, and dissolved oxygen averaged 95% of saturation. Photoperiod was adjusted to 12/12 h light/dark cycles. In both systems, the initial stocking density of fish, sea urchin, and *Ulva lactuca* were 6.9, 19.5, and 1.9 kg/m^3 , respectively. Fish were fed daily with a commercial diet of crude protein (42%) and crude fat (18%). Sea urchins were fed twice a week with the seaweed harvested from the IMTA system itself. During the trial, every other week, a water sample was collected from each tank, 0, 1, 3, 6, and 12 h after feeding, for monitoring ammonia (NH_4/NH_3), nitrites, and phosphates levels, using commercial kits. At the beginning and end of the trial period, fish were group weighed and the diameter of the sea urchins was measured. The seaweed was also weighed at the beginning of the trial and every 7 days; during this time total biomass was readjusted whenever needed to maintain the initial density. Total feed intake of fish was measured daily.

The experiments were approved by CIIMAR ethical committee for Managing Animal Welfare (ORBEA), in compliance with the European Union directive 2010/63/EU and the Portuguese Law (DL 113/2013).

Hands-on Experimental Activity for Students

A small scale IMTA system was built similarly to the aforementioned experimental units, using four 5 L aquaria. Three of them were used to place each IMTA species (Figure 1) and were provided with aeration (air pumps). The fourth aquarium was used as a water storage tank. A water pump was placed in this aquarium to allow recirculation of the water originating

from the seaweed aquarium back to the aquarium containing the fed species. The three aquaria with the cultured species were placed on uneven levels so that water could circulate by gravity, successively from the first to the second aquarium and from there to the third one (Figure 2). Marine fish were placed in the first aquarium (highest level), mussels or sea-urchins in the second aquarium and the seaweed in the third. The most relevant aspect was the adjustment of the biomass density in each aquarium, which was set to $7\text{--}10 \text{ kg/m}^3$ for fish species, $20\text{--}25 \text{ kg/m}^3$ for sea-urchin/mussels and $1\text{--}2 \text{ kg/m}^3$ for seaweeds. The activity started by assembling this IMTA system, and afterward students followed the effects of excretion/consumption of some organic and inorganic wastes on the levels of nutrients in the aquaria. Firstly, water circulation through the IMTA system was turned on and the system was left to stabilize for 2 h. After this period the water pump was switched off. This was taken as time zero of



FIGURE 1 | Demonstration prototype of an integrated multi-trophic aquaculture system. A log sheet was developed along with this experimental activity (Supplementary Material) to provide students with a structured form to record their experiment and analyze and discuss their results.



FIGURE 2 | Assembling of the hands-on Integrated Multi-trophic Aquaculture system. A video with more details on the assembling is also available through the program CIIMAR at School (<http://www.ciimar.up.pt/oCIIMARnaEscola/OCEANLAB.php>).

the experiment. Water parameters of each aquarium were then measured at time zero and every half an hour for the next 90 or 120 min. Ammonia (NH₄/NH₃), nitrates, phosphates, and pH were measured with simple kits available at pet shops. Other colorimetric methods available in schools can also be used.

Assessment of the Hands-on Experimental Activity

The hands-on activity was tested with school students ($n = 60$) from basic and high-school (secondary) learning cycles. The students were 12–18 years old and belonged to schools from Porto district in Northern Portugal. The students visited CIIMAR during the school period or holiday science sessions between 2016 and 2017. Students were enrolled in the activity by their teachers or their parents, depending on whether they participated during the school period or during the holiday science sessions, respectively. In either case, the activity was announced in the CIIMAR at School (the outreach program of CIIMAR to teachers and students) website, through regular media channels of CIIMAR and directly (by email) to all 92 school clusters of the Porto metropolitan area. On average each cluster is composed of three schools. Interested teachers or parents enrolled the youngsters to visit CIIMAR and perform the activity. No selection of students or teachers was done by the researchers within the scope of the study. The aim was to obtain a random sample of students performing the activity to gain understanding about the usefulness of the IMTA prototype and identify aspects for improvement.

A quantitative assessment of the IMTA hands-on activity was carried out using a brief questionnaire given to students before and after the activity. The questionnaire items were adapted from available literature about ocean literacy (Steel et al., 2005; Guest et al., 2015). The aim was to assess students' relationship with the ocean and their knowledge about some concepts related to environmental sustainability in aquaculture (Table 1). This simple and anonymous questionnaire was prepared in Portuguese and, besides basic demographic data, was composed of items with Likert scale response (1 – Not important; 2 – Somewhat important; 3 – Important; 4 – Very important; 5 –

TABLE 1 | Questionnaire items about the ocean and concepts related to aquaculture environmental sustainability.

Questionnaire items	
Likert scale	
How important is the Ocean to you?	
Categorical response	
In which ways can Humanity benefit from the Ocean?	
Do you know what biodiversity is?	
Do you know what an ecosystem is?	
Do you know what fishing stocks are?	
Open response	
Who produces the oxygen that comes from the ocean?	
Multiple-choice	
What is eutrophication?	

Extremely important), multiple-choice questions, open response, and categorical responses.

A qualitative assessment of the IMTA hands-on using simple questions expressed without preconceived category or hypothesis was also done. For this, at the end of the activity, three general questions were asked orally to the participating students, namely “What are your thoughts about the experiment you did?”, “What impressed you most?”, “What do you think about aquaculture products?”. Students were asked to talk freely as much as they wanted to express their subjective views and impressions.

Statistical Analysis

Possible differences in the frequency of responses to questionnaire items given by students before and after doing the IMTA hands-on activity were analyzed using the McNemar Test (2×2 tables) or the Chi-square Goodness-of-Fit Test ($2 \times K$ tables).

RESULTS

Experimental IMTA

The values of ammonia, nitrite, and phosphate levels measured in the two IMTA systems at 0, 1, 3, 6, and 12 h after fish feeding are presented in Table 2. The water quality of the laboratorial IMTA system was maintained throughout the 70 days of the experimental trial and no water exchange was needed.

