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# Macroinvertebrates Associated with Macroalgae within Integrated Multi-Trophic Aquaculture (IMTA) in Earthen Ponds: Potential for Accessory Production

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**Abstract:** The present work aims to evaluate the macroinvertebrate community associated with macroalgae in earthen pond systems to better understand their potential in detritus recycling and as an accessory production. Sampling took place on the settling pond of an aquaculture research station, where macroalgae permanently occurred at high densities. The results suggest differentiation between seasons but not between sites within the settling pond. Seasonal variation was observable in terms of macroinvertebrate density, biomass, and diversity. Two non-indigenous species of invertebrates were found, the crustaceans *Grandidierella japonica* and *Paracerceis sculpta* Amphipods were the most abundant group, and their high nutritional value can be exploited. Detritus and the epiphyte layer are the main food items for the invertebrates, reinforcing the advantages of these organisms being present to enhance the recycling of excess detritus and to transfer organic matter to upper trophic levels. These species, naturally present in aquaculture facilities, can improve the water quality and increase the variability of food nutrients for reared species.

**Keywords:** macroalgae; amphipods; detritivores species; non-indigenous species; feed for reared species

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## 1. Introduction

Integrated multi-trophic aquaculture (IMTA) systems come together as a way of cultivating species using techniques that are both ecologically efficient and environmentally friendly, whilst remaining profitable [1]. This is attained by combining the cultivation, in optimal proportions, of fed species with extractive species, which can be reared at different locations, as long as they remain connected through the water in terms of nutrient and energy transfer [1,2]. Autotrophic species such as seaweeds extract inorganic compounds, filter feeders remove the excess suspended organic materials, and deposit feeders extract the particulate organic matter that accumulates at the bottom [3,4]. Although IMTA might resemble a polyculture, it is actually a precision aquaculture system with significantly higher nutrient utilization efficiency [2] and higher economic gains [3].

IMTA has been implemented in different production systems, such as in suspended cages in estuaries or offshore, as well as in tanks and earthen ponds [3]. Earthen ponds present specific characteristics and conditions, making them comparable with lagoon systems with low environmental variability as long as the water renewal is high [5]. However, the intensification of production and poor management techniques lead to the accumulation of excess organic matter followed by the degradation of the bottom quality and the associated benthic community, although the water column may keep the appropriate quality for fish growth [6,7].

Macroalgae are an important food source to many invertebrates [8] both directly (to herbivores) and indirectly by providing a substrate for the attachment of other organisms and the creation of a periphytic material [9]. Additionally, the increasing physical complexity created by macroalgae increases the available surface area and the density and diversity of associated fauna [10,11]. Therefore, variations in the surface area covered by macroalgae could affect the animal composition associated with them.

Besides their importance as a food source, algae can aid in the treatment of aquaculture effluents by the bioremediation of nitrogenous and phosphorous compounds generally present in those waters whilst enriching them by releasing $O_2$ [12]. Therefore, the integration of macroalgae within aquaculture systems is beneficial both for the algae and the organisms present. The effect of bioremediation is particularly efficient in static systems such as those present in decantation ponds [13].

Macroinvertebrate fauna contributes to high secondary production in estuaries and coastal lagoons, which can be used, for example, by reared fish [14]. This is particularly important in the early stages of development, and the most used ones have been the small rotifers and copepods and the larger *Artemia*, which can grow up to 12 mm long [15]. However, these can prove to be either of low nutritional value or hard to produce, hence creating the need to procure different alternatives [16,17]. Other macroinvertebrates have been used to study their potential in aquaculture, both as live and formulated feeds [18–20].

The main objective of this work was to acquire knowledge regarding the invertebrate community in macroalgae within a bio mitigation pond of an IMTA system of earthen ponds. The community was studied to understand the role of macroalgae for small invertebrates and the perspective of using the latter as an accessory production. A previous study, performed at EPPO, showed that the macrobenthic community in fish earthen ponds is diverse and mainly composed of polychaeta, amphipods, and insects [21]. However, no information exists on macroinvertebrates associated with macroalgae in fish aquaculture earthen ponds. Macroalgae frequently attain high densities and biomasses in reared ponds, making their manual removal necessary [22].

