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# Perspectives for Exploitation of *Sabella spallanzanii*'s Biomass as a New Integrated Multi-Trophic Aquaculture (IMTA) By-Product: Feeding Trial on *Amphiprion ocellaris* Using Sabella Meal

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**Abstract:** This paper is part of a series of studies aimed at understanding the potential exploitation of the biomass of the polychaete worm *Sabella spallanzanii* (Gmelin, 1791), which is obtained as a by-product of an innovative Integrated Multi-Trophic Aquaculture (IMTA) system. IMTA systems are designed according to an ecosystem approach with the aim to reduce marine monoculture impact while further increasing production via exploitation of valuable by-products. *S. spallanzanii* can remove large amounts of suspended matter by filtering large volumes of water per hour and performs well as an extractive organism under IMTA; however, it currently lacks any economic value, thus hindering its sustainable large-scale implementation. However, *S. spallazanii* has the potential to become competitive as a newcomer in fish bait, as an ornamental organism, and in fish feed markets. Notably, sabella meal has already been successfully tested as an attractant in an innovative fish feed. Here, we refer to the use of sabella meal as the main component (60%) in the formulation of a novel aquarium fish feed. Following the biochemical analysis of farmed sabella meal, the experimental feed was formulated by adding spirulina (25%) and dry garlic (15%) in such proportion as to be isoproteic and isoenergetic to the commercial control feed. After preliminary observations of the palatability of sabella meal for several tropical fish species, the novel experimental feed was tested on ocellaris clownfish, *Amphiprion ocellaris* (Cuvier, 1830), by evaluating their growth response in a 70-day feeding trial. The fish seemed to enjoy the experimental feed at least as much as the control, and both the control and treatment groups showed no significant differences in weight gain ($p = 0.46$), specific growth rate ($p = 0.76$), and feed conversion ratio ($p = 0.48$), reinforcing the suitability of *S. spallanzanii* as a viable source of animal proteins to be employed in the fish feed industry in a circular economy perspective.

**Keywords:** sustainable aquaculture; IMTA by-products; fish feed; circular economy



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## 1. Introduction

The mariculture industry has been growing steadily for the past few decades and is likely going to replace fisheries in the next few years as the major supplier of fish and seafood for ever-increasing global demand [1]. Such an expansion has been and inevitably will be taking its toll on the marine environment, mainly in the form of the organic enrichment of both water and sediment compartments, with detrimental effects on biological communities [2–5]. Furthermore, global mariculture has focused predominantly on the farming of high-value carnivorous fish species needing high protein intake, which is currently being met by fish meal. Yet, the overfishing of pelagic fish to produce fish meal for

mariculture feed is a paradox hindering industry sustainability. For these reasons, efforts were made on one hand to develop new aquaculture techniques that would minimize the impact of fish waste, and on the other to search for alternative protein sources for fish meal and/or less protein-demanding fish species to farm [6–8].

The replacement of monoculture by polyculture as Integrated Multi-Trophic Aquaculture (IMTA) systems may be one of the sustainable solutions. IMTA systems are designed according to an ecosystem approach and aim to reduce fish waste accumulation while further increasing production via exploitation of valuable by-products [9,10]. Within the European project "REMEDIA Life", an innovative IMTA system has been developed in the Mar Grande of Taranto (Ionian Sea), with the employment of a new set of extractive organisms, such as polychaetes and sponges, together with mussels and macroalgae, to greatly enhance the overall bioremediation performance [11].

Among the novel extractive species, the polychaete worm *Sabella spallanzanii* (Gmelin, 1791) is one of the promising candidates to help achieve a circular economy. *S. spallanzanii* is a fast-growing sessile species, naturally abundant in the fouling communities of the study area [12,13]. It is able to filter large volumes of water by feeding on suspended organic matter [14–16], and a great amount of organic matter is even compacted with mucus during tube building, thus being removed from the system [17]. Furthermore, the species was proved to be easily culturable at high densities on vertical collectors [11], with evidence for environmental restoration [18].

Notwithstanding its bioremediation capability, *S. spallanzanii* currently lacks any economic value, thus hampering its large-scale implementation as an extractive species in IMTA systems. However, it has the potential to become competitive as a newcomer in fishing bait, as an ornamental organism, and in fish feed markets. Regarding the latter field, previous biochemical analysis performed on wild specimens of *S. spallanzanii* revealed a very interesting aminoacidic composition, quite similar to fish meal, with high protein content [19], and sabella meal was already successfully tested as attractant in an innovative fish feed on juvenile European sea bass *Dicentrarchus labrax* (Linnaeus, 1758) [20].

