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## Research Article

# Assessing Vegetable Diets on the Growth of Reared Juvenile *Paracentrotus lividus*: Implications for Population Restoration Initiatives

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The demand for the purple sea urchin *Paracentrotus lividus* has led to the decline of its wild populations, increasing interest in cultivating this species for commercial purposes and ecological restoration purposes. Consequently, there is a need to expand knowledge about the optimal conditions for sea urchin aquaculture, particularly regarding food regimes. Here, we evaluate the effects of several vegetable diets on the survival and growth of early juveniles of *P. lividus*. Using an outdoor experimental assay, we fed early juveniles (5–6.5 mm) various diets: fresh algae (*Ulva* sp.), aquaculture pellets, and vegetables (carrot, spinach, and maize) presented as individual or mixed diets (equal mass proportions). All diets were provided *ad libitum* in equal mass proportions. After 120 days, the pellet-fed urchins showed good growth but a high mortality rate (>40%). The *Ulva* sp. diet, considered the control regime mimicking wild sea urchin diet, resulted in low mortality but poor growth performance (maximum of  $10.92 \pm 1.94$  mm in diameter and  $0.68 \pm 0.26$  g in weight). In contrast, plant-based diets, especially those containing maize, led to the highest growth and lowest mortality rates (<5%). Notably, the triple mixed diet (carrot–spinach–maize) resulted in a threefold size increase, as the *P. lividus* juveniles reached 15 mm in 4 months. This study demonstrates the effectiveness of inexpensive, ready-to-use vegetable-based diets in optimizing rearing protocols for early juveniles of *P. lividus*. It offers preliminary insights into dietary preferences to rapidly attain an adequate release size for purple urchin aimed at restocking wild populations and balancing aquaculture and ecological restoration needs.

**Keywords:** aquaculture; diets; ecological restoration; growth performance; sea urchin hatchery

## 1. Introduction

*Paracentrotus lividus* (Lamarck, 1816), also referred to as the purple sea urchin, is a member of the Parechinidae family and is found throughout the Mediterranean Sea and along the European Atlantic coast. Highly valued in several European countries for the taste of its gonads, *P. lividus* faces significant fishing pressures, which have led to population declines in many areas and threaten the sustainability of fishing activities [1–3]. Moreover, rising sea temperatures linked to global

warming further increase the vulnerability of the populations of this sea urchin, which have already led to high mortality episodes linked to warmer water [4].

Currently, efforts are underway to promote the sustainable exploitation of this urchin. Stock preservation generally involves setting minimum harvest sizes, harvest quotas, and closed seasons during critical reproductive periods; however, these methods have not been effective enough to ensure the repopulation of *P. lividus* in the Mediterranean Sea [5]. As the sea urchin's reproduction cycle involves emitting its



gametes into the environment and an external fertilization [6], this urchin's breeding success depends directly on population density [7]. Thus, urchin populations are particularly susceptible to the Allee effect, lowering the chances of successful populations' restoration through restrictive measures alone [8]. In many cases of *P. lividus* overfishing, the depletion of initial stocks through commercial exploitation has led to local collapses in fishing activity [1, 9], yet even complete closures of fishing for several years have not succeeded in restoring initial population densities [8].

In recent years, the development of echiniculture has provided a new tool to address these issues. Sea urchins can be reared to meet increasing demand without harvesting from natural populations [10]. Currently, such facilities are rare and mostly experimental, and they often struggle to be economically viable [11]. However, rearing juvenile *P. lividus* for eventual release into the environment to help restore depleted populations is a possible solution. As gametes and larvae are the life stages most susceptible to mortality and dispersal in the water column [7, 12], releasing fully developed sea urchins could enhance local population densities and restore reproductive efficiency and ecological functions as well as fishing stocks. The concept of restocking wild populations is particularly relevant given recent legislation on environmental management and biodiversity conservation. Indeed, population restocking could play a crucial role in ecological restoration, as emphasized at the recent 15th meeting of the Conference of the Parties to the UN Convention on Biological Diversity [13]. Population restocking can significantly help meet the objectives of the Nature Restoration Law, which mandates the restoration of at least 20% of the European Union's degraded ecosystems by 2030 [14]. Juvenile release has already been used successfully in Japan for ecological restoration projects for the sea urchins *Strongylocentrotus intermedius* and *Strongylocentrotus nudus* [15, 16].

