

Seagrass detritus as a food source for *Pinna nobilis*: implications for species ecology and ex-situ conservation

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Abstract

For critically endangered aquatic species, restorative aquaculture and ex-situ conservation may represent the last opportunity to halt extinction trajectories; however, spawning induction, larval rearing, and feeding, depend on understanding species-level ecology. *Pinna nobilis*, a marine bivalve currently on the brink of extinction, has never yet been successfully kept for multiple generations in captivity. We tested the impact of diet on captive growth of juveniles recovered from mussel farms and maintained in a recirculating aquaculture system, comparing three diets: mixed live microalgae, commercial optimized mix of microalgae, and conditioned seagrass detritus. Seagrass detritus produced the highest mean growth rates in both small (150–190 mm) and large (190–220 mm) size classes, with significantly higher growth observed in the large size class. These results indicate that seagrass detritus constitutes a significant natural food source for this species, providing experimental evidence of a recently hypothesized direct trophic link mediated by heterotrophic bacteria in seagrass meadows. Our findings contribute to both the ecological understanding of *P. nobilis* and practical guidance for the formulation of optimal diets in captive rearing. This knowledge is essential for the establishment of effective ex-situ conservation programs aimed at promoting growth and reproduction of this iconic Mediterranean species.

Introduction

Climate change, increasing anthropogenic pressure, and habitat degradation are placing a growing number of species at risk of extinction (Lucente et al. 2021). Among the various conservation strategies, restorative aquaculture can play a crucial role in the recovery of critically endangered aquatic species. Through artificial breeding, rearing, and release programs, this approach may represent one of the last viable options to prevent extinction (Anders 1998; Bath et al. 2023). Although aquaculture is primarily aimed at food production by controlling species' life cycles in captivity, the biotechnological knowledge it provides can also be a powerful tool for biodiversity conservation. Over the past decades, aquaculture has increasingly contributed to the conservation of a wide range of endangered invertebrate species (Bath et al., 2023; Ferranti et al., 2022; Guallart et al., 2020; Jones et al., 2024; Junus et al., 2018; Katow et al., 2015; Markovic et al., 2018; Nakamura et al., 2019; Price et al., 2017).

In this context, *Pinna nobilis*, a critically endangered Mediterranean endemic and one of the largest bivalves worldwide (Zavodnik et al., 1991), could greatly benefit from ex-situ conservation programs based on aquaculture. Since 2016, the species has been affected by a mass mortality event (MME) that rapidly spread across the entire Mediterranean basin, leading to the collapse of most coastal populations (Acarli et al., 2022; Cabanellas-Reboredo et al., 2019; Pensa et al., 2022; Sarić et al., 2020; Zotou et al., 2020). This species is already protected under Annex IV of the Habitats Directive since 1992, and after the dramatic recent population decline *P. nobilis* was assessed as Critically Endangered on the IUCN Red List in 2019 (Kersting et al., 2019).

Currently, only a limited number of enclosed ecosystems, often characterized by estuarine conditions, are known to host residual populations (Acarli et al., 2022; García-March et al., 2020; Lattos et al., 2023; Nikolaou et al., 2024; Peyran et al., 2022; Prado et al., 2021; Silvestri et al., 2022; Zotou et al., 2020). Alarming, even these last refugia are showing signs of further decline (Foulquié et al., 2024; Nikolaou et al., 2023).

Recent studies have demonstrated the feasibility of inducing spawning in *P. nobilis* using wild-collected broodstock (Ferranti et al., 2024; Hernandis et al., 2023; Trigos et al., 2018). However, larval rearing and metamorphosis remain poorly understood, and filling this knowledge gap is essential for achieving full control of the species' life cycle under aquaculture conditions. A prerequisite for this goal is the availability of mature broodstock. Given the severe depletion of natural populations, collecting mature individuals from the wild poses serious risks to the already fragile residual populations. Consequently, the collection of juveniles through natural recruitment represents a more sustainable

alternative for broodstock establishment. This strategy is particularly promising considering that *P. nobilis* can reach sexual maturity within approximately three years (Kožul et al., 2024).

To date, two effective and sustainable approaches have been developed to collect juveniles without negatively impacting remaining populations: targeted recruitment using specifically designed spat collectors, and the recovery of juveniles from aquaculture facilities, where *P. nobilis* may occur as a biofouling by-product (Theodorou et al., 2015). Nevertheless, a major limitation for successful ex-situ rearing remains the limited understanding of the species' trophic ecology. Previous captive breeding trials reported significantly lower growth rates compared to those observed in the wild, highlighting the need for further investigation into feeding requirements and diet optimization for captive maintenance (Prado et al., 2020).