The present paper is part of a series of studies aimed at understanding the potential exploitation of *S. spallanzanii* in different fields. Here, we refer to the evaluation of sabella meal as aquarium fish feed. Following the biochemical analysis of the farmed worms, sabella meal was utilized as the main component in the formulation of a novel aquarium fish feed and tested on ocellaris clownfish, *Amphiprion ocellaris* (Cuvier, 1830), by evaluating their growth response in a 70-day feeding trial.

## 2. Materials and Methods

### 2.1. Sampling Activities

The utilized worms came from the farming conducted within the "REMEDIA life" project in the Mar Grande of Taranto (see [11] for a detailed description of the IMTA system).

In October 2019, a total of 196 bare collectors for fouling recruitment (10 m long coconut fibre ropes) were placed on the floating long-lines around fish cages for the second farming cycle. In April 2021, at the end of the farming cycle, one entire collector bearing about 1500 worms was sampled (Figure 1). The worms were removed from the collectors and transferred to the laboratory.

### 2.2. Biochemical Analysis

Twenty randomly selected worms were taken out of the tubes and the branchial crowns were removed. The worms were then rinsed with fresh water, frozen at −80 °C, and subsequently lyophilized. Approximately 10 mg of each sample's dry weight (DW) was reduced to ash in a muffle furnace for five hours at 450 °C (Relp 2H-M9). The percentage of ash-free dry weight (AFDW) was then calculated as the difference between DW and the weight of the ash [21]. AFDW was used to normalize the lipid, protein, and carbohydrate data.


(a) (b)

**Figure 1.** (**a**) Underwater picture showing several specimens of *S. spallanzanii* on the collectors; (**b**) phase of collecting worms.

Lipid content: the total amounts of lipids were quantified colorimetrically using 5 mg of DW. The tissue powder was resuspended in 3 mL of chloroform:methanol (2:1) following the Barnes and Blackstock procedure [22], then transformed by Rossi et al. [23]. The calibration line was made using cholesterol as standard and the final reaction was performed using vanillin.

Carbohydrate content: carbohydrates were quantified following the colorimetric method used by Dubois et al. [24]. Glucose was used as standard. Each sample (10 mg of DW) was homogenized in 3 mL of distilled water and total carbohydrates were extracted with a solution of phenol and sulfuric acid (1:5). Results were expressed in µg/mg AFDW.

Protein content: each lyophilized sample was beaten with liquid nitrogen and 3 mg of DW were homogenized in 240 µL of Thiourea/urea/CHAPs buffer (2M thiourea; 4M urea; 4% CHAPS). Proteins quantification in each sample was evaluated using the Bradford assay [25] set for Infinite 200 PRO microplate reader (TECAN, Switzerland) and using bovine serum albumin (BSA) as a standard.

Energy content: the gross energy content was estimated using the extensive general factor system [26], assuming an energy value of 17 kJ/g for proteins, 37 kJ/g for lipids, and 15.7 kJ/g for carbohydrates.

### *2.3. Feed Formulation*

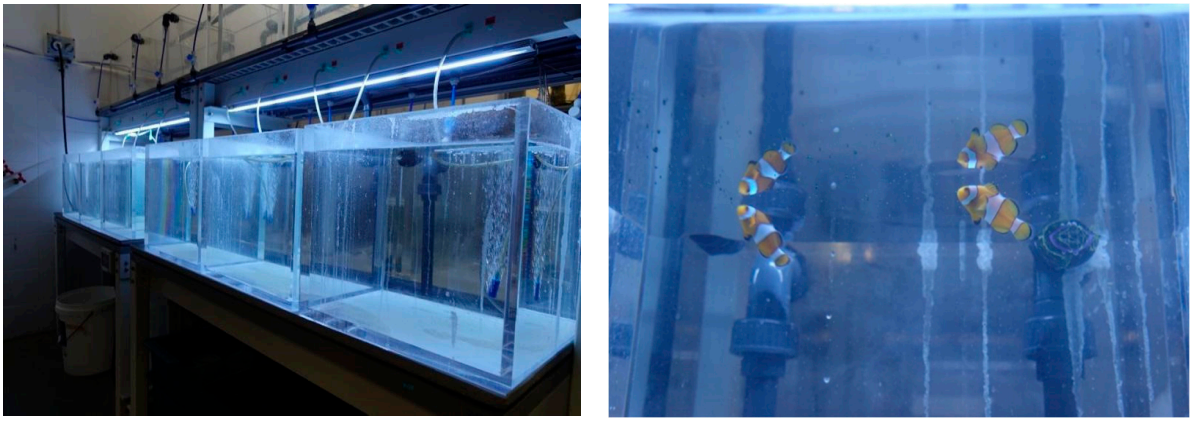
The experimental feed was composed mostly of sabella meal, adding spirulina and garlic as vegetable components (e.g., fibre sources). The relative proportions of the ingredients were computed taking into account the lipid, protein, carbohydrate, and energy content of the commercial feed used as control. The spirulina, *Arthrospira platensis* (Nordstedt) Gomont, commonly used as a dietary supplement, is a cyanobacterium with multiple nutritional and therapeutic properties, rich in proteins (60–70%), vitamins (4%), essential amino acids, minerals, essential fatty acids, carotenoids, chlorophylls, and phytosterols [27]. Dry garlic, which was also present in the control feed, was added as a preservative [28].

