

Article

Isopods in the Bioremediation of Invasive Seaweeds? First Experience with the Seaweed *Rugulopteryx okamurae* Mass Dumped on Beaches

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Abstract: Since 2015, the invasive seaweed *Rugulopteryx okamurae* has triggered the most serious marine macrophyte invasion in Europe. Its huge coastal biomass dumped on beaches generates health problems, strong odors, impacts on tourism, and high clean-up costs, but it also constitutes a new potential marine resource that offers significant prospects for exploitation. In this sense, as a circular economy strategy, seaweed bioremediation with isopods (Isopoda, Crustacea, Arthropoda) was experimented on for the first time. Specimens of *Porcellio laevis* (native terrestrial isopod) were obtained from urban parks and kept in terrariums with adequate humidity and temperature control. A sample of 150 adult specimens was divided into six batches of 25 animals. Three batches were fed with 100 g of mulch of *Quercus pyrenaica* leaves (control) and three with a diet composed of 100 g of mulch of algae (treatment). *P. laevis* consumed up to 1.5 times their weight per day on the algae diet, with little or no weight loss and adequate reproduction rates. The weight of the isopods averaged 1.6 g in the seaweed group and 2.5 g in the control group. However, high mortality was observed in both manca and adults in the treatment group. In this sense, the average number of mancas per cm² was 0 in the algae group and 325 in the control group. Despite this, the results are promising, and consequently, we propose to encourage research with isopods due to their high voracity, high prolificacy, and resistance to invasive algal recycling. Future work should explore what percentage of *R. okamurae* prevents diterpene mortality of isopods and other invertebrates. This is a preliminary step towards the massive bioremediation of spring and summer blooms of *R. okamurae*. This work contributes to highlighting this abundant marine resource.



Academic Editor: Azizur Rahman

Received: 26 November 2024

Revised: 18 December 2024

Accepted: 25 December 2024

Published: 26 December 2024

Citation: Patón, D.; García-Gómez, J.C. Isopods in the Bioremediation of Invasive Seaweeds? First Experience with the Seaweed *Rugulopteryx okamurae* Mass Dumped on Beaches. *J. Mar. Sci. Eng.* **2025**, *13*, 12. <https://doi.org/10.3390/jmse13010012>

Correction Statement: This article has been republished with a minor change. The change does not affect the scientific content of the article and further details are available within the backmatter of the website version of this article.

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Keywords: *Porcellio laevis*; *Rugulopteryx okamurae*; bioremediation; biological invasion; algal blooms

1. Introduction

In 2015, the invasive alga *Rugulopteryx okamurae* burst into the Strait of Gibraltar (Figure 1), with unprecedented impact compared to other macrophyte bioinvasions that have occurred in European waters [1]. This species has also spread along the Spanish Mediterranean coast, southern France, and continental marine areas of Portugal [2] as well as in the Azores, Madeira, and Canary Islands [3]. The relentless competitive capacity for space of this alga, its rampant growth, and its ecosystem-scale impact have been

well documented [2,4]. The algae biomass dumped on beaches of thousands of tons per year generates important health and economic problems (cleanliness and tourism) that do not find effective ways to be alleviated. In fact, we can affirm that we are facing a new, very abundant marine resource that invites us to try new paths for its possible sustainable exploitation.



Figure 1. Massive *Rugulopteryx okamurae* biomass dumped on the beaches of the coast of Tarifa (Strait of Gibraltar) in June 2023. In the lower right photo, note the accumulated volume of algae, above the knees, of those collecting samples for this study.