## 2. Materials and Methods

### *2.1. Sampling Area*

This work was conducted at IPMA's (IPMA—Instituto Português do Mar e Atmosfera) aquaculture research station (EPPO—Estação Piloto de Piscicultura de Olhão), located in Olhão, Portugal. The station is composed of a water reservoir connected to the Ria Formosa, a pumping system which provides water for 17 production ponds, and a settling pond where water outflows back to the natural environment. The study was performed on the settling pond, rich in nutrients, with an approximate area of 3700 $m^2$. In this pond, macroalgae are abundantly present throughout the year and no removal actions take place, making it the appropriate place to study the macroinvertebrate community associated with macroalgae.

### *2.2. Sampling and Sorting of Macrofauna*

Sampling was undertaken in three different periods: Autumn, Winter, and Spring. For each sampling period, four replicates were performed, distributed evenly across the pond. Sampling was always carried out during the morning, between 9 a.m. and 12 p.m. At each sampling occasion, using a Hanna multiparameter waterproof meter, the following parameters were measured: water temperature, salinity, dissolved oxygen (%), and pH.

Macroalgae and associated macroinvertebrate quantitative sampling was carried out using an O-shaped sweep net with a 20 cm opening mouth and a mesh size of 500 µm. The net was raised vertically from a depth of about 35 cm to the surface, resulting in a total sampled water volume per replicate of 0.011 $m^3$. The samples were subsequently filtered with the help of a mesh sieve (500 µm) to retain macroinvertebrates and macroalgae.

All invertebrates were handpicked and preserved in 70% ethanol for later taxonomic analysis. Furthermore, the macroalgae collected were wet-weighed (g) followed by drying at approximately 70 °C for 24 h to obtain their dry weight (g). All measurements were carried out using a precision balance with two decimals.

Benthic macroinvertebrates were sampled during the same sampling occasions, with a PVC corer of a 0.011 $m^{-2}$ internal diameter, following the same methodology as in previous studies [23]. Four replicate samples were taken in the pond. The sediment was sieved with a 1 mm mesh, and the organisms were sorted out and preserved in 70% ethanol until identification and quantification. The identification of species was made to the lowest taxonomic level possible. The sediment granulometry and organic matter content were assessed on the first sampling occasion, from three replicate samples taken in the first two cm of sediment. The sediment particles' sizes were analysed by laser diffraction and the organic matter of the sediment by ignition loss (EN 13039).

Invertebrates associated with macroalgae were wet-weighed using a precision balance after the removal of excess water with absorbent paper. The total biomass of each taxon was estimated by multiplying the average individual weight by the total number of individuals.

The total number of invertebrate species, density (individuals per volume as well as individuals and per algae dry weight), biomass (g $m^{-3}$), and species richness were estimated. Seasonal variations in dried algae biomass (galgaeDW) and density were analysed. Regarding benthic macroinvertebrates, estimations on density and diversity were carried out. To evaluate the possible seasonal or site differentiation of invertebrate communities associated with macroalgae, multidimensional scaling (MDS) was carried out using the Bray–Curtis dissimilarity index after square root transformation, utilizing Primer-e software (V 6.1.13).

## 3. Results

### 3.1. Environmental Parameters

Dissolved oxygen was always high, above 84%. Temperature and salinity had higher mean values during Spring, following seasonal variations (Table 1).

**Table 1.** Mean values of water temperature, salinity, dissolved oxygen, and pH by season of sampling in the settling pond of EPPO.

| | Autumn | Winter | Spring |
|---|---|---|---|
| Temperature (°C) | 17.4 | 16.4 | 24.3 |
| Salinity | 35.8 | 35.7 | 37.1 |
| Dissolved Oxygen (%) | 108.9 | 100.2 | 101.0 |
| pH | 8.1 | 8.0 | 8.3 |

### 3.2. Macroalgae

Two types of algae were collected, 11 samples of Ulva spp. and 1 sample of Chaetomorpha sp., the latter sampled on the fourth Winter replicate (Table S1). The algae biomass decreased from Autumn to Spring (Figure 1). The dominant algae, *Ulva*, can occur in various species and hence, it is difficult to differentiate among them (*Ulva clathrata*, *U. flexuosa*, *U. intestinalis*, *U. prolifera*, and *U. torta*) [24]. The scatter distribution of *Ulva* spp. dry and wet weight showed a positive linear relationship (DW = 0.0814 WW), with a Pearson correlation coefficient = 0.95 ($p < 0.001$) (Figure S1).