To produce sabella meal, the branchial crown was removed once the worms were taken out of the tubes, since *S. spallanzanii* is known to accumulate heavy metals in this compartment as a defence against predation [29]. The worms were then rinsed with fresh water, frozen at −80 °C, and subsequently lyophilized. Dry worms were then pulverized with a grinder. The dry ingredients were mixed in the right proportions 12:5:3 (60% sabella meal, 25% spirulina, and 15% garlic) along with water (final moisture = 10%) in order to obtain a homogeneous dough, which was then pressed through a sieve to obtain pellets of 0.8 mm.

### 2.4. Feeding Experiment

Some preliminary observations on the palatability were carried out on different species of tropical fish thanks to the collaboration of the aquarium shop "ANIMALI D'AUTORE" of Daniele Rizzo. The fish species *A. ocellaris* was selected for the feeding trial. Fish of the genus *Amphiprion*, commonly known as clownfish or anemonefish, belong to the Pomacentridae family and account for approximately 43% of the total traded species [30]. In particular, ocellaris clownfish are the most popular fish among aquarists thanks to their behaviour and beautiful colours, especially since the release of the movie *Finding Nemo* (Nemo is an ocellaris clownfish) by Disney and Pixar in 2003, the so-called "Nemo effect" [31].

A total of 18 individuals of *A. ocellaris* were randomly divided into 6 tanks to perform the feeding trial (3 individuals for each tank). Three tanks (treatment group) were fed with the experimental feed while the remaining three (control group) were fed with commercial fish feed (D-Allio Plus Granulat, Tropical®). The tanks were each 40 L in volume and equipped with aeration (Figure 2). Tanks were all connected within a single water recirculation system equipped with a heater to keep the water temperature constant at 27.00 ± 0.50 °C. Partial water changes of about 30% were performed to compensate for losses due to fish waste removal and evaporation. Salinity and pH were kept, respectively, at 29.04 ± 0.72 psu and 8.09 ± 0.15. Ammonium and nitrite concentrations remained below 0.5 mg/L, while nitrate concentration did not exceed 10 mg/L. Water temperature, salinity, and pH were measured daily using a multiparametric probe (IDROMAR, IP050D). Colorimetric tests (SERA Italia s.r.l.) were used every week for ammonium, nitrite, and nitrate measurements [32].



(**a**) (**b**)

**Figure 2.** (**a**) Empty fish tanks in preparation; (**b**) clownfish feeding on the experimental feed.

The feeding trial started one week after fish acclimatization. Each fish was fed twice a day for 10 weeks with a daily ration feed equal to 4% of their average biomass [33]. The weight of each fish was measured every two weeks until the end of the experiment. Survival rate, weight gain, % weight gain, specific growth rate, and feed conversion ratio were computed to evaluate the growth response according to the following equations:

- Survival rate (%) = (final number of fish/initial number of fish) × 100;
- Weight gain (g) = final individual weight/initial individual weight;
- Weight gain (%) = (weight gain/initial individual weight) × 100;
- Specific growth rate (%) = [(ln final weight − ln initial weight)/feeding days] × 100;
- Feed conversion ratio = total weight feed given/weight gain/feeding days.

Student's *t*-test was performed to test for differences in fish survival rate and growth parameters between control and treatment groups. Prior, Levene's test was performed to verify the homogeneity of variances. When the assumption of homogeneity of variances

was not met, Welch *t*-test was applied. Significance was set at a critical level of 95% ($p < 0.05$). The analyses were performed using STATISTICA 10.0 software package.