At the end of the trial, no mortality of fish or sea urchins was registered. Fish growth and feed utilization efficiency were within

TABLE 2 | Water quality parameters measured over time in the two experimental laboratorial scale IMTA systems.

		Time (hour)				
Tank		0	1	3	6	12
Ammonia (mg/L)						
Fish	I	0.18	0.22	0.40	0.37	0.28
	II	0.12	0.28	0.50	0.22	0.22
Sea urchin	I	0.19	0.26	0.52	0.39	0.18
	II	0.22	0.34	0.48	0.31	0.12
Seaweeds	I	0.12	0.28	0.20	0.14	0.12
	II	0.18	0.12	0.31	0.26	0.09
Nitrites (mg/L)						
Fish	I	0.27	0.42	0.51	0.72	0.52
	II	0.13	0.38	0.59	0.58	0.48
Sea urchin	I	0.37	0.33	0.34	0.61	0.57
	II	0.22	0.37	0.66	0.60	0.54
Seaweeds	I	0.18	0.24	0.29	0.46	0.34
	II	0.22	0.36	0.41	0.34	0.26
Phosphates (mg/L)						
Fish	I	0.96	1.6	1.8	1.1	0.19
	II	0.64	1.45	1.9	0.85	0.21
Sea urchin	I	1.3	1.3	1.2	1.3	0.24
	II	1.7	1.8	1.3	1.1	0.15
Seaweeds	I	0.80	1.4	0.75	0.54	0.23
	II	1.1	1.0	1.1	0.38	0.13

the expected for this species when reared at 18°C. Sea urchin had a total diameter gain of 38%, relatively to the initial diameter and a total fresh algae intake of 28 g per kg of sea urchin per day was observed (Table 3).

Hands-on Experimental Activity for Students

The hands-on experimental activity was tested with 60 school students. At the beginning of the activity, the students had a brief introduction to aquaculture and were given the questionnaire to fulfill. The IMTA demonstration prototype (Figure 1), already assembled and fully working, was then shown. After discussing the objectives and work to be done in their experimental activity, students switched off the water pump (time zero) and started to measure the levels of ammonia, nitrates, phosphates and pH, every 30 min for the next one and a half or 2 h, depending on the time available. The values were recorded in the experimental log sheet provided to them (Supplementary Material). In between measurements, they assembled a new IMTA system and subsequently discussed the concept, its ecosystem approach, and benefits in terms of environmental sustainability and safety of aquaculture products. At the end of the 2-h measurements, they analyzed and discussed the results obtained and worked on the experimental log sheet.

From the observation of their results, of which an example is given in Table 4, they noted that, after switching off the water pump, some parameters remained stable over time (e.g., temperature). In contrast, there was a progressive build-up of ammonia in the fish and the seaweed aquaria (Table 4), as a result of fish metabolism and excretion. Depending on the biomass level, these changes could be accompanied by alterations in pH (acidification) and the accumulation of phosphates (Table 4).

TABLE 3 | Performance of European seabass, sea urchin and seaweed obtained for the two experimental laboratorial scale IMTA systems.

Tank	I	II
<i>European seabass</i>		
Initial body weight (g)	59.6	60.6
Final body weight (g)	87.5	104
Weight gain (% IBW)	46.7	72.3
Daily growth index ¹	0.76	1.12
Feed intake (g DM kg ⁻¹ ABW day ⁻¹)	8.29	12.6
Feed efficiency ²	0.65	0.60
<i>Sea urchin</i>		
Initial Diameter (cm)	3.60	3.68
Final Diameter (cm)	5.08	5.00
Diameter gain (% initial diam.) ³	41.1	35.9
Seaweed intake (g FW kg ⁻¹ day ⁻¹)	28.1	24.5
<i>Ulva lactuca</i>		
Relative growth rate (1/day) ⁴	0.038	0.025

DM: dry matter; FW: fresh weight; ABW: Average body weight = [(initial body weight (IBW) + final body weight (FBW))/2]. ¹DGI: [(FBW^{1/3} - IBW^{1/3})/time in days] × 100. ²FE: wet weight gain/dry feed intake. ³Diameter gain = [(final test diameter - initial test diameter)/initial test diameter] × 100. ⁴RGR (1day⁻¹) = (ln final fresh biomass - ln initial fresh biomass)/time in days.

After completing the experimental log sheet, the students were asked to fulfill the questionnaire again.

Assessment of the Hands-on Experimental Activity

Socio-demographic information (school grade, age, and education of the participant's parent or guardian) obtained for the 60 students is presented in Table 5. Overall 88.3% of the students were attending the 3rd cycle of the national basic education system (7th grade: 35.0%; 8th grade: 30.0%; 9th grade: 23.3%). Their ages ranged between 12 and 14 years. The remaining students were attending the secondary/high-school level. Participant's parents or guardians were mostly aged between 36–45 years old (41.7%) and 46–55 years old (53.3%). The majority of the participants' parents or guardians (88.3%) had a university education (Table 5).

TABLE 4 | Water quality parameters measured by school students in the IMTA prototype.

Aquarium	Time (min)				
	0	30	60	90	120
Temperature (°C)					
Fish	21.0 ± 0.2	21.0 ± 0.2	21.0 ± 0.2	21.0 ± 0.2	21.0 ± 0.2
Seaweeds	21.0 ± 0.2	21.0 ± 0.2	21.0 ± 0.2	21.0 ± 0.2	21.0 ± 0.2
pH					
Fish	8.0 ± 0.0	8.0 ± 0.0	7.9 ± 0.2	7.7 ± 0.2	7.5 ± 0.0
Seaweeds	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0
Ammonia (mg/L)					
Fish	0.50 ± 0.00	1.0 ± 0.0	1.1 ± 0.3	2.0 ± 0.0	2.3 ± 0.5
Seaweeds	0.50 ± 0.00	0.86 ± 0.21	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.3
Nitrates (mg/L)					
Fish	5.0 ± 0.0	4 ± 2	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0
Seaweeds	4 ± 2	5.00 ± 0.0	5.0 ± 0.0	5.0 ± 0.0	5.0 ± 0.0
Phosphates (mg/L)					
Fish	0.22 ± 0.08	0.3 ± 0.1	0.53 ± 0.08	0.7 ± 0.1	0.75 ± 0.00
Seaweeds	0.22 ± 0.08	0.28 ± 0.08	0.4 ± 0.1	0.50 ± 0.00	0.50 ± 0.00

Values presented as means ± standard error, n = 9.