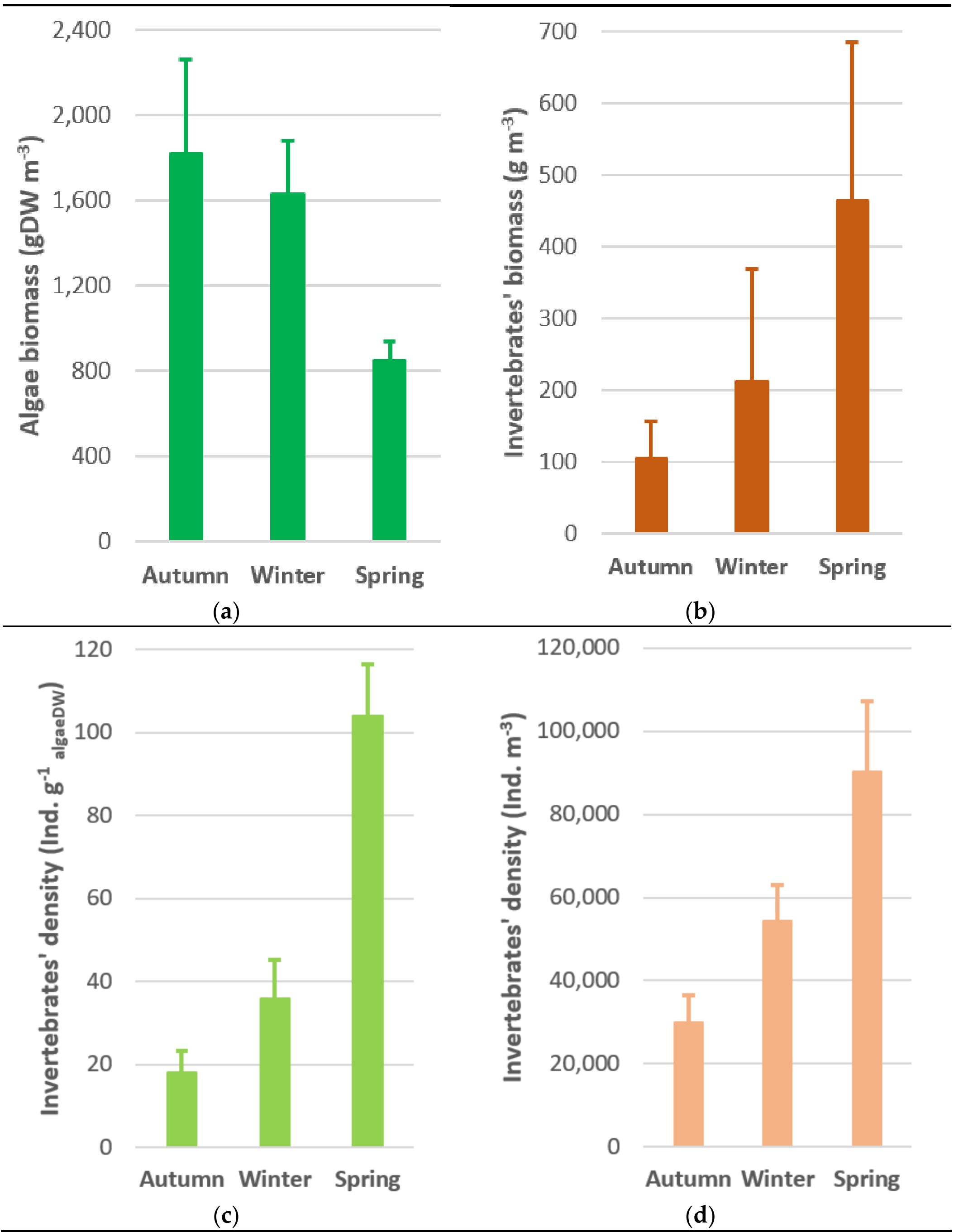



**Figure 1.** Seasonal variation plots with standard error for (**a**) dried algae biomass by cubic metre of water; (**b**) invertebrates' biomass by cubic metre of water; (**c**) invertebrates' density by gram of algae dry weight; (**d**) invertebrates' density by cubic metre of water.