For *P. lividus*, experimental releases of juveniles (<1 mm) have been conducted in the western Mediterranean Sea, but they have not significantly improved local population densities [17]. A major challenge is the difficulty of growing large quantities of sea urchins larger than 1 mm, as zootechnical practices during rearing hinder effective releases [17]. In aquaculture, feeding strategy is one of the most fundamental leverage to consider as it affects survival rates, physiology, behavior, and the economic and environmental sustainability of production [18]. Rearing protocols for ecological restoration should optimize production cost efficiency while ensuring urchins can adapt to their natural habitat after release and maintain reproductive capacities. Because smaller urchins usually experience higher mortality rates [7, 12], feeding protocols for *P. lividus* primarily focus on maximizing growth rates while minimizing production costs [19, 20]. Fishmeal-based pellets are frequently used, as high protein levels enhance growth and fertility [21], allowing more energy to be devoted to gonad development [22]. However, plant-based diets may offer a cheaper, more sustainable, and more natural alternative to pellets. Moreover, as the urchins' microbiomes depend on diet [23], using plant-based foods during rearing could improve the postrelease survival of

TABLE 1: Estimated relative nutritional value of the different tested foods.

| Proximate composition | Carrot | Spinach | Maize | Pellet | Fresh <i>Ulva</i> |
|-----------------------|--------|---------|-------|--------|-------------------|
| Proteins (%)          | 0.60   | 3.40    | 2.90  | 52.00  | 1.29              |
| Lipids (%)            | 0.00   | 0.60    | 2.40  | 18.00  | 0.03              |
| Fiber (%)             | 2.8    | 2.60    | 3.8   | 0.60   | 6.09              |
| Carbohydrates (%)     | 5.30   | 2.00    | 12.00 | 0      | 5.98              |

Note: Values were determined from the seller's labeling provided for the vegetables, the technical sheet for the pellets, and Azenha et al. [29] for the *Ulva* sp. nutritional information.

juvenile sea urchins. For example, maize has been successfully used in echiniculture as a dietary option for *P. lividus* [24]. A maize and spinach mix has also been experimentally identified as an effective diet for echiniculture because of its energy supply and high carotenoid and antioxidant content, which is optimal for growth and gonad development in *P. lividus* [25, 26].

To date, experiments on sea urchin feeding have focused primarily on adult individuals or late juveniles, mostly in recirculating aquaculture systems (RASs) or in controlled low-density experimental setups (e.g., [10, 21, 24, 25, 27, 28]). Knowledge about rearing protocols for early juveniles for ecological restoration is scarce; therefore, this study aims to evaluate the effects of different diets on early juvenile sea urchins intended for environmental reintroduction. An outdoor experimental setup is used, which is a more cost-effective rearing option than RAS systems or indoor structures. The main objective is to assess the effect of eight different vegetable-based diets on the survival and growth of *P. lividus* early juveniles.

## 2. Materials and Methods

The outdoor trial tested eight diets on early *P. lividus* juveniles (5–6.5 mm) over 120 days. For the plant-based diets, we selected carrot, spinach, and maize because of their previous use in sea urchin rearing and their low cost, nutritional value, and worldwide availability (Table 1). We also compared these with an artificial diet of aquaculture pellets and a diet based on the alga *Ulva* sp., which served as a control diet because it closely resembles the natural diet of sea urchins and is often used as a reference diet in other similar studies [28, 30].

**2.1. Experimental Design.** In April 2022, we obtained a batch of *P. lividus* juveniles using gametes from wild adult specimens captured in Albo (42°48'27" N, 9°19'57" E; Corsica, France) at the UAR CNRS 3514 Stella Mare (Biguglia, France) laboratory. The juveniles were then fed *Ulva* sp. during early life stages, following the established rearing protocol.

After 266 days, in January 2023, all juveniles had reached full development. We selected 1620 sea urchins measuring 5–6.5 mm and divided them into 27 baskets, each containing 60 individuals. We used oyster baskets (SEAPA, Adelaide), having a 15 L capacity and a 3 mm mesh, to be able to stack the baskets. The 27 baskets were placed in an outdoor raceway consisting of a 4320 L tank filled with filtered disinfected

seawater (50 µm filtration through a sand filter and UV disinfection). We regularly displaced the batches in the tank to avoid potential position-based bias.

All urchins were not fed for 1 week prior to the start of the experiment to ensure that their response to the offered food would not be affected by a sense of satiety.

**2.2. Feeding Protocol.** Eight diets were tested. Four individual diets included *Ulva* sp. (the control diet), carrots (Ca), spinach (Sp), and a pellet diet (Pe); the pellets contained fish and squid powder, fish oil, wheat flour, and tapioca starch (MAR 13 Vitalis Repro, Skretting, France). The four mixed diets comprised three two-ingredient diets (Mixed diet-2): carrot–spinach (Ca–Sp), carrot–maize (Ca–Ma), and spinach–maize (Sp–Ma), and one three-ingredient diet (Mixed diet-3): carrot–spinach–maize (Ca–Sp–Ma). Each mixed diet consisted of equal mass proportions of the ingredients (i.e., 50% and 33.3% of each for Mixed diets-2 and -3, respectively).

Three random batches of 60 urchins were assigned to each diet treatment. During the experiment, all batches were fed *ad libitum* and in equal mass proportion for both individual and mixed diets. All vegetable diets' components were presented chopped and manually prepared and selected for distribution. All batches were regularly monitored and replenished weekly to ensure continuous food availability in the baskets.