TABLE 5 | Characterization of students doing the IMTA hands-on activity (N = 60).

Socio-demographic characteristics	Percentage (%)
School grade	3rd cycle of Basic Education 88.3
	High-school 11.7
Age	
Mean ± SD	13 ± 1
Range	(12–18)
Parent's/Guardian's age	26–35 1.67
	36–45 41.7
	46–55 53.3
	56–65 3.33
Parent's/Guardian's Education	Basic 1.67
	Secondary 10.0
	University 88.3

Importance of the ocean to students

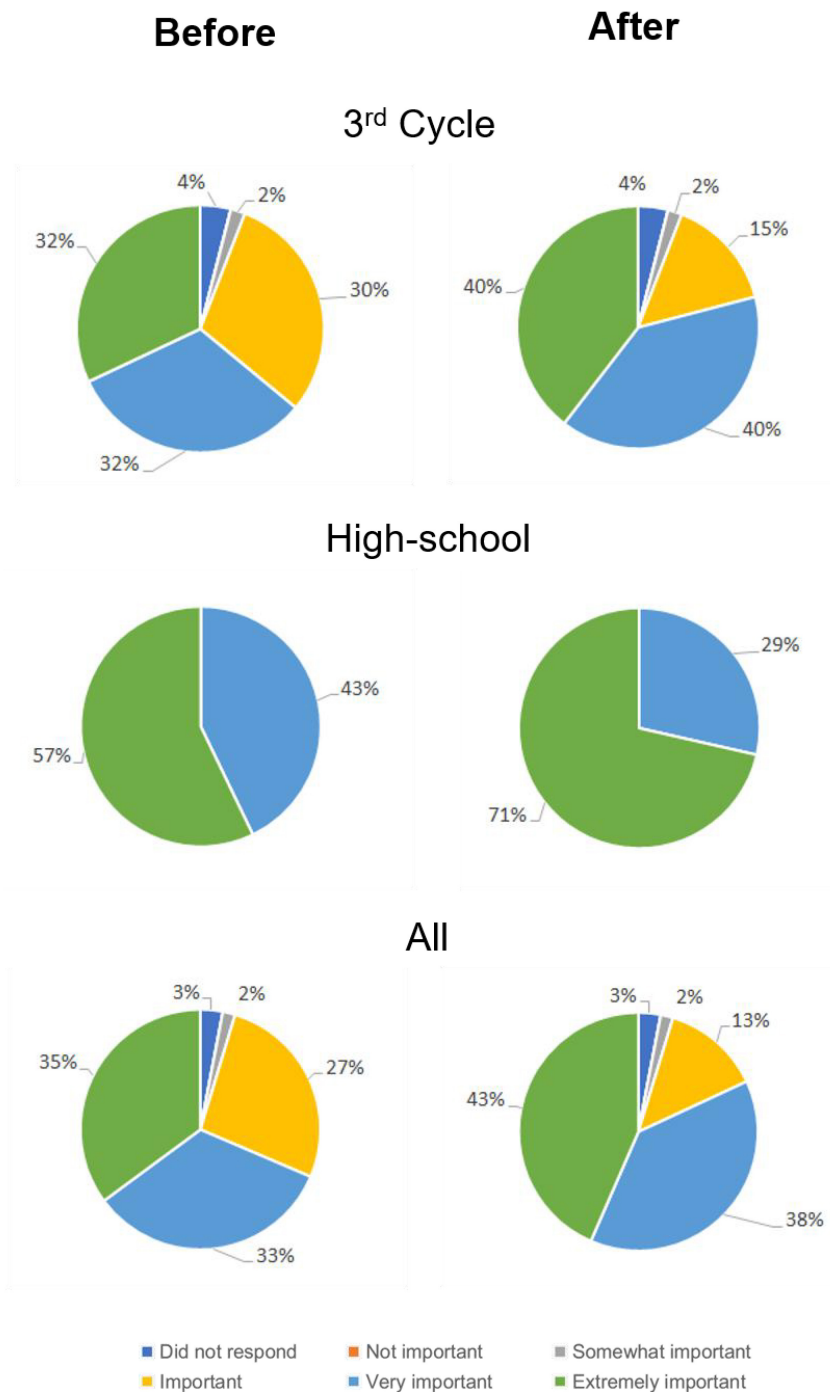
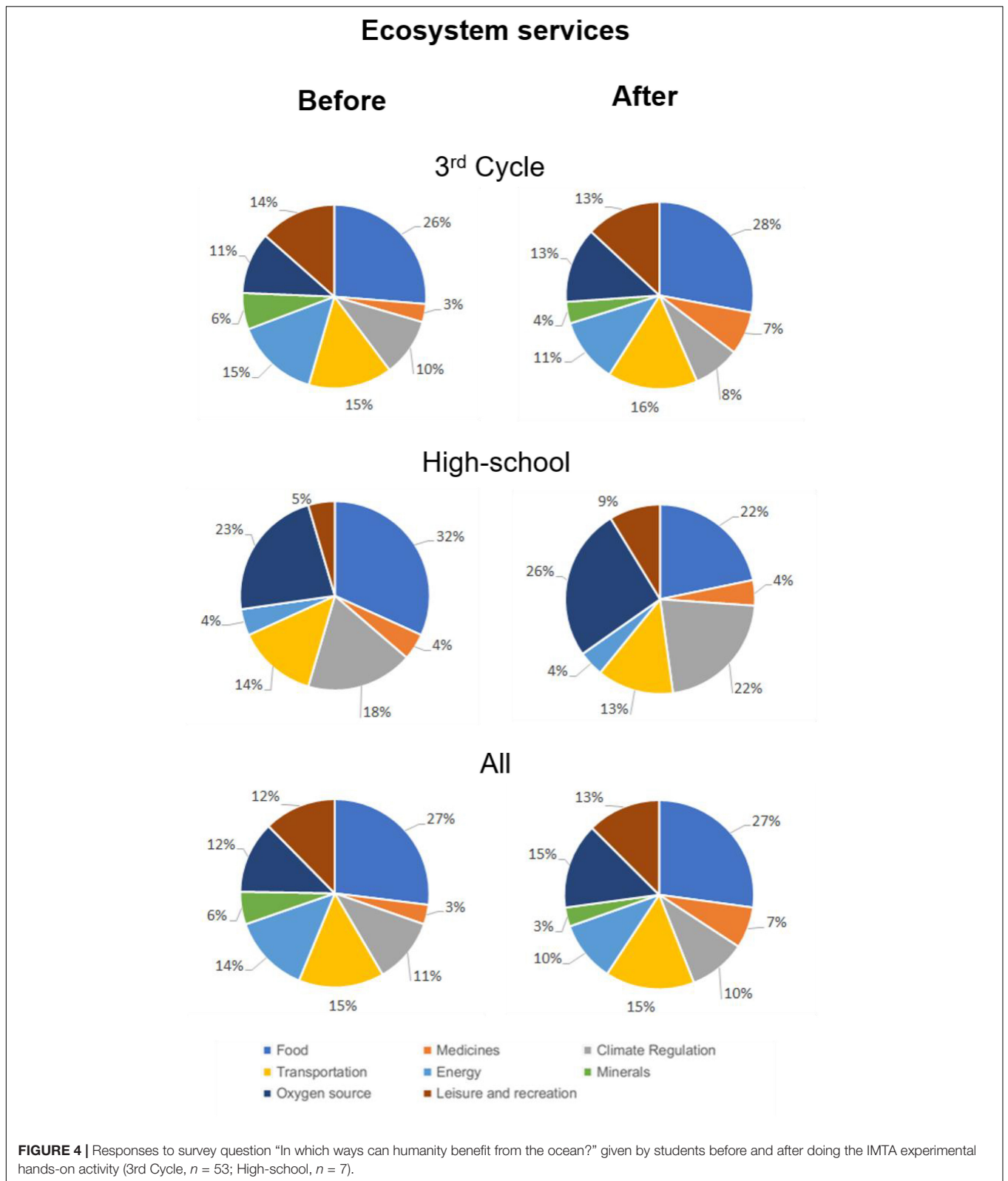