In the field of circular economy, the composting of algae offers well-founded perspectives to mitigate the problem, but *R. okamurae*, given the potential toxicity by diterpenes, salinity, and an extreme resistance to the decomposition of its outcrops, represents a real challenge for science. The composting of this seaweed would allow us to take advantage of its massive biomass dumped on beaches from an economic point of view by creating companies to process this waste. In this regard, different composting strategies of the aforementioned species with free-living microorganisms and invertebrates have been tested [5]. Although different composting techniques have been used with *R. okamurae* algae, the use of invertebrates is highly advantageous since it produces an increase in compost quality that is not achieved with more traditional techniques [5]. In the invertebrate bioremediation, the diterpenes of the algae cause earthworm mortality, and only the insects seem to tolerate the toxicity of *R. okamurae*. These other insect bioremediation systems have proven to be effective, although they are difficult to implement in the long term because they are very sensitive to temperature. An advantage of isopods is to be able to guarantee their bioremediation under more extreme environmental conditions. However, bioremediation

with isopods (Isopoda, Crustacea, Arthropoda), which in principle could be interesting due to the proverbial ability of these invertebrates to recycle a wide variety of substrates, has not yet been tested. In fact, this is the first time that bioremediation with isopods on seaweed has been tested, and the results are promising. Terrestrial isopods are invertebrates with very peculiar functional characteristics that must be known in order to optimize their management and exploitation, especially the potential of different species to process various organic wastes or the husbandry and handling requirements of some species on an industrial scale.

The species studied in this paper, *Porcellio laevis*, is native to Europe [6–8] and was accidentally introduced through the plant trade; it is behaving as an invasive species [9]. This isopod is found under natural conditions in humid and shady microhabitats of the Iberian Peninsula and island areas [10,11].

Terrestrial isopods, such as *P. laevis*, are attracting increasing attention for organic waste treatment or for their role in bioremediation [12]. The trophic versatility of terrestrial isopods, their high reproductive rate, low maintenance cost, and ease of breeding make them ideal candidates for these roles [13]. Another functional characteristic that makes the isopod *P. laevis* perfect for waste recycling is its broad-spectrum detritivorous foraging behavior [14,15].

In the present research, we explore the bioremediation potential of *P. laevis* in the recycling of the toxic algae *R. okamurai*. The algae's toxicity is primarily due to its diterpene content, specifically dillkamural [5]. This work explores for the first time the potential of the Order Isopoda (Crustacea, Arthropoda), with the objective of contributing to offer sustainable exploitation solutions, through bioremediation by the terrestrial isopod *P. laevis* for the tons of biomass of the invasive seaweed *R. okamurai* thrown onto the beaches. In particular, in the present work, we studied the consumption, growth, and reproductive capacity of *P. laevis* subjected to standard and seaweed diets. Our results allow us to conclude that it is an appropriate species for processing *R. okamurai* waste.

2. Materials and Methods

The isopods under study were obtained during 2018 in the “Parque del Príncipe” in the city of Cáceres (southwestern Spain; lat 39.472943° lon −6.383403°) under stones and logs. The animals were transferred to the Environmental Biotechnology Laboratory of the Faculty of Sciences of the University of Extremadura, Spain, where they were kept until their complete acclimatization and rearing, which lasted about three months.

From the date of capture of the initial population until 2022, thousands of captive-bred specimens were obtained and distributed in six 60 × 40 × 32 cm plastic boxes with lids and mesh-sealed slots, to which we added 250 g of blatticompost (cockroach compost), drinkers, and bark to create the necessary microclimatic conditions. The function of the blatticompost is to serve as an absorbent substrate for the excess moisture that will be gradually released as the box environment dries out. This maintenance of a high level of environmental humidity is basic for rearing many species of isopods, but it is important not to reach waterlogging [16]. The drinking troughs were developed with plastic bottles equipped with a cotton wick that allows water to ooze slowly, creating the necessary humidity gradient for this species, recommended by professional breeders.

Three replicates of the control group (without the toxic alga *Rugulopteryx* and with a 100 g mulch of *Quercus pyrenaica*) and three replicates of the treatment group (with 100 g *Rugulopteryx*) were prepared. *Q. pyrenaica* leaves were selected because they form part of the habitat of *P. laevis* under natural conditions. Every three days, mulch and bark were removed from each box, and the blatticompost substrate was sprayed with water until completely moist but not waterlogged. Then, mulch and bark were added again, as they

serve as moist, dark shelter for the isopods. In each box, 25 adult isopods bred in our laboratory from the original wild strain were added. Once a week in each box, 5 g of dog feed (Royal Cannin) was provided to provide some additional protein (~20%), and every 15 days, 5 g of finely powdered eggshell to add the necessary calcium since these animals are crustaceans and their exoskeleton presents high levels of calcium carbonate [17].