**2.3. Monitoring During the Experiment.** Sea urchin mortality and growth in body size and weight were monitored over 120 days, specifically on day 0, day 30, day 60, and day 120. On day 0, we weighed all urchins and measured their diameters using a caliper (resolution 0.1 mm) for each treatment. On day 30, we counted all individuals in each basket and measured urchins drawn from the baskets until weight and mean size were no longer significantly affected by new draws, establishing a sampling threshold of 25 individuals. From day 30 onward, we randomly drew samples of 25 sea urchins from each basket for measurement and weighing, which was sufficient for estimating the average weight and size in the batches. At the end of the experiment on day 120, we counted all live sea urchins to determine the mortality rate.

We monitored the tank temperature throughout the experiment and maintained dissolved oxygen saturation in the water above 75% using an Oxyguard Handy Polaris 2 (Oxyguard International, Farum, Denmark). Ammonium, nitrite, and nitrate levels were monitored every 2 days using Visicolor ECO Ammonium 3, Nitrite, and Nitrate colorimetric test kits (Macherey-Nagel, Düren, Germany). Concentrations of those nitrogen compounds were continuously kept below detection limits of the kits (i.e., 0.2, 0.02, and 1 mg·L<sup>-1</sup>, for ammonium, nitrites, and nitrates, respectively) through seawater renewal adjustments.

**2.4. Data and Statistical Analysis.** All statistical analyses and data visualization were performed using R software ([31]; version 4.0.2). Data were verified to ensure that they met the conditions required for applying a linear model. We verified the absence of outliers and tested data normality and equal variance between groups using the Shapiro–Wilk

and Levene tests, respectively. We analyzed growth differences between diets over time using a two-factor mixed ANOVAs, followed by a post hoc pairwise comparison of means. The post hoc test used the estimated marginal means method, calculated with the *lme4* [32] and *emmeans* [33] R packages, with a significance threshold of 0.05. In these analyses, time was treated as a qualitative variable, and we modeled the effect of repeated measures within baskets as a within-subject factor, justifying the use of a mixed model. We tested differences in mortality rates between diets at the end of the experiment using a single-factor analysis of variance (ANOVA) after transforming the data ( $\log [x + 1]$ ) to meet ANOVA conditions. Significant differences ( $\alpha = 0.05$ ) were further assessed using a post hoc Tukey HSD test. To evaluate potential bias from repeated measures within the same treatment, we estimated the share of size and weight variance associated with these repeated draws by calculating the intraclass correlation within baskets.

### 3. Results

**3.1. Mortality Rate.** At the end of the experimental period, the overall mortality rate remained low, never exceeding 5%, except for two groups. In the Sp–Ma treatment, mortality reached 7% by day 120, whereas the Pe treatment group had the highest mortality, approximately eight times greater than the other diets (Figure 1). By day 120, up to 41% of the pellet-fed urchins had died. The ANOVA test confirmed a significant effect of the treatment on mortality rate ( $F = 6.068$ ,  $p = 0.002$ ), with the Pe group significantly higher than the other treatments (except Sp–Ma), according to pairwise comparisons (Pe vs. *Ulva* sp.:  $p = 0.046$ ; Pe vs. Ca:  $p = 0.008$ ; Pe vs. Sp:  $p = 0.002$ ; Pe vs. Ca–Sp:  $p = 0.001$ ; Pe vs. Ca–Ma:  $p = 0.029$ ; Pe vs. Sp–Ma:  $p = 0.167$ ; Pe vs. Ca–Sp–Ma:  $p = 0.015$ ).

**3.2. Growth in Size and Weight.** We observed a significant effect of the diet on growth in size and weight. The batch influence on the urchin's size and weight was negligible, accounting for less than 1% of the residual variance explained by the random effect on batches. ANOVA indicated that time, diet, and the interaction of both factors significantly affected growth in terms of size ( $F = 8431.0$ ,  $F = 78.5$ , and  $F = 36.0$ , respectively,  $p < 0.001$  in each case) and weight ( $F = 7658.4$ ,  $F = 117.9$ , and  $F = 71.6$ , respectively,  $p < 0.001$  in each case).

Over the 120-day experiment, each diet treatment's size consistently increased in a similar trend, as shown in Figure 2. Initially sized between 5 and 6.5 mm, sea urchin mean size varied from  $10.92 \pm 1.94$  mm for the *Ulva*-fed group to  $14.83 \pm 1.43$  mm for the Ca–Sp–Ma treatment at the end of the experiment. On day 30, no significant effect of diet on size was detected. At day 60, the mean size was greatest for urchins fed with mixed diets Ca–Sp–Ma ( $9.4 \pm 1.17$  mm), Ca–Ma ( $9.2 \pm 1.20$  mm), and Sp–Ma ( $8.9 \pm 1.36$  mm). At the midpoint of the experiment, the size of urchins fed with Sp, Sp–Ma, Ca–Ma, and Ca–Sp–Ma significantly exceeded those fed with Ca only ( $8.01 \pm 1.06$  mm at day 60:  $p = 0.002$ ,  $p = 0.001$ ,  $p = 0.001$ , and  $p = 0.0004$ , respectively) and *Ulva* sp.