### *3.3. Macroinvertebrates Associated with Macroalgae*

Arthropoda was the most abundant phylum, with a mean density of $42 \times 10^3$ individuals per volume of water (ind. $m^{-3}$, ≈72%), followed by Mollusca with $14 \times 10^3$ ind. $m^{-3}$ (≈24%) and Cnidaria with $2.3 \times 10^3$ ind. $m^{-3}$ (≈4%). The other groups, Annelida, Chordata, and Echinodermata, contributed less than 0.3% (Table S2). Total species richness varied between 16 (Winter), 19 (Autumn) and 22 (Spring) (Table S3).

Both the density of individuals by volume of water and by algae dry weight (DW) attain higher values for Spring replicates (Figure 1). Although there was a decrease in dry algae biomass throughout the sampling periods, from Autumn to Spring, the biomass and density of invertebrates per dry weight of algae and by volume of water increased. Variation in density by algae DW seems to be more accentuated between Winter and Spring, whereas density by volume of water is smoother.

The seasonal scatter distribution, represented in Figure 2, of taxa richness and the number of individuals per algae DW was used to study the relationship between these variables. The number of species sampled increased with the quantity of algae sampled. A positive relationship is observable between the nr of individuals and algae DW (g), although the goodness of fit is low for Autumn and Winter (20.27% and < 0.01%, respectively) and high for Spring (76.72%).

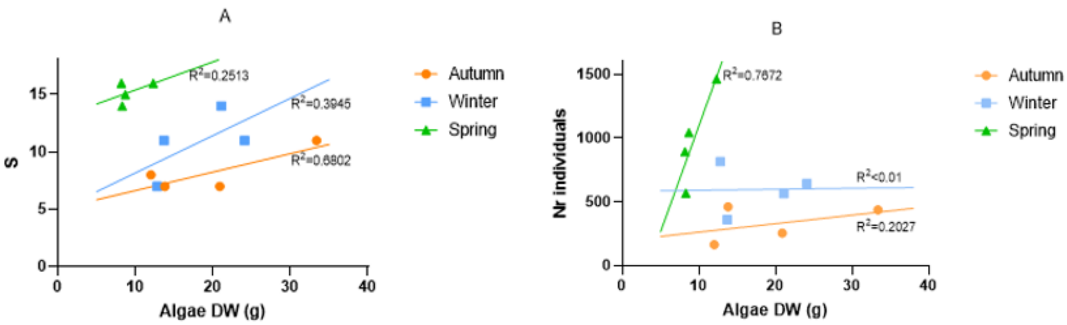
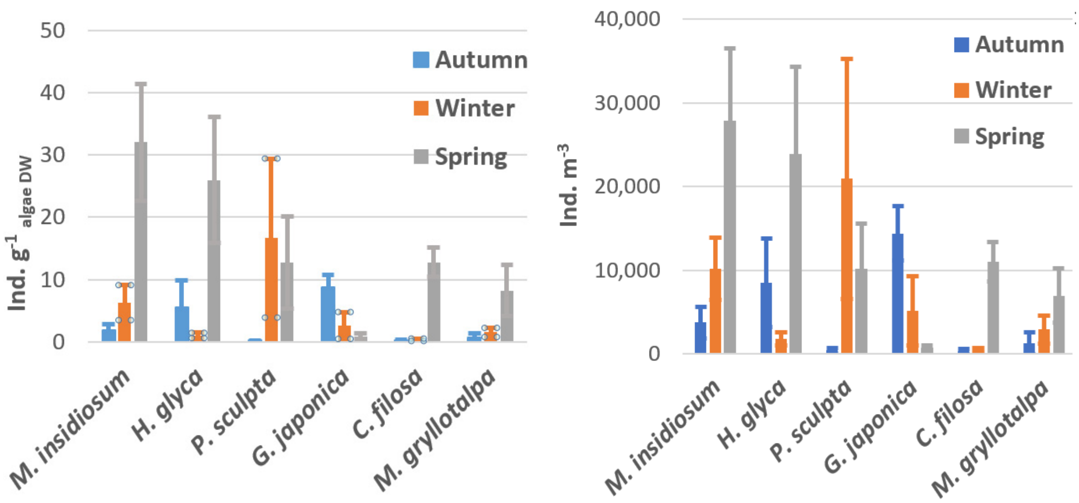



**Figure 2.** Scatter plots of taxa richness (**A**) and number of individuals (**B**) per algae dry weight (DW) based on season. A: Autumn (Y = 0.1608x + 5.030); Winter (Y = 0.3252x + 4.921); Spring (Y = 0.2446x + 12.96). B: Autumn (Y = 6.681x + 196.0); Winter (Y = 0.7874x + 582.9); Spring (Y = 166.2x − 565.5).