The residue, consisting of a mixture of blatticompost, degraded mulch, and isopod excrements, was also weighed. The mulch and residue were weighed fresh. A 2 g sample was extracted from the mulch and residue, and the dry weight was calculated using an OHAUS MB35 halogen balance equipped with a 120, 240 VAC 50/60 Hz infrared desiccation lamp. The desiccation temperature ranged from 41 to 104 °C, and the sample was held until weight stabilization, which took about 10 min. The dry weight value was extrapolated to the entire sample to determine the total dry weight of the mulch and remnant.

When sieving the substrate, the mancae pass through it, so the remnant with white mancae was spread on a dark tray and photographed. Subsequently, the manual particle counter of the image analysis program ImageJ 1.54j [18] was used, and the data were transferred to density per square centimeter (Figure 2). Once these weighing and counting operations were completed, everything was put back in the same order: (1) blatticompost with the mancas was added; (2) this substrate was moistened; (3) mulch (leaves or algae) and bark were added; and (4) adult isopods were added. Mulch and compost biomass data over days were plotted in graphs in the control and treatment groups. Polynomial regressions of degree 2 were determined in both groups by calculating the R^2 values, F value, and significance of the regression parameters. Feed conversion rate (FCR) was determined in both groups (with and without algae) thanks to inter-weekly differences in adult weight, substrate, and mulch. The mean differences in isopod weight in both groups as well as the survival of adults throughout the study period were established, applying a linear regression model with the R^2 statistic, F-ratio, and significance of the regression terms. The density of mancas per square centimeter was determined in both groups. The data were transferred to spreadsheets (two groups: control and algae) and analyzed using the Wilcox test [19] in R [20].



Figure 2. The counting of *Porcellio laevis* mancas (right) on the substrate using ImageJ's particle counting tool.

3. Results

During the study period, a more pronounced decrease in the percentage of mulch was observed in the oak leaf treatment than in the algae treatment (Figure 3). As the leaf substrate almost disappeared on day 40 after the start of the study (t0), we discontinued the trial. The equations of both degree two polynomial models are shown in Table 1. The same model was used in the group with and without algae for comparative criteria. However, in the algae treatment, a simpler linear regression model would have been equally appropriate (Figure 3) because although both models (polynomial and linear) fit well, the significant terms are only those of degree one and the constant (Table 1).

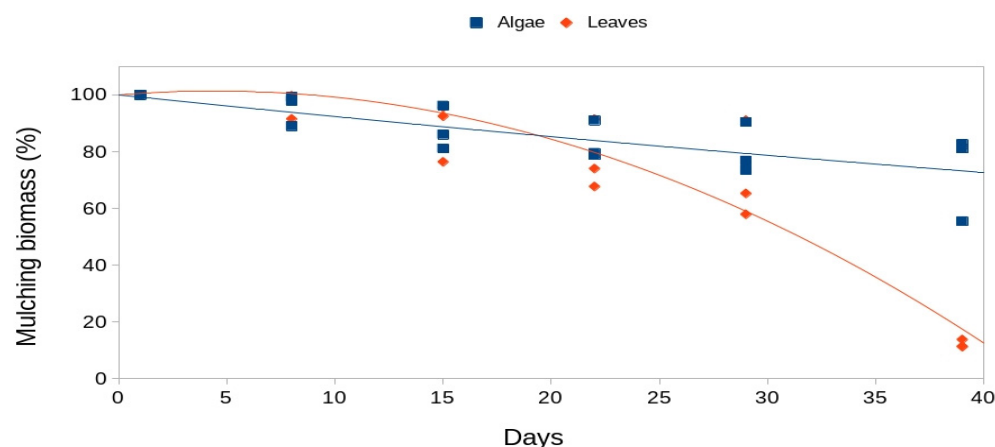


Figure 3. The decrease in the biomass in percent after consumption by *Porcellio laevis* in both types of mulch.