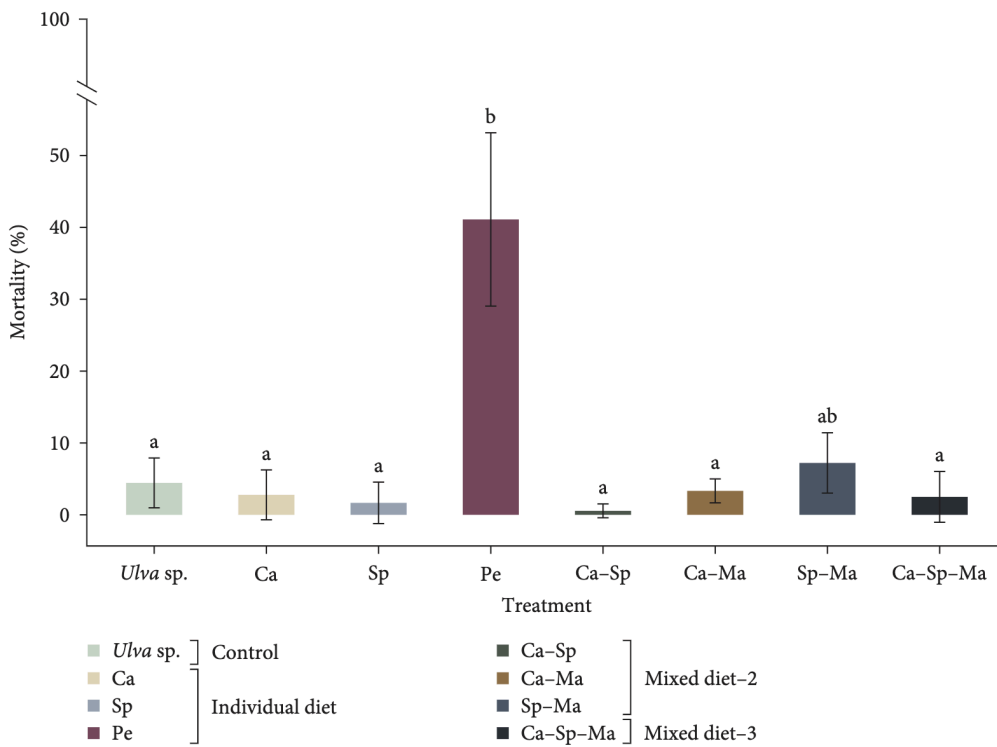


FIGURE 1: Mean mortality rate of juvenile *P. lividus* (%) ± SD for each of the eight diet treatment groups at the end of the experimental period (day 120). Significant differences between regimes are indicated by different letters.

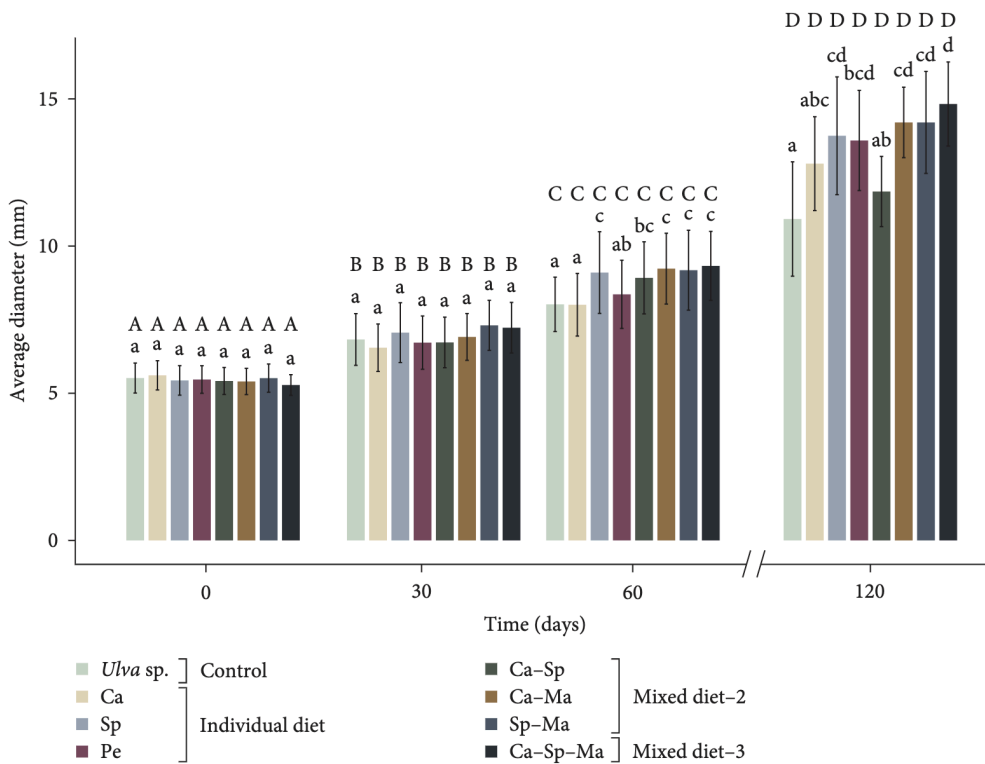


FIGURE 2: Evolution of the mean size (mm) ± SD of juvenile *P. lividus* for each diet treatment during the 120-day experiment. Statistically significant differences are represented by different letters. Capital letters indicate significant differences between sampling times within regimes, whereas lowercase letters indicate statistically significant differences between regimes within the same sampling time.