FIGURE 3 | Responses to survey question “How important is the ocean to you?” given by students before and after doing the IMTA experimental hands-on activity (3rd Cycle, *n* = 53; High-school, *n* = 7).

The responses of the students to the questionnaire items are presented in **Figures 3–5**. Statistically significant differences between students’ responses given before and

after doing the IMTA hands-on activity were found for four out of the seven questionnaire items. These were related to the importance of the ocean to the students and

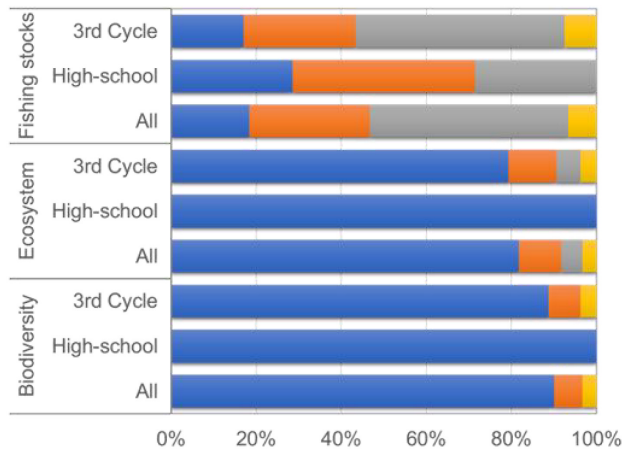


with knowledge and concepts related to aquaculture and its environmental sustainability; fishing stocks, oxygen production and eutrophication (Figures 3, 5).

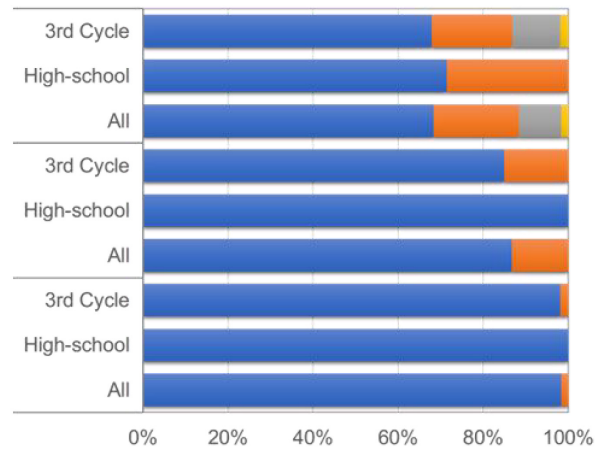
The overall frequency of students considering that the ocean was “very important” or “extremely important” (against “important” or “somewhat important”) to them

IMTA related concepts

Before

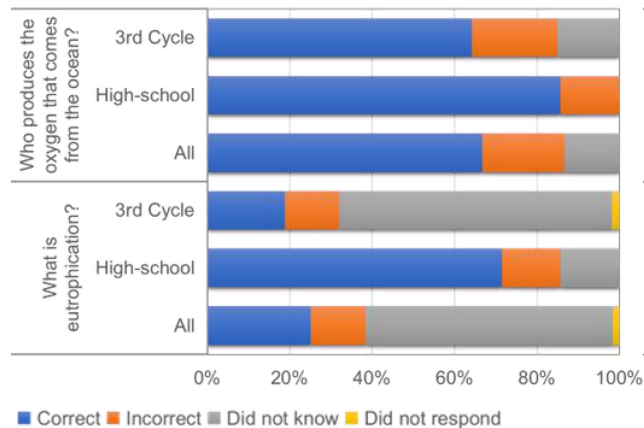


After

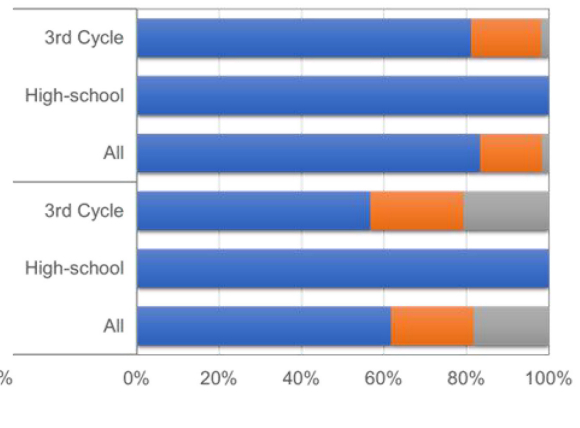


■ I know what it means ■ I've heard of it but I do not know what it means ■ I've never heard of it ■ Did not respond