Table 1. Degree two polynomial models for changes in mulch biomass over days of exposure. **: p -value < 0.01; ***: p -value < 0.001.

Parameter	Algae	Leaves
Model	$\text{Biomass}(\%) = 86.61 - 22.04 \times \text{Days} + 1.73 \times \text{Days}^2$	$\text{Biomass}(\%) = 74.43 - 65.06 \times \text{Days} - 28.24 \times \text{Days}^2$
Fitted R^2	0.986	0.933
F-statistic	179.7 ***	35.84 **
Significant terms	Intercept, one degree term	All

Regarding the blatticompost substrate, consumption is very similar in both groups (with and without algae) (Figure 4, Table 2). This indicates that the presence of algae does not condition substrate consumption. In this case, polynomial models of degree three were applied, indicating a sharp drop in substrate volume during the first two weeks, a stabilization phase, and a slight drop during the last week (Figure 4). These models were significant in all polynomial terms for both types of mulching.

Regarding the FCR data, the Wilcoxon test showed non-significant differences ($W = 33$, p -value = 0.371) for both groups of mulching (leaves and algae). However, looking at Figure 5, we see that these differences are not significant because of the high dispersion of the FCR data in the leaf mulch group. Higher FCR values are shown when the food is of lower nutritional value [21]. This is confirmed by the analysis of the weight of the animals between both types of mulch, as there are significant differences ($W = 6$, p -value = 0.023, Figure 6).

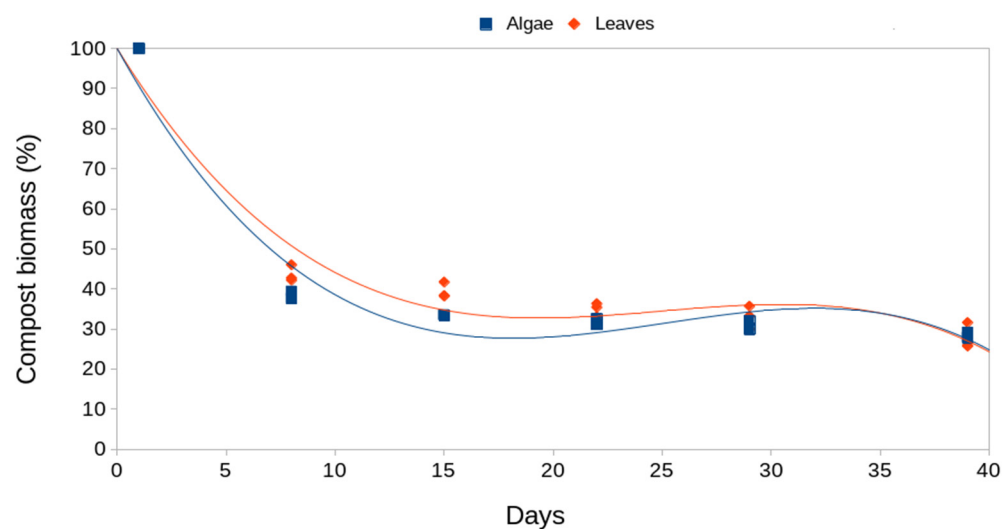


Figure 4. Decrease in percentage of blatticompost substrate over time for both types of mulch (with and without algae).

Table 2. Polynomial models of degree three for changes in biomass of the blatticompost substrate over the days of exposure and for both types of mulch (with and without algae). **: p -value < 0.01; ***: p -value < 0.001.