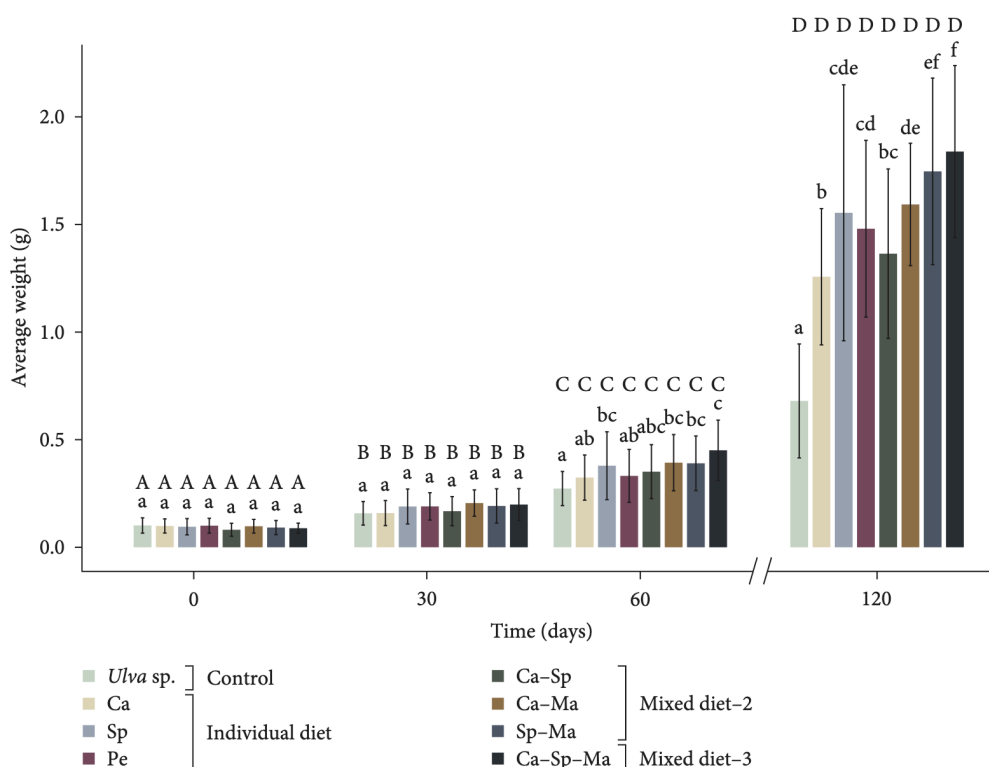


FIGURE 3: Evolution of the mean weight (g) ± SD of juvenile purple sea urchins (*P. lividus*) for each diet regime during the 120-day experiment. Statistically significant differences are represented by different letters. Significant differences between sampling times within regimes are indicated by capital letters, whereas lowercase letters indicate statistically significant differences between regimes within the same sampling time.

(8.02 ± 0.92 mm at day 60:  $p = 0.039$ ,  $p = 0.019$ ,  $p = 0.016$ , and  $p = 0.001$ , respectively).

At day 120, the mean size was highest for urchins fed with Ca-Sp-Ma (14.8 ± 1.43 mm). Groups fed with Sp-Ma and Ca-Ma mixed diets closely followed (14.20 ± 1.73 mm and 14.20 ± 1.19 mm, respectively). The mixed diet (Ca-Sp) and all individual diets (Sp, Pe, Ca, and *Ulva* sp.) produced smaller sizes, particularly that of the *Ulva* sp. diet, which had the lowest growth. Despite these size differences, only the Ca-Sp-Ma diet had a significant effect on urchin size relative to Ca ( $p = 0.034$ ), Ca-Sp ( $p = 0.002$ ), and *Ulva* sp. ( $p < 0.0001$ ). No significant size differences were noted between the Sp-Ma, Ca-Ma, Sp, Pe, and Ca diets, whereas all diets, except Ca ( $p = 0.052$ ) and Ca-Sp ( $p = 0.681$ ), produced significantly larger urchin sizes than the *Ulva*-based diet.