The amphipods *Monocorophium insidiosum* (Crawford, 1937), *Cymadusa filosa* (Savigny, 1816), and *Microdeutopus gryllotalpa* (Costa, 1853) were increasingly abundant from Autumn to Spring, whereas *Grandidierella japonica* (Stephensen, 1938) showed the opposite trend. The gastropod *Hydrobia glyca* (Servain, 1880) was almost absent in Winter, reappearing in Spring, and the isopod *Paracerceis sculpta* (Holmes, 1904) peaked in Winter samples. Both units of density show similar results, although the error observed is smaller for density per algae DW (Figure 3). *G. japonica* and the isopod *P. sculpta* are non-indigenous species.




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

**Figure 3.** Variation, per season, in the six most abundant species (*Monocorophium insidiosum*, *Hydrobia glyca*, *Paracerceis sculpta*, *Grandidierella japonica*, *Cymadusa filose* and *Microdeutopus gryllotalpa*). (**a**) Density per gram of dry algae and (**b**) density per cubic metre.

Analysis through multidimensional scaling (MDS) shows a seasonal separation of the samples. It was possible to observe a grouping of samples from the same season due to their higher similarity (Figure 4). Sample Win4 was very distant from the others, probably as a reflection of it being the only sample of *Chaetomorpha* sp. present in the study.

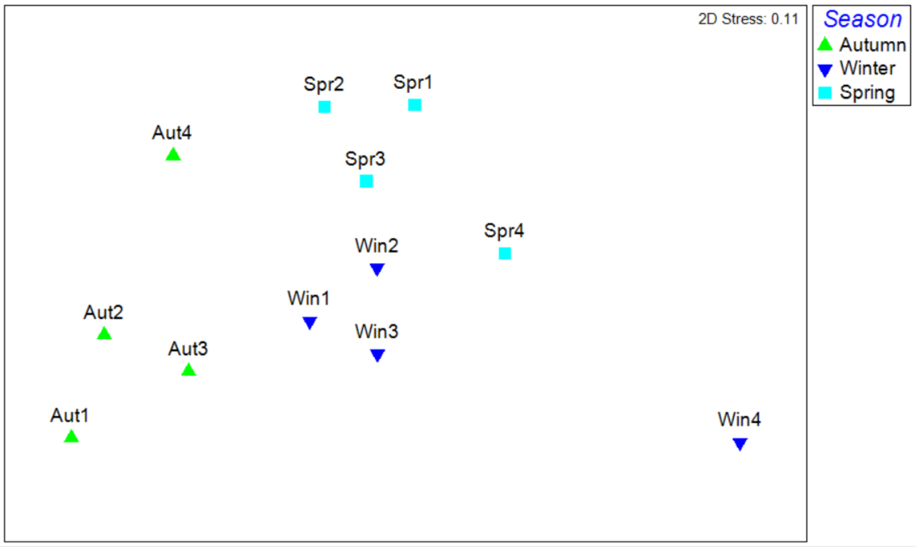
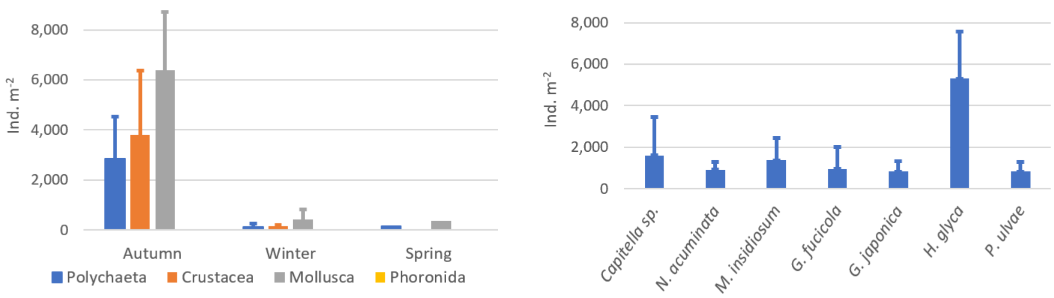



**Figure 4.** Multidimensional scaling (MDS) carried out with macroinvertebrates associated with a macroalgae abundance dataset using Bray–Curtis similarity index and root transformation. Four samples were collected for each season: Autumn (Aut1–4), Winter (Win1–4), and Spring (Spr1–4).