Parameter	Algae	Leaves
Model	$\text{Biomass}(\%) = 43.71 - 77.11 \times \text{Days} + 59.66 \times \text{Days}^2 - 40.93 \times \text{Days}^3$	$\text{Biomass}(\%) = 46.44 - 80.55 \times \text{Days} + 51.21 \times \text{Days}^2 - 36.21 \times \text{Days}^3$
Fitted R^2	0.958	0.954
F-statistic	130.20 ***	119.00 **
Significant terms	All	All

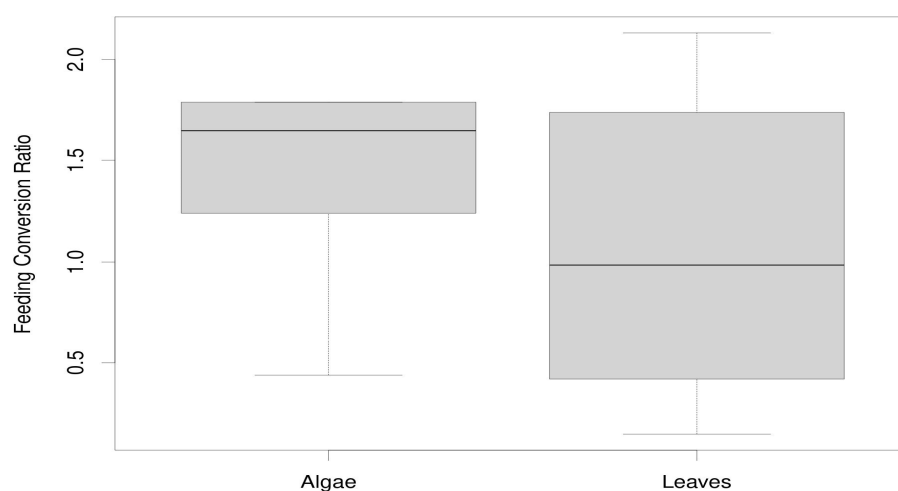


Figure 5. Box plots of feed conversion rate (FCR) for both types of mulch.

For both types of mulch, adult survival fits reliable linear regression models (Figure 7 and Table 3). Algal mulch produces lower adult survival (Figure 7). If we extend the regression line, 100% of adult individuals would die in the algal mulch around day 49 (only 9 days after the duration of the experiment). This would correspond (in the leaf mulch) to 108 days after the start of the experiment, which is the expected longevity for the species.

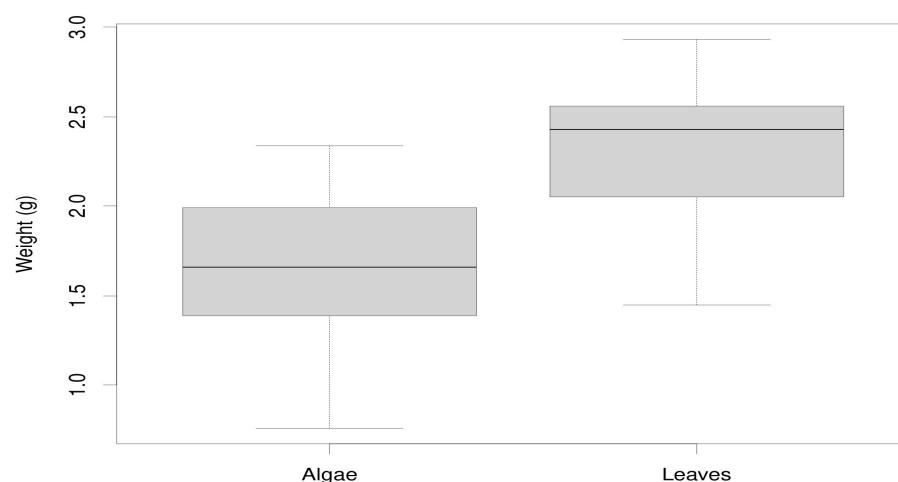


Figure 6. Differences in isopod weight for both types of mulching.