Urchin weight also increased during the experimental period, as illustrated in Figure 3. Similar to size growth, we found no significant diet effect on weight evolution on day 30. Over the course of the entire study period, urchin weight increased from 0.09 ± 0.03 g (day 0) to 0.68 ± 0.26 g in the *Ulva*-fed group and 1.84 ± 0.40 g in the Ca-Sp-Ma treatment (day 120), representing the lowest and highest growth, respectively. The Sp-Ma and Ca-Ma diets produced mean weights slightly less than those of the Mixed diet-3 (1.75 ± 0.43 g and 1.59 ± 0.28 g by day 120, respectively). Individual and Mixed diet-2 groups (Sp, Pe, Ca-Sp, and Ca) had similar

final weights (1.55 ± 0.60 g, 1.48 ± 0.41 g, 1.37 ± 0.39 g, and 1.26 ± 0.32 g, respectively), whereas the *Ulva*-based diet produced significantly lower values, remaining under 1 g at the experiment's end (0.68 ± 0.26 g). All groups had significantly higher weights than the *Ulva*-fed group ( $p \leq 0.0001$  in each case). Post hoc tests showed the Ca-Sp-Ma diet produced significantly higher juvenile weights by day 120 than the *Ulva* sp., Ca, and Sp-Ca diets ( $p \leq 0.0001$  in each case), as well as the Pe ( $p = 0.001$ ), Sp ( $p = 0.006$ ), and Ca-Ma diets ( $p = 0.017$ ). However, there was no significant difference between the Ca-Sp-Ma and Sp-Ma diets ( $p = 0.670$ ).

## 4. Discussion

**4.1. Effect of Diet on the Survival and Growth of Sea Urchin Juveniles.** The present study described the survival and growth of early juveniles of *P. lividus* fed eight diets based on *Ulva* sp., aquaculture pellets, and vegetables, that is, carrot, spinach, and maize, which were proposed under the form of mono and mixed diets. Most previous studies on sea urchin feeding focused on adults, making it difficult to compare with our research on early juveniles.

We found that an *Ulva*-based diet did not support high growth performance in *P. lividus* juveniles. *Ulva*-fed urchins had a mortality rate under 5%. These rates align with previous studies on similar species and diets; for example, Daggett et al. [34]

reported mortality rates below 4.2% for various macroalgal diets, including *Ulva lactuca* and *Ulva linza* fed to *Strongylocentrotus droebachiensis* (O.F. Müller, 1776) juveniles. However, compared to the other studied diets, *Ulva*-fed sea urchins in our study had the lowest growth results both in size ( $10.92 \pm 1.94$  mm after 120 days) and weight ( $0.68 \pm 0.26$  g after 120 days), resulting in growth rates of  $\sim 0.045$  mm·day<sup>-1</sup> and  $4.83 \times 10^{-3}$  g·day<sup>-1</sup>, respectively. Other studies on adults present contrasting results regarding macroalgae-based diets. For instance, Sartori and Gaion [25] found that macroalgal diets performed poorly for *P. lividus*, particularly noting a low ingestion rate of *U. lactuca* compared to other tested diets. Contrasting feeding behaviors have been observed in other sea urchin species fed *Ulva* sp. and other macroalgae-based diets [34–36]. For example, similarly to our results, Daggett et al. [34] found that *Ulva*-based diets yielded good size growth in *S. droebachiensis*, as juveniles experienced growth rates of  $\sim 0.042$  mm·day<sup>-1</sup> for a mixture of *Ulvaria obscura* and *Ulva lactuca* and about 0.056 mm·day<sup>-1</sup> when fed with *U. linza*. However, these rates were lower than those of juveniles fed other algae and prepared diets (e.g.,  $0.059 \pm 0.001$  mm·day<sup>-1</sup> for *Porphyra purpurea* and  $0.056 \pm 0.001$  mm·day<sup>-1</sup> for the prepared diet). Likewise, on the same species, Eddy et al. [35] found that most tested artificial diets outperformed a natural kelp-based diet for the growth of urchins, both in size and weight. The kelp diet produced a growth rate of about 0.081 mm·day<sup>-1</sup> in a 216-day trial, whereas growth using the best-prepared diet reached 0.090 mm·day<sup>-1</sup>. The weight gain of juveniles for this kelp diet was  $4.35 \pm 0.13$  g over 216 days ( $\sim 0.020$  g·day<sup>-1</sup>), compared to  $6.00 \pm 0.24$  g for juveniles fed the best-prepared diet ( $\sim 0.028$  g·day<sup>-1</sup>). This lower growth for the kelp-based diet may be attributed to its lower ingestion rate by the urchins, as the commercial diets offer better palatability for urchins and result in relatively higher consumption rates than other feed [10, 25, 37]. Indeed, Cárcamo [36] found that *Loxechinus albus* (Molina, 1782) ingested *Ulva* sp. the least among tested algal diets. For *P. lividus*, Schlosser et al. [38] reported lower consumption and energy efficiency for a *U. lactuca* diet relative to other offered algal and artificial diets. Given the high cost of *Ulva* sp. and its relatively poor growth performance, observed as well for other alga-based diets, we conclude it is not optimal for commercial rearing purposes.