## 3. Results

### 3.1. Biochemical Analysis

The protein, lipid, and carbohydrate content of farmed worms, expressed as percentage of ash-free dry weight (AFDW), is reported in Table 1 in comparison with the literature's available data on wild specimens. Farmed *S. spallanzanii* showed a high protein content in line with previous table values of wild specimens. The lipid and ash content differs considerably among the three value sets. Farmed worms were much fatter and with much less ash content than the wild ones.

**Table 1.** Mean content ± S.D. of proteins, lipids, and carbohydrates as % ash free dry weight (% ASDW) in farmed and wild specimens of *S. spallanzanii*.

| | **Farmed *S. spallanzanii*** | **Wild *S. spallanzanii* *** | **Wild *S. spallanzanii* **** |
|---|---|---|---|
| Proteins (% AFDW) | 51.65 ± 5.06 | 54.80 ± 5.80 | 47.20 ± 0.30 |
| Lipids (% AFDW) | 14.42 ± 3.91 | 8.00 | 11.70 ± 1.70 |
| Carbohydrates (% AFDW) | 18.44 ± 2.15 | Not available | 41.10 ± 1.40 |
| Ash (% DW) | 15.20 ± 5.33 | 30.00 | 53.10 ± 4.80 |

* [19]; ** [34].

### 3.2. Experimental Feed Composition

The experimental feed was composed of sabella meal (60%), spirulina (25%), and dry garlic (15%). This composition derives from the need to obtain an isoproteic and isoenergetic feed for the control feed (Table 2).

**Table 2.** Percentage composition and gross energy content (kJ/g) of experimental feed and its ingredients (sabella meal, spirulina, garlic) and control feed.

| | **Sabella Meal** | **Spirulina** | **Garlic** | **Control Feed** | **Experimental Feed** |
|---|---|---|---|---|---|
| Proteins % | 51.65 | 60.00 | 16.55 | 47.00 | 47.03 |
| Lipids % | 14.42 | 1.00 | 0.73 | 7.00 | 7.56 |
| Carbohydrates (fibres) % | 18.44 | 19.80 (7.00) | 72.73 (9.00) | 27.00 (4.00) | 25.65 (3.10) |
| Ash % | 15.20 | 6.23 | 3.54 | 9.00 | 9.76 |
| Moisture % | - | 12.97 | 6.45 | 10.00 | 10.00 |
| Energy (kJ/g) | 17.06 | 13.68 | 14.50 | 14.82 | 14.82 |

### 3.3. Laboratory Experiment

Preliminary observations on several tropical fish species testing sabella meal revealed that the new meal was welcomed by freshly imported fish, which were still acclimatizing and having difficulties in feeding, particularly ocellaris clownfish; therefore, we chose it for the long-term feeding trial.

In the case of the laboratory experiment, observations on fish feeding behaviour showed that they appreciated the experimental fish feed as much as the traditional one. During the experiment, a single event of mortality was observed, occurring in the control group. Both control and treatment groups displayed similar growth trends without significant differences in survival rate and growth parameters ($p > 0.05$). Survival rate ($t_{(2)} = 4.30$, $p = 0.42$), initial ($t_{(4)} = 2.78$, $p = 0.81$) and final ($t_{(4)} = 2.78$, $p = 0.77$) weight, weight gain ($t_{(2)} = 4.30$, $p = 0.46$), % weight gain ($t_{(3)} = 3.18$, $p = 0.66$), specific growth rate ($t_{(2)} = 4.30$, $p = 0.76$), and feed conversion ratio ($t_{(3)} = 3.18$, $p = 0.48$) are reported in Table 3.

**Table 3.** Mean values ± S.D. of survival rate, initial and final weight, weight gain, specific growth rate, and feed conversion ratio of control and treatment groups.

| | Survival Rate (%) | Initial Weight (g) | Final Weight (g) | Weight Gain (g) | Weight Gain (%) | Specific Growth Rate (% per Day) | Feed Conversion Ratio (g/g per Day) |
|---|---|---|---|---|---|---|---|
| Control | 88.89 ± 19.24 | 0.80 ± 0.30 | 0.92 ± 0.29 | 0.11 ± 0.01 | 14.58 ± 5.92 | 0.19 ± 0.07 | 23.98 ± 2.36 |
| Treatment | 100.00 ± 0.00 | 0.86 ± 0.27 | 0.99 ± 0.31 | 0.13 ± 0.04 | 12.71 ± 3.23 | 0.17 ± 0.02 | 21.25 ± 5.31 |