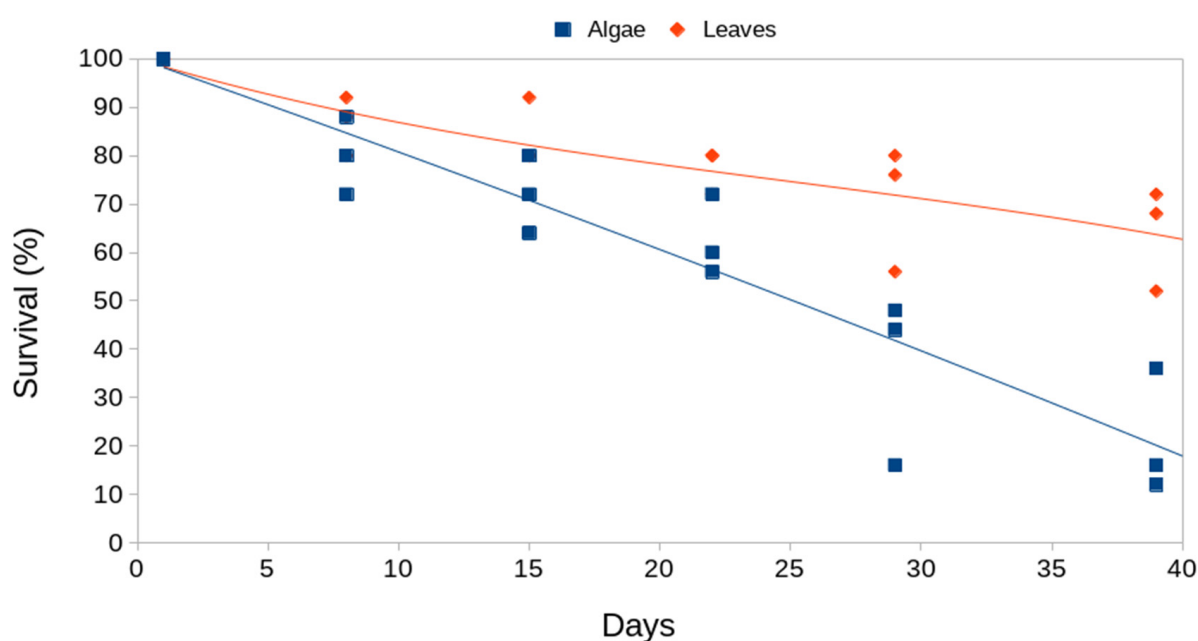


Figure 7. The survival of *Porcellio laevis* adults throughout the study period for both types of mulches.

Table 3. Linear regression models for changes in adult isopod survival over days of exposure and for both types of mulch. ***: p -value < 0.001.

Parameter	Algae	Leaves
Model	Survival (%) = $101.05 - 2.06 \times \text{Days}$	Survival (%) = $97.50 - 0.90 \times \text{Days}$
Fitted R^2	0.875	0.717
F-statistic	119.9 ***	44.03 ***
Significant terms	All	All

Regarding the appearance of mancas, these were only observed in small numbers in the boxes with seaweed mulch from day 29 of the trial. On the same day, a large number of mancas appeared in the boxes with leaf mulch. Differences in the number of mancas between the two groups were highly significant according to the Wilcoxon test ($W = 0$, p -value = 0.005). Our results for leaf mulching indicate a really high number of mancas per square centimeter (Figure 8).