After 60 days, the pellet-based diet led to the highest mortality rate for the juvenile purple sea urchins and produced a lower weight and size increase than the vegetable Mixed diet–3 treatment. Our results contrast with several studies that demonstrated good growth and survival rates using comparable pellets for similarly sized urchins [10, 19, 22]. The differences likely stem from our rearing structure, which significantly differed from those in the other studies. The sea urchins did not ingest pellets efficiently, and low consumption rates for pellet feed have been noted in other studies [25]. In our case, despite regular cleaning, the rapid degradation and stagnation of food in the fine mesh baskets likely created small anoxic zones, contributing to the higher mortality observed. Although pellet diets offer a protein-rich supply, our experimental setup did not favor their use. Pellets may be suitable in other setups, including those with an effective waste management protocol and low-density populations. The type of pellet used in our study is designed for adult

fish growth, likely making it unsuitable for rearing sea urchins because of its underwater resistance time. In addition, since pellets used are not specifically designed for sea urchins, significant higher mortality observed under this diet could be due to potentially excessive protein provision compared to sea urchins' requirements, even though at juvenile stage, Grosso et al. [21] reported that feed containing 40% animal supply, that is, about 37% protein, can benefit growth.

Finally, several studies have tested the effects of formulated diets on the growth, gonad development, gamete production, and fecundity of *P. lividus* [10, 25–27]. Most of these studies involved adult sea urchins, making comparisons with our research more difficult. Nonetheless, most demonstrated that artificial diets based on terrestrial food, particularly maize and spinach, are effective for heightening growth and gonad development, outperforming natural macroalgae and aquacultural pellets in promoting *P. lividus* growth [22, 25, 26]. In our study, juvenile sea urchins thrived and maintained a high survival rate (>95%) when fed diets based on carrots, maize, and spinach, indicating that these vegetables provide essential nutrients for sea urchins' growth and survival at juvenile stage too. Among the vegetarian options, the best results generally came from the three maize-containing diets (Ca–Sp–Ma, Ca–Ma, and Sp–Ma). Maize is rich in protein and carbohydrates and has been recognized as an excellent food source for sea urchins [24–27]. Mean size and weight were generally higher in groups fed mixed-vegetable diets, although the difference was not always statistically significant.

Feeding regimes must also be tailored to the specific organisms (i.e., life stage, culture conditions, and rearing objectives) as ingestion and dietary needs vary among species, developmental stage, size, culture conditions, food quality, and seasonality (especially for algal-based diets) [36, 39]. Given this need, we conclude that terrestrial plants present an economical, practical, and effective option for ensuring the growth and survival of early juvenile sea urchins in a rearing environment. In terms of economic consequences, vegetable options can reduce costs up to 14 times that of *Ulva* sp. (*Ulva* sp. price is 35 €·kg<sup>-1</sup> versus 6 €·kg<sup>-1</sup> for maize, 5 €·kg<sup>-1</sup> for carrots, and 2.5 €·kg<sup>-1</sup> for spinach; personal observation—France 2022). Therefore, we argue that incorporating maize into mixed diets could significantly enhance the food quality and growth performance of sea urchins at a relatively low cost [24].

**4.2. Perspectives on Research and Implications for Aquaculture and Restocking Programs.** The hatchery rearing of a species for economic or ecological purposes faces several challenges. From an economic perspective, it is crucial to ensure reasonable production costs associated with the selected rearing protocol relative to the selling price of the reared species. Our results indicate that *Ulva* sp. does not provide a favorable production-to-cost ratio because of its high price and the low growth performance for *P. lividus* juveniles reared on this alga. Our study offers preliminary insights into the hatchery rearing of early juvenile sea urchins, nevertheless, future studies should adopt a multidisciplinary approach, including

detailed evaluations of consumption efficiency (ingestion, assimilation, and absorption rates) and comprehensive economic analyses. Moreover, the quality of the final product is critical in the hatchery rearing of commercially valuable species such as *P. lividus* for human consumption. Several studies have shown that artificial pellets, formulated diets, and algae-based feeds can adversely affect the color, firmness, texture, and taste of sea urchin gonads compared to wild-captured specimens, compromising their commercial value [20, 35, 40]. Therefore, exploring the organoleptic qualities is valuable to assess whether artificial diets negatively influence the gustatory quality and, consequently, the commercial value of hatchery-reared sea urchins in the long term.