## 4. Discussion

The biochemical analysis performed on farmed worms, obtained as by-products of the IMTA system, confirmed the very high protein content of *S. spallanzanii*, which was consistent with previous studies on wild specimens [19,34]. However, farmed worms have a substantially higher amount of lipids and a lower ash content (Table 1). It is well known that farmed fish, having a feed-based diet with generally higher (10–25%) lipid content [35], are fatter than their wild counterparts [36–38]. The feed employed in the IMTA system (i.e., Royalmarine, 4fish®) has a lipid content of 18% DW, a value consistent with that of farmed worms. Higher lipid levels were also reported for mussels farmed close to fish cages [39]. It is conceivable to assume that the higher lipid content of farmed worms may be related to the different diet composition compared with wild ones, being reflected in a different biochemical composition. Moreover, as a fouling species, wild *S. spallanzanii* preferably lives in enclosed environments under eutrophic and polluted conditions [40], with lower seston quality. By contrast, the worms farmed under IMTA were grown feeding mostly on fish faeces and uneaten fish feed, which were likely energy-richer and with a higher organic content than their natural diet, translating into a less inorganic ash content and higher biomass quality. However, it is fair to point out that Pan et al. [34] performed the biochemical analysis on the whole animal without removing the tubes, thus increasing the inorganic proportion of the sample.

Fish meal production is currently one of the main bottlenecks in the sustainable development of the aquaculture industry, promoting the search for high-quality alternative protein sources [6–8]. It is well known that the considerable content of high-quality proteins with all the essential amino acids makes fish meal a very good ingredient that cannot be replaced, for example, with crop plants, which lack most of these amino acids, such as lysine, methionine, threonine, and tryptophan [41]. Therefore, insect meal has been suggested as a fish meal alternative due to its high protein content; however, it results in a less palatable feed for marine fish [42]. On the other hand, *S. spallanzanii* has a fish-meal-like aminoacidic composition and great amounts of specific amino acids, such as glutamic acid, arginine, and glycine, which may intensify feed flavour, making it more palatable for fish [19]. Moreover, sabella meal was recently proved to have even antioxidant and metal-chelating properties that may reduce heavy metal accumulation in farmed fish [34,43]. Hence, sabella meal was suggested as an attractant in aquaculture feed formulations [20,34]. At present, the amount of sabella meal obtained from biomass produced in the IMTA system is still not enough to meet the demand for feeding aquaculture fish. However, it can substitute the smaller quantity of fish meal utilized in the formulation of aquarium fish feeds.

Information on the nutritional requirements of ornamental fish is scarce despite the economic importance of the aquarium sector [44]; however, the minimal use of feed and the relatively low growth rate are available for some fish species of the family Pomacentridae, such as *A. ocellaris* [32,33,45]. *A. ocellaris* is an omnivorous species [46], and preliminary works on co-family members indicated that clownfish eat up to 63% of algae in proportion to the volume of food consumed, with the ingestion of algae promoting a pH of 2.7 in the stomach, allowing them to break down cell walls [47,48]. Therefore, during the formulation of the experimental feed, spirulina was added as a carbohydrate/fibre source.

In the present trial, ocellaris clownfish seemed to enjoy the experimental feed at least as much as the control one. Both control and treatment groups showed very similar growth trends without significant differences in the tested growth parameters, suggesting that

the experimental sabella-meal-based feed may be a valid substitute for the commercial one. Moreover, during the 70-day feeding trial, clownfish showed growth rates consistent with previous experiments testing clownfish in the same conditions [32,33]. A single mortality event was observed in the control group, which could have had a large influence on the results, given the small number of experimental fish ($n$ = 3). Indeed, the number of fish tested in the present experiment is quite small, increasing the risk of not sufficiently covering the clownfish's individual variability. However, the survival rate showed no significant differences between the two groups. The mortality event had no obvious causes; however, it can be easily assumed that it was not related to the fish diet, given the good health conditions of the remaining fish.

The results of the feeding trial corroborate the suitability of *S. spallanzanii* as a viable alternative source of animal proteins and lipids for the fish feed industry, with the potential to help resolve the paradoxical situation of overfishing pelagic fish to produce fish feed. Furthermore, providing an economic dimension to sabella meal as aquarium feed would encourage the large-scale utilization of *S. spallanzanii* under IMTA systems, with both environmental benefits and economic returns in a circular economy perspective for the sustainability of the whole industry.

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**Conflicts of Interest:** The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this manuscript.

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