Before



After



■ Correct ■ Incorrect ■ Did not know ■ Did not respond

FIGURE 5 | Students' responses to survey questions more related to aquaculture and its the environmental sustainability given before and after doing the IMTA experimental hands-on activity (3rd Cycle, $n = 53$; High-school, $n = 7$).

increased from 68 to 81% ($p < 0.001$) after doing the activity (Figure 3). For the youngest students (from the 3rd Cycle and the most representative in the sample) this increase was done at the expense of students previously considering the ocean as 'important' that appear to have changed their opinion after carrying out the activity.

While no significant differences were found in the frequency of responses given before and after the experiment, globally "Food," "Transportation," and "Leisure and recreation" were

considered the most relevant ecosystem services provided by the ocean (Figure 4).

Statistically significant differences were also found for the responses given before and after the activity to the items related to fishing stocks, oxygen production and eutrophication. For the fishing stocks, the percentage of students considering they knew the concept increased by 50% ($p < 0.0001$) after doing the IMTA experimental activity (Figure 5). This global trend was reflected in the responses of 3rd Cycle students who showed a 51% ($p < 0.0001$) increase in their acknowledgment of the

concept. The frequency of students answering correctly to the question about oxygen production increased significantly by over 15% after doing the IMTA hands-on activity, when analyzing the responses of all students ($p < 0.05$) and those attending the 3rd Cycle ($p < 0.05$). Finally, about eutrophication the frequency of correct answers significantly increased from 25 to 62% ($p < 0.0001$) globally and from 20 to 58% in students attending the 3rd Cycle ($p < 0.0001$), after they carried out the IMTA hands-on activity (Figure 5).

After the students ended their experiment log sheet, they were requested to express themselves freely about their results and the activity. To prompt dialogue about their views and the perception gained, the discussion was triggered by the three general questions relating to their thoughts and impressions about the experiment. Overall, the students enjoyed the activity for the possibility of having a hands-on experiment and getting more information about aquaculture products and their quality. Some of them had questions at the beginning of the sessions. These were related to important aspects from a consumer viewpoint, such as (i) the quality and taste of aquaculture fish relative to their equivalents originating from fisheries; (ii) how to know one would be buying “good” aquaculture fish in the market in what concerns its safety and nutritional value to the human diet; or (iii) about the use of antibiotics during the growth process. They considered the experiment was useful to improve their knowledge about environmental sustainability of aquaculture, recent culture methods developed and the use of natural solutions to protect the environment and increase economic viability. They also showed a willingness to share with family and friends their experience and the different views acquired about aquaculture products.

Teachers and researchers working with the students in this experiment expressed that they got very interested and focused in their measurements and in assembling the IMTA kit. They also appreciated the discussions and new knowledge acquired about the quality of aquaculture products and the many developments that have been done to promote fish health and decrease the environmental impacts.

DISCUSSION

Implementation of IMTA systems contribute to the sustainable development of aquaculture and, by using environmentally responsible practices, also contribute to increasing the acceptability of the aquaculture sector. However, awareness about IMTA benefits is still limited, so that educating stakeholders about this practice is of critical importance. The hands-on experimental IMTA prototype presented herein may give a valuable contribution to tackle such educational and awareness needs.

Firstly, a laboratorial-scale IMTA was designed to test the feasibility to maintain in the long term different species, under production conditions, in a closed seawater system. The results showed that the simultaneous production of seabass and sea urchins is feasible, providing an adequate amount of seaweed stocking. It was also shown that the integration of a recirculating

water system (RAS) for fish production, with other crops as sea urchin and seaweed, was efficient to maintain water quality and to reduce the waste load to the environment. The association of these two aquaculture production systems, RAS and IMTA, is relatively new, but has several advantages, allowing a tight control and monitoring of water quality, as recently demonstrated by Chang et al. (2019).

Based on the laboratory IMTA presented, a hands-on experimental activity was developed to address aquaculture and its environmental sustainability with school students. The students carrying out this experiment found it to be attractive because of the possibility to perform a lab activity and the interest of the IMTA system itself. Overall, the results of questionnaires presented to students before and after doing this experiment suggest that it can be effective to improve their connection to the ocean, as well as their knowledge about key concepts such as eutrophication, the role of macroalgae in the production of atmospheric oxygen or fishing stocks. The fact that “Food” was the most frequent response to the questionnaire item related to the services provides to mankind, may be indicative of the high importance that the participants are likely to attribute to Fisheries and Aquaculture. The students were also interested in the possibility of knowing more about aquaculture and clarifying their doubts about the safety of its products, nutritional value or how to buy good quality aquaculture fish.

It is noteworthy, however, that this is a small sample, which may not reflect the wider population of Portuguese students. While the purpose was to obtain a random sample, and no selection of participating students was done, inadvertently the great majority of parents of the students engaged was university educated. This probably happened because most of the participating schools were located in the main urban area of Porto. Urban schools are closer to CIIMAR and usually have more resources for transportation of students than rural schools. The main urban area also concentrates the best employment opportunities for higher educated people. On the other hand, higher educated parents usually have better access to information easily enrolling their children in activities with a limited number of participants. This may have influenced the results of the initial survey that were much better than expected in terms of base knowledge of the participating students. Hence, a more detailed questionnaire should now be prepared to evaluate other relevant aspects related to aquaculture and its acceptability, and conduct an assessment of the activity under an appropriate test-retest evaluation design (Boateng et al., 2018) with a larger sample representative of both the rural and urban environment.