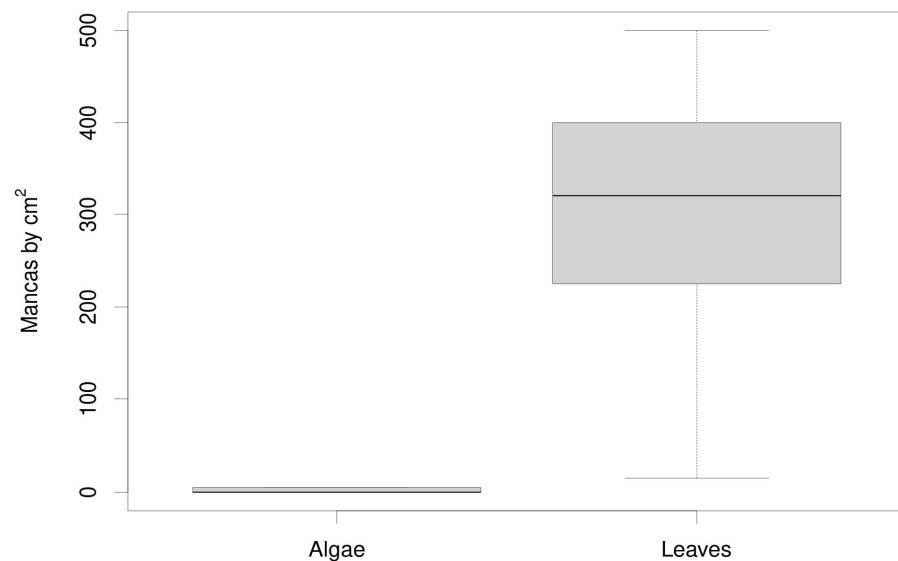


Figure 8. The effect of the mulch type on mancas production per square centimeter in the isopod *Porcellio laevis*.

4. Discussion

In this work, the isopods studied were divided into two groups: treatment (algae) and control, with three replicates in each, which is the minimum number to obtain averages. Nevertheless, the results were conclusive. The present work analyzes the consumption by *Porcellio laevis* of the invasive toxic alga *Rugulopteryx okamurae* [22]. The consumption of mulch (algae or leaves), the consumption of blatticompost, the growth of isopods, and their reproduction have been determined. The results obtained clearly indicate that *P. laevis* consumes more leaf substrate, which is its natural diet, but the batch with the algae also consumes it, although in smaller quantities. However, algae do not affect compost consumption, which is similar in both groups (treatment and control). We can also conclude that the seaweed provides more nutrients than the dry leaves, and therefore, less is consumed. This makes sense since the seaweed is supplied fresh, i.e., with all its nutrients. Most likely this is due to the fact that while they have an organic substrate (in our case, blatticompost), the isopods feed on it, and only when it becomes scarce do they begin to consume the algae. We should not forget that isopods are detritivores, i.e., they consume partially degraded food. This would also explain why they use less fresh material. When these consumption data are converted to the feed conversion rate (FCR), we observed greater variability in the leaf mulch group. It is possible that this mulching is not uniform at leaf maturity and hence the greater variability in the data. Adult weights are notably higher in the leaf-feeding group. Undoubtedly, the seaweed is difficult to digest because of its terpenes, but despite them, it is consumed, so we can conclude that toxicity has a slight effect on consumption. Diterpenes are a group of highly variable compounds, but those with an antifeedant effect dominate [23]. In this sense, the diterpene dilkamural present in *Rugulopteryx* is extraordinarily toxic according to previous studies, showing increased mortality in sea urchins [24]. It is quite possible that the isopods balance the trade-off between toxicity and nutrient supply from the algae, resulting in the lower consumption observed.

The survival data show the same tendency and that there is an effect of the algae on the viability of the adults. The major effect of the algae is on the mancas, which are noticeably smaller in number in the algal batch. Decreased reproductive rates and increased adult mortality may be attributed to the toxic components (diterpenes) of the algae and/or their salinity, which is less than 1 g per kilogram [24]. In this regard, a previous study by [25] with

a similar species, *Porcellio scaber*, indicates that salt itself is not a problem for the viability of these animals at rates up to 5 g per kilogram, so it is questionable whether this could be the cause of the observed loss of reproductive viability. Therefore, the consumption of seaweed is conditioned to the use of adults only, which could hinder the use of isopods in industrial farms to compost the seaweed. Despite this, isopods are one of the invertebrates most resistant to algal toxicity, as previous studies with other invertebrates, such as black soldier flies, mealworms, or compost cockroaches, have shown [5]. This toxicity of the algae is due to its richness in diterpenes and specifically in dillkamural, which has an important antifeedant effect [24].