From an ecological restoration standpoint, the primary factors to prioritize are the survival, feeding, and reproduction capabilities of hatchery-reared sea urchins upon their reintroduction into the wild. A key aspect to consider is rearing time: a short rearing period offers both economic and ecological advantages. Brundu, Farina, and Domenici [41] demonstrated that the longer *P. lividus* sea urchins are kept in captivity, the more their behavior is negatively affected, jeopardizing their successful introduction into the wild. Thus, rapid growth is preferable for restocking projects. In the study mentioned, sea urchins fed *U. lactuca* reached 15 mm, a suitable size for release into the wild, after 16 months [41]. In our study, *P. lividus* juveniles reached 10–15 mm after 12 months, with a marked increase in size toward the end (90–120 days) of the experiment, which represent remarkable growth rates. Despite the seasonal temperatures during our experiment being the lowest of the year, individuals tripled their initial size over 4 months, particularly when fed the triple-content mixed diet. As all juveniles were fed *Ulva* sp. for the first 8 months before the experiment, an earlier adoption of the triple-content mixed diet may produce threefold faster growth and hence even larger sea urchins (i.e., >2 cm after 12 months). A larger size for juvenile urchins is crucial for successful reseeding, as it reduces predation, dispersal, and vulnerability to environmental change [15, 42, 43]. This size advantage coupled with the abovementioned negative effects of prolonged captivity on sea urchin readaptation to life in the wild favors introducing alternative diets that reduce rearing times and lower economic costs as a positive “side effect” [41]. Therefore, our study provides valuable insights into alternative diets for promoting size growth, especially through mixed-vegetable diets for optimizing growth rates and shortening the hatching period for urchins intended for ecological restoration projects.

Another key question is to determine whether hatchery-reared sea urchins can adopt a natural diet after being released into the wild. Food preference studies should be carried out and ideally compare these urchins with wild-caught individuals of similar age and size to understand the species’ behavioral dynamics and ensure effective restoration programs.

In the tested diets, maize, carrots, and spinach have higher carbohydrate and lipid contents than the algae eaten by urchins in their natural environment, which may alter the behavior or digestive abilities of reared urchins. Some authors have argued that a high supply of protein and lipids hinders

gonadal growth in urchins because of the energetic costs associated with absorbing excess nutrients, particularly from rich pellet-based diets, which may have long-term adverse effects on later life stages [25]. It is also crucial to examine the ability of sea urchins to reproduce in nature, as this contributes to population restocking. Future research should determine whether artificial diets hinder normal development or compromise reproductive capability. Previous studies indicate that low-cost artificial diets composed of maize and spinach can support gonadal development and produce healthy mature gametes [24–27]. Nevertheless, Ruocco et al. [28] found that a pellet-based artificial diet promotes gonadal growth in adult *P. lividus* but results in significantly lower gamete production than macrophyte-based diets, which are more similar in composition to the sea urchins’ natural food. Our study and the existing research indicate that maize, carrots, and spinach are promising food options for *P. lividus* juveniles from a strictly zootechnical standpoint. However, to ensure their relevance to ecological restoration programs, particularly the long-term effects on reared juveniles, more detailed studies on diet and rearing conditions during later developmental stages and postrelease are essential [44].

## 5. Conclusion

In this study, we re-evaluated rearing protocols for restoring sea urchin populations using hatchery-reared *P. lividus* early juveniles. We tested several diets, including fresh algae (*Ulva* sp.), aquaculture pellets, and vegetables (carrot, spinach, and maize in individual and mixed diets), and compared how the diets affected juvenile mortality rates and growth. All diets, except that of pellets, produced good juvenile urchin survival rates. All artificial diets outperformed the control *Ulva*-based diet, likely because of their higher nutritional quality and better ingestion and absorption rates. Specifically, the triple mixed diet (carrot–spinach–maize) produced the highest growth increase in juvenile sea urchins, that is, a threefold size increase to 15 mm in 4 months. The observed growth of the plant-fed urchins coupled with the vegetable-based diets’ similarity to natural feed—as well as its low cost, accessibility, and suitability for high-density farming—suggest that plant-based diets, particularly those containing maize, provide an effective feed option for rearing *P. lividus* juveniles. These diets could significantly benefit ecological restoration projects by enabling faster growth, quicker attainment of release size, reduced rearing time, and minimized adverse effects of captivity, providing both economic and ecological advantages for restocking the wild populations of *P. lividus* using hatchery-reared juveniles.

## Data Availability Statement

The data sets generated or analyzed during this study are available from the corresponding authors upon reasonable request.

## Conflicts of Interest

The authors declare no conflicts of interest.



## Author Contributions

**Martin Simonoviez:** formal analysis, data curation, writing—original draft, visualization. **Viviana Ligorini:** validation, writing—original draft, writing—review and editing, visualization, supervision. **Léa Piacentini:** validation, formal analysis, writing—review and editing, supervision. **Mikaël Demolliens:** conceptualization, methodology, investigation, resources, writing—review and editing, supervision. **Alban Delmas, Alizée Boussard, and Sally Pugliese:** conceptualization, methodology, investigation, resources. **Romain Bastien:** resources, supervision, project administration, funding acquisition. **Jean-José Filippi and Alexandre Vela:** validation, resources, writing—review and editing, supervision, project administration, funding acquisition. **Sonia Ternengo:** validation, writing—review and editing, supervision. **Antoine Aiello:** resources, writing—review and editing, project administration, funding acquisition. Martin Simonoviez and Viviana Ligorini contributed equally to this work.

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