The teachers accompanying their students in this experiment showed their interest in it because of the opportunity to provide their students with state-of-the-art knowledge and developments about aquaculture and important environmental issues. The experiment log sheet was considered useful to consolidate the IMTA experiment, by equating the testing hypothesis, data plotting, and analysis. For natural sciences and biology teachers, this provides also a much-requested tool to address the scientific method, quantitative interpretation, and rigor to prepare their students for their final exams (Saiote et al., 2014). The activity gains additional interest as a

multidisciplinary experiment if involving also the chemistry and mathematics teachers. Chemistry teachers can provide other methods and deeper understanding of water quality parameters, allied to their core biological importance to the cultured species. Math teachers can take advantage of the data gathered by the students to introduce them to inferential statistics, by calculating average values, their corresponding standard deviations, and performing simple statistical hypotheses tests. Philosophy and social science teachers can also be involved by discussing the importance of environmental sustainability for the future of our planet and the social benefits of IMTA, in particular for poorer, marginalized peoples, or those living under tough climate change impacts. In fact, seaweed farming requires low capital and technological investment and can be easily associated to already existing aquaculture production of feed species (Barceló-Villalobos et al., 2017). Furthermore, it works well under small-scale familial farms, where all members of the household can be involved, including still-active elderly (Valderrama et al., 2013). Such an integrated model can further reduce the production costs, increasing employment opportunities for families in deprived areas, enhancing their income, and economic status, while working toward environmental sustainability. Employment opportunity is heightened within the aquaculture value chain by the production of high value products besides human food. A good example is the increasing interest in bioactive compounds extracted from seaweed for the production of products protecting or improving the human health. This includes their use in the cosmetic industry as e.g., photoprotective sunscreen; in medical areas as antioxidants for treatment of disorders related to oxidative stress; or in the dietary supplement industry for inclusion in nutraceutical compounds (Barceló-Villalobos et al., 2017 and references therein). These represent further opportunities for production, processing, distribution, and trading, based on which associations or cooperatives of people not integrated in the big classical business companies can thrive. Further social and environmental benefits of IMTA that can be discussed by the teachers are its recognized importance to increasing resilience to climate changes. IMTA has been identified as an effective strategy to adapt to climate changes in countries experiencing high coastal vulnerability (Ahmed and Glaser, 2016; Ahmed et al., 2019). The combination of ever more frequent extreme weather events (e.g., droughts, floods, cyclones, intense rainfall) with increased salinity, temperature and ocean acidification, which affect marine organisms and ecosystems, can have strong detrimental effects on coastal aquaculture (Ahmed et al., 2019). Coastal IMTA, in particular, is considered a sound ecosystem-based solution by a number of reasons that contribute to counteracting these detrimental impacts (Ahmed and Glaser, 2016). Among those listed by Ahmed and Glaser (2016) are: (i) the oxygen production and absorption of carbon dioxide by the cultured seaweeds; (ii) the ability of shellfish to sequester carbon in the shells; (iii) the culture of euryhaline species, tolerating wide ranges of salinity; and (iv) the ability of shellfish and seaweeds to withstand water temperature variation and clear the water (by

accumulation of pollutants, sediments, and suspended particles) through biofiltration. Globally, compared to industrialized aquaculture, the social dimension of IMTA is much higher. IMTA allows for a better capitalization of natural resources, creates new sustainable products, value chains and markets in support of coastal communities while enhancing resilience to climate changes. Using local resources, IMTA creates new economic niches, increasing the opportunities for local investment and employment, supporting rural development and counteracting the fading of coastal communities. These are all valuable aspects of discussion with students in an interdisciplinary setting.

Overall, the hands-on experiment presented herein is versatile and also easily adapted to the available time. In case of time limitations in the classroom or training sessions, it can be easily used in a show-cooking fashion, as presented in this study, where a system is already assembled and stabilized so that water quality parameters may be immediately measured while in the meantime another system is assembled. The assembling and measurement of water quality parameters and biomass increase can also be planned to be done on different days allowing the students to maintain an interesting exhibition set and investigate the impact of different factors on fish, filter feeders and macroalgae growth. This experiment can also be used in lifelong professional training of teachers and marine educators to provide them with a resource to discuss aquaculture importance and benefits. This IMTA kit is also of expeditious assembling and easy use in museums, public science centers and aquariums during exhibitions or workshops. It helps to introduce the subject of sustainable aquaculture, with the reduction of the environmental problems associated with fish culture (e.g., excess nutrients), while providing additional economic and social benefits to the farmers and the end-users. From our own experience, recognizing its different components and assembling the prototype provides a deeper understanding of ecosystem functioning, ocean sustainability, the blue economy and nature-based solutions to societal challenges. It also promotes easy grasping of basic concepts and principles related to biodiversity, eutrophication, ocean acidification, and water circular economy. For this, it covers Essential Principles of Ocean Literacy (*Understanding the ocean's influence on you and your influence on the ocean*), namely Essential Principle 5 (*The ocean supports a great diversity of life and ecosystems*) and Essential Principle 6 (*The ocean and humans are inextricably interconnected*) (Ocean Literacy, 2013). Both the laboratory IMTA and the hands-on experiment fit also the objectives of the Decade of Ocean Science for Sustainable Development (2021–2030), proclaimed by the United Nations. This initiative is intended to nurture actions aiming at reversing the decline in ocean health and to bring together ocean stakeholders from all over the world to work on a common framework able to ensure that ocean science can fully support the creation of enhanced conditions for sustainable development of the Ocean. Furthermore, IMTA as an environmentally sustainable food production system, addressing the problem of food production, food security, and improved nutrition, is directly related to various Sustainable Development Goals (SDG) of the United

Nations 2030 Agenda; namely, SDG2 (Zero hunger), SDG3 (Good health and well-being), SDG 4 (Quality education), SDG 13 (Climate action) and SDG14 (Life below water). Finally, spreading scientific knowledge on aquaculture, its state of the art methods as well as its safety and quality, is crucial to increase the public perception and acceptability of its products and increase their consumption.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The animal study was reviewed and approved by the CIIMAR Orbea and Direção-Geral de Alimentação e Veterinária (DGAV).