If we want to develop a realistic *R. okamurai* recycling large-scale strategy, we must consider the waste as a whole. In a scenario of thousands of tons of annual algae biomass dumped on beaches, it is not feasible to pre-treat the algae. Given that in the feeding of isopods, the seaweed has been mixed with blatticompost, whose salt contents are much lower (<2%) [26], we consider that salt should not be the problem. Rather, we believe that the diterpenes in the algae is responsible for inhibiting the reproduction of adults and increased mortality of the manca. We know that most terpenes have an antifeedant effect, and dillkamural present in seaweed is no exception [24]. This conclusion is supported by the fact that we have observed similar phenomena in *Tenebrio molitor* larvae fed with this invasive alga [5]. Therefore, isopods present us with an interesting scenario in recycling, provided that we mix the algae with other wastes to lower their toxicity. Pre-composting could help to lower toxicity and reduce the volume of the alga due to water loss, which would greatly simplify subsequent bioremediation with isopods. These would give an added value to the compost, increasing its quality compared to other more conventional treatments. Another advantage of using these invertebrates is that they consume up to 1.5 times their weight per day. We have not observed these consumptions in insects fed with the algae, let alone with earthworms, so we assume that isopods could be a better alternative for *Rugulopteryx* recycling. On the other hand, as *P. laevis* is a native species, they do not pose problems with regulations for the protection of biodiversity nor are they within the laws of animal welfare, which are advantages for their exploitation. Furthermore, the role of isopods as accumulators of heavy metals is far superior to that of insects or earthworms, which supports their use to further reduce the toxicity of algae, especially if they come from polluted areas [12]. We cannot forget that isopods are much more rustic in their feeding than other invertebrate species [13], so we could use them in mixed diets of very fibrous waste (wood, leaves, paper, cardboard, crop residues, etc.) and algae. It should also be noted that isopods have very short cycles and high reproductive rates, so they would be viable alternatives to more productive insects, such as cockroaches and black soldier flies [27]. Despite these good prospects, isopods are largely unknown in their role as recyclers in industrial facilities, but many field studies indicate that their effect as detritivorous is very high in natural environments [26,28] in regard to other invertebrates [29]. We believe that more research is needed on the composting of *R. okamurai* algal blooms [30] and thus give economic viability to this abundant marine resource. To ensure this viability, we propose the creation of invertebrate farms where isopods can be massively used to recycle algae waste. Stacked tray systems similar to those used with mealworms would be the most advisable. These industrial systems generate a large volume of processing per unit area and have been shown to be commercially viable.

5. Conclusions

Finding ways to convert the tons of biomass from the invasive algae *Rugulopteryx okamurai* that periodically wash up on beaches into exploitable marine resources is a priority objective within the circular economy inherent in the use of invasive marine algae waste. In

this sense, the use of terrestrial isopod crustaceans is an alternative to consider. Although young specimens are sensitive to algae toxins, adults consume them, grow, and reproduce quickly. Therefore, in order to possibly exploit them, further research is necessary to advance our knowledge of their strengths and weaknesses. Our contribution opens the way for the possible future creation of bioremediation farms that can use these invertebrates—taking advantage of their reproductive capacity and high consumption rates—to use and value the marine waste considered here.

Author Contributions: Conceptualization, D.P.; Methodology, D.P.; Software, D.P.; Investigation, D.P.; Writing—original draft, D.P.; Writing—review & editing, J.C.G.-G.; Supervision, D.P.; Funding acquisition, J.C.G.-G. All authors have read and agreed to the published version of the manuscript.

Funding: Financial assistance was obtained from the Autoridad Portuaria de la Bahía de Algeciras (APBA), Fundación CEPESA y Red Eléctrica de España (REE).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: We appreciate the assistance and facilities provided by the Puerto Deportivo La Alcaidesa (La Línea) and the Aquarium of Seville. Key information for isopod breeding was provided by Rus Wilson of Aquarimex. Financial support: Red Eléctrica Española (REE), Fundación CEPESA, Autoridad Portuaria de la Bahía de Algeciras (APBA).

Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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