### 3.4. Benthic Macroinvertebrates

The benthic macroinvertebrates sampled in Autumn presented an average density of 13 $\times$ $10^3$ individuals $m^2$ but decreased sharply in Winter and Spring to approximately 0.6 $\times$ $10^3$ individuals $m^{-2}$ (Table S2). Molluscs were always the most abundant group (Figure 5). Species richness also decreased sharply in the same period, from 21 taxa in Autumn to 7 in Winter and 8 in Spring (Table S4). The sediment changed from a compact brown colour to a very dark and disaggregated sediment during the last two sampling occasions. The first sampling occasion, when granulometric analysis was carried out, revealed a silty sand sediment (41.1% sand, 40.7% silt, and 18.2% clay) with 3.5% organic matter. The gastropods were the most abundant group, represented by *Hydrobia glyca* and *Peringia ulvae* (Pennant, 1777). The polychaetes *Capitella* sp. and *Neanthes acuminata* (Ehlers, 1868) and the amphipods *Monocorophium insidiosum*, *Gammarella fucicola* (Leach, 1814), and *Grandidierella japonica* were also well represented (Figure 5b).




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

**Figure 5.** (**a**)Variation per season in the main taxonomic groups; (**b**) density of the most abundant benthic macroinvertebrates in Autumn sampling (*Capitella* sp., *Neanthes acuminata*, *Monocorophium insidiosum*, *Gammarella fucicola*, *Grandidierella japonica*, *Hydrobia glyca*, and *Peringia ulvae*).

## 4. Discussion

### *4.1. Sample Characterization and Species Composition*

The measured water parameters did not vary greatly, except water temperature, which showed some differences in values, ranging from an average of 16.36 °C in Winter to 24.27 °C in Spring. The dissolved oxygen saturation was high, probably caused by the abundance of algae in the pond and the time of sampling (between 10 a.m. and 12 p.m.).

Macroalgae, present within all EPPO IMTA's earthen ponds, work as a substrate for macroinvertebrates. In the settling pond, a total of 7678 individuals were sampled, with an average density of $58 \times 10^3$ ind. $m^{-3}$, showing a community composed mainly of Arthropoda and Mollusca, which is in accordance with another study performed on different macroalgae as a substrate for invertebrates [25]. Previous studies, performed on the benthic community in earthen ponds [21,23], show slightly different results, which is expected due to differences in environment and substrate.

Some similarities were found between the macroinvertebrate community associated with macroalgae and the benthic community. There were species common to both habitats, such as the gastropod *Hydrobia glyca*, which represented 19.5% of the total organisms found in the macroalgae habitat and 40.6% of total benthic invertebrates. Some amphipods were also well represented in both habitats, such as *Monocorophium insidiosum* and *Grandidierella japonica* (24% and 11.6% in the macroalgae habitat and 9.8% and 6.1% in the benthic habitat, respectively).

Some of the dominant species in the benthic macroinvertebrate community were also found to be the dominant species in previous studies in the same earthen pound, such as the polychaetes *Capitella* sp., *Neanthes acuminata* and *Microdeutopus gryllotalpa*. However, some taxa were not found, such as Chironomid larvae, cited as abundant in previous studies [23,26]. On the first sampling occasion (Autumn), the density of organisms was comparable with the density found by Gamito [23] in the old saltern that originated the actual EPPO research station (Site A in Gamito's publication). However, the sharp decrease observed in the community density and species richness in the next two sampling occasions (Winter and Spring) may reveal a degradation of the bottom quality, due to the excessive accumulation of organic matter. Interestingly, the macroalgae invertebrate community, present only a few cm above the bottom, seemed not to be affected by this degradation. It is important to note that the settling pond does not have aeration which, coupled with the large quantity of algae and organic matter, may result in events of anoxia at the bottom during the night.