REFERENCES

- Aarset, B., Beckmann, S., Bigne, E., Beveridge, M., Bjørndal, T., Bunting, J., et al. (2004). The European consumers' understanding and perceptions of the "organic" food regime—The case of aquaculture. *Br. Food J.* 106, 93–105. doi: 10.1108/00070700410516784
- Ahmed, N., and Glaser, M. (2016). Can "Integrated Multi-Trophic Aquaculture (IMTA)" adapt to climate change in coastal Bangladesh? *Ocean Coast. Manag.* 132, 120–131. doi: 10.1016/j.ocecoaman.2016.08.017
- Ahmed, N., Thompson, S., and Glaser, M. (2019). Global aquaculture productivity, environmental sustainability, and climate change adaptability. *Environ. Manag.* 63, 159–172. doi: 10.1007/s00267-018-1117-3
- Alexander, K. A., Angel, D., Freeman, S., Israel, D., Johansen, J., Kletou, D., et al. (2016a). Improving sustainability of aquaculture in Europe: stakeholder dialogues on Integrated Multi-trophic Aquaculture (IMTA). *Environ. Sci. Policy* 55, 96–106. doi: 10.1016/j.envsci.2015.09.006
- Alexander, K. A., Freeman, S., and Potts, T. (2016b). Navigating uncertain waters: European public perceptions of integrated multi trophic aquaculture (IMTA). *Environ. Sci. Policy* 61, 230–237. doi: 10.1016/j.envsci.2016.04.020
- Azeredo, R., Machado, M., Afonso, A., Fierro-Castro, C., Reyes-Lopez, F. E., Tort, L., et al. (2017). Neuroendocrine and immune responses undertake different fates following Tryptophan or Methionine dietary treatment: tales from a teleost model. *Front. Immunol.* 8:1226. doi: 10.3389/fimmu.2017.01226
- Bacher, K. (2015). "Perceptions and misconceptions of aquaculture: a global overview," in *GLOBEFISH Research Programme*, Vol. 120 (Rome: FAO).
- Barceló-Villalobos, M., Figueroa, F. L., Korbee, N., Álvarez-Gómez, F., and Abreu, M. H. (2017). Production of mycosporine-like amino acids from *Gracilaria vermiculophylla* (Rhodophyta) cultured through one year in an integrated multi-trophic aquaculture (IMTA) System. *Mar. Biotechnol.* 19:246–254. doi: 10.1007/s10126-017-9746-8
- Boateng, G. O., Neilands, T. B., Frongillo, E. A., Melgar-Quinonez, H. R., and Young, S. L. (2018). Best practices for developing and validating scales for health, social, and behavioral research: a primer. *Front. Public Health* 6:149. doi: 10.3389/fpubh.2018.00149
- Buck, B. H., Troell, M. F., Krause, G., Angel, D. L., Grote, B., and Chopin, T. (2018). State of the art and challenges for offshore integrated Multi-Trophic Aquaculture (IMTA). *Front. Mar. Sci.* 5:165. doi: 10.3389/fmars.2018.00165
- Bureau, D. P., Kaushik, S. J., and Cho, C. Y. (2002). "Bioenergetics," in *Fish Nutrition*, 3rd Edn, eds J. E. Halver, and R. W. Hardy (Cambridge, MA: Academic Press), 1–59.

AUTHOR CONTRIBUTIONS

IA, HP, AO-T, CA, and LG conceived and designed the experiments and analyzed the data. MC and RM performed the experiments and all analytical measurements. All authors contributed to the writing of the manuscript and the reviewing and approval of its final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2020.00156/full#supplementary-material>

- Chang, B. V., Liao, C. S., Chang, Y. T., Chao, W. L., Yeh, S. L., Kuo, D. L., et al. (2019). Investigation of a farm-scale multitrophic recirculating aquaculture system with the addition of *rhodovulum sulfidophilum* for Milkfish (*Chanos chanos*) coastal aquaculture. *Sustainability* 11:1880. doi: 10.3390/su11071880
- Chopin, T., Cooper, J. A., Reid, G., Cross, S., and Moore, C. (2012). Open-water integrated multi-trophic aquaculture: environmental biomitigation and economic diversification of fed aquaculture by extractive aquaculture. *Rev. Aquacult.* 4, 209–220. doi: 10.1111/j.1753-5131.2012.01074.x
- Food and Agriculture Organization [FAO] (2009). "Integrated mariculture - A global review," *FAO Fisheries and Aquaculture Technical PAPER No.529*, 194 (Rome: FAO).
- Food and Agriculture Organization [FAO], (2018). *The State of World Fisheries and Aquaculture-Meeting the Sustainable Development Goals*. Rome: FAO.
- Freeman, S., Vigoda-Gadot, E., Sterr, H., Schultz, M., Korchenkov, I., Krost, P., et al. (2012). Public attitudes towards marine aquaculture: a comparative analysis of Germany and Israel. *Environ. Sci. Policy* 22, 60–72. doi: 10.1016/j.envsci.2012.05.004
- Gatlin, D. M., Barrows, F. T., Brown, P., Dabrowski, K., Gaylord, T. G., Hardy, R. W., et al. (2007). Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquacult. Res.* 38, 551–579. doi: 10.1111/j.1365-2109.2007.01704.x
- Girard, P., and Payrat, T. D. (2017). *Challenges and Opportunities in Using New Technologies to MONITOR SUSTAINABLE FISHERIES*. OECD, *Green Growth Studies*. Paris: OECD Publishing, 28.
- Gormaz, J. G., Fry, J. P., Erazo, M., and Love, D. C. (2014). Public health perspectives on aquaculture. *Curr. Environ. Health Rep.* 1, 227–238. doi: 10.1007/s40572-014-0018-8
- Guest, H., Lotze, H. K., and Wallace, D. (2015). Youth and the sea: ocean literacy in Nova Scotia Canada. *Mar. Policy* 58, 98–107. doi: 10.1016/j.marpol.2015.04.007
- Hughes, A. D., and Black, K. D. (2016). Going beyond the search for solutions: understanding trade-offs in European integrated multi-trophic aquaculture development. *Aquacult. Environ. Interact.* 8, 191–199. doi: 10.3354/aei0174
- Magalhães, R., Lopes, T., Martins, N., Díaz-Rosales, P., Couto, A., Pousão-Ferreira, P., et al. (2016). Carbohydrases supplementation increased nutrient utilization in white seabream (*Diplodus sargus*) juveniles fed high soybean meal diets. *Aquaculture* 463, 43–50. doi: 10.1016/j.aquaculture.2016.05.019
- Mangano, M. C., Ape, F., and Mirto, S. (2019a). The role of two non-indigenous serpulid tube worms in shaping artificial hard substrata communities: case