Two non-indigenous species were found: the amphipod *Grandidierella japonica* and the isopod *Paracerceis sculpta*. The latter has already been reported in the Iberian Peninsula and the Ria Formosa, near Faro [27]. The species is known as the most widespread species within the genus and shipping and recreational boating are referred to as the main vectors [27]. In contrast, and to the best of our knowledge, this is the first record of *G. japonica* in the Ria Formosa. The species was reported to be spreading on the west coast of France [28,29] and present in the Adriatic Sea [30]. The main vector of introduction is suggested as the commercial transfer and production of *Crassostrea gigas* [30,31]. Since this oyster species is present in the research station and widespread in the Ria Formosa, we can assume this may be the vector of introduction.

### *4.2. Macroalgae as a Substrate and Habitat for Macroinvertebrate Epifauna*

The macroalgae was dominated by amphipods, with 4082 organisms ($31 \times 10^3$ ind. $m^{-3}$) distributed by 10 species. The corophiid *Monocorophium insidiosum* was the most abundant of them. This species is characterized by building tubes of mud on macroalgae and being a selective deposit feeder of suspended particles [32]. Sheader [32] suggested two generations per annum for this species, reflected by peaks of recruitment in June and February, with most individuals in Autumn being in a "resting" immature state. The same can be proposed for the population in our study since the peaks found for this species occurred in Winter samplings (February–March) and especially in Spring (June).

Seasonal influence within the macroalgae invertebrate community can be observed. Autumn had the lowest values of biomass, density, and diversity, whereas Spring was characterized by the highest values of those indicators. For most of the seven more abundant species, it is also possible to infer that some of them have seasonal variation, reflected in changes in the dominant species per time of the year. MDS ordination plots confirmed the seasonal variation in the macroinvertebrate community and the lack of spatial differentiation since the pond was evenly covered with macroalgae.

This study showed that macroalgae are valuable substrates for epifauna, with a diverse community. From the two species of macroalgae collected, *Ulva* spp. was the most abundant over the course of all samples taken on the pond. The relationship between algae dry weight and macroinvertebrate abundance, per season, shows a positive relationship, although with low values of goodness of fit ($R^2$) for Autumn and Spring. A study of macroalgae complexity in the epifaunal community [33] shows that the relationship between algae dry weight and the number of individuals varies depending on the species of algae, with a positive relationship between macroalgae dry weight and epifaunal taxa richness. The same tendency was observed in this study since a larger quantity of sampled seaweed resulted in a sample with higher species variability and a greater number of organisms.

Algae was observed as a direct source of shelter for species such as *C. filosa* which, at the time of sampling, was usually seen wrapped around the edges of the *Ulva* spp. collected, in accordance with observations on species tube building in *Ulva lactuca* [34]. However, algae may not only be useful as a structure for the settlement and growth of these organisms but also as a source of food, both directly and indirectly. The present study shows amphipod species known for occupying a large spectrum of feeding habits in trophic pathways, although most of them are detritivores [35,36].

### *4.3. Invertebrates as Feed for Aquaculture*

There are few studies on the culture of these small invertebrates for feeding [37]. Their protein, lipid, and carbohydrate contents are adequate for feeding reared fish, crustaceans, and molluscs [38]. Species present in this study such as *P. ulvae*, *M. palmata*, *Corophium* spp. and other gammarideans have already been reported in the diet of the sole (Solea senegalensis) from their nursery area in the Tagus estuary [39], proving that some of these organisms can be a nutritional source of live feed for relatively small individuals. Benthic macroinvertebrates, as well as the macroinvertebrates associated with macroalgae, have been reported as being selected by common fish from the Ria Formosa lagoon and of interest to aquaculture, such as the gilthead seabream *Sparus aurata*, which preferentially selects hard-bodied prey such as gastropods and bivalves, although the juveniles prefer soft-bodied prey such as crustaceans, while other seabreams (*Diplodus vulgaris* and *D. annularis*) preferentially select crustaceans such as amphipods, isopods, and also gastropods [40,41].

Several other studies show the importance of macroinvertebrates in the diet of many wild fish and cephalopods of interest to aquaculture. Some examples are sole and seabass (*Dicentrarchus labrax*), two species well established in the industry, as well as some ornamentals such as seahorses and even emerging species such as cuttlefish and octopus [20,42–44]. These studies also show that different species of invertebrates are predated at different development stages of the predator. Keeping in mind the possible accessory production of invertebrates, it is essential to know the community and its variations. This may also aid in the planning of uses for these invertebrates, taking into consideration the needs of the targeted fish/cephalopod species being reared, as well as the harvest of such invertebrates, according to different development stages of the reared species.

Macroalgae are often collected and discarded when in excess [22]. Future research could explore the feasibility of retaining macroinvertebrates by filtering the water used to wash the collected macroalgae. Farmers producing macroalgae in IMTA could utilize this byproduct instead of discarding it. In southern Portugal, farmers have reported enhanced growth and improved flesh quality in gilthead seabream in semi-intensive pond farming when macroalgae are supplied alongside with artificial feed. This improvement could be attributed to both the macroalgae and the associated macroinvertebrate community, but further detailed studies are needed to confirm this.

### *4.4. Macroinvertebrate Role in Nutrient Recycling and in the Trophic Web*

Amphipods have been referred to as a group of organisms occupying several levels of the trophic web, but detritus remains the principal food item in the majority of species, especially detritus from animal matter and epiphytes, strengthening the advantages of these organisms being present with macroalgae [36]. Hence, amphipods are not only relevant as a nutritious source included in fish's diets but also in the uptake of organic waste as biofilters, emphasizing their potential as an accessory production in integrated multi-trophic aquaculture where they can be used to enhance water quality and later converted into a source of protein [45].

Most macroinvertebrates associated with macroalgae are thus detritivores, helping to recycle excess detritus in the pond and accumulating biomass on their bodies that can be transferred to upper trophic levels such as other macroinvertebrates or fish and birds. In a multi-trophic production pond, these invertebrates may be an important source of feed for some species, and the high densities of macroalgae increase nutrient absorption and prevent phytoplankton development in excess [6], improving water transparency and quality.

In recent IMTA experiments, it was inferred that macroalgae present in the rearing ponds have a positive impact on fish growth and production and may point towards a more sustainable aquaculture [6,7]. Furthermore, the invertebrates associated with macroalgae can be an additional source of food for some reared fish species in multi-trophic aquaculture.

## 5. Conclusions

The dominant group of organisms in the macroalgae community were amphipod species, characterized by direct development with low individual biomass and high growth rates, characteristic of r-strategists or opportunistic species, allowing them to cope with the high anthropogenic organic loads typical of aquaculture systems and resist to a sharp degradation of the bottom due to the excess accumulation of organic matter. Most of the macroinvertebrates are detritivores, being an important link in the trophic web by recycling excess detritus and transferring organic matter to higher trophic levels.

The periodic removal of macroalgae from culture ponds, a usual management action in earthen ponds, needs to be conducted carefully, and some of the macroalgae and associated macrofauna should be left behind to allow important ecosystem services to be performed, such as nutrient uptake, the recycling of detritus, and the transfer of organic matter to upper trophic levels.

The macroinvertebrate associated with macroalgae strengthens the idea of a multi-trophic approach where organisms previously undervalued could be important both in environmental impact reduction and later potentiated into a protein source. Seasonal variation in organisms was observable both in terms of species composition and particularly in terms of densities. For example, Spring was characterized by a fivefold increase in density in comparison to Autumn. If exploited, these populations could lead to more than one production per annum, with different species harvested depending on the time of the year. Another advantage is the fact that different species of invertebrates could be used in the different lifecycle stages of reared species or be an additional feed in multi-trophic aquaculture.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse12081369/s1. Table S1 and Figure S1. Macroalgae sampled in each replicate (0.011 $m^3$) and sampling occasion and weight wet/dry weight relationships. Table S2. Total number of macroinvertebrates sampled organized by Phyla, in both the associated macroalgae and the bottom (benthic) macroinvertebrate communities. Average density (ind. $m^{-3}$) in the macroalgae community and average density (ind. $m^{-2}$) in the benthic community. Table S3. Macroinvertebrates in macroalgae—the number of individuals sampled in each replicate (0.011 $m^3$)

and sampling occasion along with their individual wet weight, as well as species richness for each sampling period. Table S4. Benthic macroinvertebrates—the number of individuals sampled in each replicate (0.011 $m^2$) and sampling occasion, as well as species richness for each sampling period.

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