- study of a fish farm in the central Mediterranean Sea. *Aquacult. Environ. Interact.* 11, 41–51. doi: 10.3354/aei00291
- Mangano, M. C., Giacoletti, A., and Sarà, G. (2019b). Dynamic energy budget provides mechanistic derived quantities to implement the ecosystem based management approach. *J. Sea Res.* 143, 272–279. doi: 10.1016/j.seares.2018.05.009
- Moutinho, S., Martínez-Llorens, S., Tomás-Vidal, A., Jover-Cerdá, M., Oliva-Teles, A., and Peres, H. (2017). Meat and bone meal as partial replacement for fish meal in diets for gilthead seabream (*Sparus aurata*) juveniles: growth, feed efficiency, amino acid utilization, and economic efficiency. *Aquaculture*. 468, Part 1, 271–277. doi: 10.1016/j.aquaculture.2016.10.024
- Ocean Literacy, (2013). *The Essential Principles and Fundamental Concepts of Ocean Sciences for Learners of All Ages, Version 2. 2013*. Silver Spring, MA: National Oceanic and Atmospheric Administration.
- Oliva-Teles, A., Couto, A., Enes, P., and Peres, H. (2020). Dietary protein requirements of fish - a meta-analysis. *Rev. Aquacult.* doi: 10.1111/raq.12391
- Olsen, M. S., and Osmundsen, T. C. (2017). Media framing of aquaculture. *Mar. Policy* 76, 19–27. doi: 10.1016/j.marpol.2016.11.013
- Peres, H., and Oliva-Teles, A. (2005). Protein and energy metabolism of European seabass (*Dicentrarchus labrax*) juveniles and estimation of maintenance requirements. *Fish Physiol. Biochem.* 31, 23–31. doi: 10.1007/s10695-005-4586-2
- Rabanal, H. R. (1988). *History of Aquaculture*. ASEAN/SF/88/Tech.7. Manila: ASEAN/UNDP/FAO Regional Small-Scale Coastal Fisheries Development Project.
- Ridler, N., Wowchuk, M., Robinson, B., Barrington, K., Chopin, T., Robinson, S., et al. (2007). Integrated multi-trophic aquaculture (IMTA): a potential strategic choice for farmers. *Aquacult. Econ. Manag.* 11, 99–110. doi: 10.1080/13657300701202767
- Saiote, J., Correia, M., Larsen, K., Vasconcelos, V., and Guimarães, L. (2014). “CIIMAR at school: hands-on activities in marine and environmental sciences,” in *Hands-on Science. Science Education with and for Society*, eds M. Costa, P. Pombo, and J. Dorrio (Braga: The Hands-on Science Network), 333–337.
- Sarà, G., Gouhier, T. C., Brigolin, D., Porporato, E. M., Mangano, M. C., Mirto, S., et al. (2018a). Predicting shifting sustainability trade-offs in marine finfish aquaculture under climate change. *Glob. Change Biol.* 24, 3654–3665. doi: 10.1111/gcb.14296
- Sarà, G., Mangano, M. C., Johnson, M., and Mazzola, A. (2018b). Integrating multiple stressors in aquaculture to build the blue growth in a changing sea. *Hydrobiologia* 809, 5–17. doi: 10.1007/s10750-017-3469-8
- Shuve, H., Caines, E., Ridler, N., Chopin, T., Reid, G. K., Sawhney, M., et al. (2009). Survey finds consumers support Integrated Multi-Trophic Aquaculture. Effective marketing concept key. *Glob. Aquacult. Advocate* 12, 22–23.
- Steel, B., Lovrich, N., Lach, D., and Fomenko, V. (2005). Correlates and consequences of public knowledge concerning ocean fisheries management. *Coast. Manag.* 33, 37–51. doi: 10.1080/08920750590883105
- Tacon, A. G. J., and Metian, M. (2013). Fish matters: importance of aquatic foods in human nutrition and global food supply. *Rev. Fish. Sci.* 21, 22–38. doi: 10.1080/10641262.2012.753405
- Valderrama, D., Cai, J., Hishamunda, N., and Ridler, N. (eds) (2013). “Social and economic dimensions of carrageenan seaweed farming,” in *Fisheries and Aquaculture Technical Paper No. 580* (Rome: FAO), 204.
- Van Osch, S., Hynes, S., Freeman, S., and O’Higgins, T. (2019). Estimating the Public’s preferences for sustainable aquaculture: a country comparison. *Sustainability* 11:569. doi: 10.3390/su11030569
- Van Osch, S., Hynes, S., O’Higgins, T., Hanley, N., Campbell, D., and Freeman, S. (2017). Estimating the Irish public’s willingness to pay for more sustainable salmon produced by integrated multi-trophic aquaculture. *Mar. Policy* 84, 220–227. doi: 10.1016/j.marpol.2017.07.005
- Whitmarsh, D., and Palmieri, M. G. (2011). Consumer behaviour and environmental preference. A case study of Scottish salmon aquaculture. *Aquacult. Res.* 42, 142–147. doi: 10.1111/j.1365-2109.2010.02672.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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