Artificial feeding is therefore a critical component for broodstock maintenance, juvenile growth following artificial reproduction, and long-term ex-situ conservation in laboratories or aquaria. Advancing knowledge in this field is imperative both to prevent extinction and to develop effective restorative strategies. Building on recent findings on spatial distribution and habitat association, which suggest that seagrass detritus may represent a primary food source for *P. nobilis* (Pensa et al., 2022), this study provides a refined framework for exploring the species' trophic interactions.

Accordingly, the aim of this study was to investigate the trophic ecology of *P. nobilis* and to define suitable aquaculture diets for ex-situ breeding by comparing juvenile growth performance under captive conditions when fed phytoplankton-based diets and, for the first time, seagrass detritus artificially conditioned.

Material and methods

Experimental setting

Juveniles of *Pinna nobilis* were collected in 2023 as biofouling from mussel farms (Fig. 1a) and subsequently transported to and maintained at the Laboratory of Experimental Ecology and Aquaculture of the University of Rome Tor Vergata. Specimens were housed individually in perforated cylindrical plastic pots filled with volcanic lapillus, which was used as a substrate for byssus attachment (Fig. 1b). In June 2024, prior to the start of the feeding experiment, specimens were carefully extracted from their pots, taking care not to damage or detach the byssus, and were measured underwater in a supine position using a caliper and a ruler (Fig. 1c). Individual shell biometric data (length and width) were recorded (T_0). Individuals were then labelled and assigned to two size classes: small (150–190 mm) and large (190–220 mm). Experimental groups, each composed of 29 specimens including both size classes, were subjected to different feeding treatments. Following measurements, each organism was reinserted into its original perforated cylindrical plastic pot filled with volcanic lapillus and each group was subsequently maintained in a separate 300 L tank operating within a recirculating aquaculture system (RAS). Practical requirements for large water volumes demanded that each group was maintained in a single tank, and sample limitations prohibited and individuals were used as statistical units within each feeding group.

The RAS was equipped with a high performing mechanical, UV and biological filtration, a protein skimmer, temperature control and microfiltration systems. Water parameters were maintained at $18 \pm 0.5^\circ\text{C}$ (temperature), 32 ± 1 PSU (salinity), and 8.20 ± 0.06 (pH). Water quality parameters were maintained within ranges commonly considered non-stressful for marine invertebrates in recirculating aquaculture systems (Boyd & Tucker, 1998; Timmons & Ebeling, 2010; FAO, 2018). Target thresholds were set at ammonium ≤ 0.05 mg L⁻¹, nitrites ≤ 0.02 mg L⁻¹, nitrates ≤ 25 mg L⁻¹, and phosphates ≤ 1 mg L⁻¹, with regular water exchanges (10–100%) performed to prevent nutrient accumulation.

Diet treatments

Three dietary treatments were tested: a live microalgae diet (LM), an optimized commercial microalgae diet (CM), and a seagrass detritus-based diet (SD). The live microalgae diet (LM) consisted of a mixed culture of *Isochrysis galbana*, *Phaeodactylum tricornutum*, and *Tetraselmis suecica*, combined in a 1:1:1 optical density (OD₇₂₀) ratio. These species are commonly used in aquaculture feeding protocols (Rakaj et al., 2018). Optical density (OD) was measured at 720 nm (OD₇₂₀) as a proxy for suspended particulate load. This wavelength is commonly used in studies of microalgae and particulate suspensions because it minimizes absorbance by photosynthetic pigments, thereby providing a measurement that reflects light scattering by particles rather than pigment-specific absorption. Microalgae were cultured in 2 L plastic bag photobioreactors using Guillard's f/2 medium (Guillard & Ryther, 1962) at 21°C under a 12:12 h light–dark photoperiod. Only cultures in the exponential growth phase were used for feeding. Daily feeding volumes were adjusted using live microalgal cultures until a target optical density of 0.05 ± 0.01 was reached, corresponding to approximately 300–2000 mL of mixed culture per 300 L tank.

The commercial microalgae diet (CM) consisted of Shellfish Diet 1800[®], a concentrated solution composed of five marine microalgae (*Isochrysis* sp., *Pavlova* sp., *Tetraselmis* sp., *Thalassiosira weissflogii*, and *Thalassiosira pseudonana*), with a constant dry weight of 8% and an approximate concentration of 2×10^9 cells/mL. The product was diluted in system water and filtered prior to administration following the manufacturer's instructions. Also in this case, daily feeding volumes were adjusted to reach the same target optical density (OD₇₂₀ = 0.05 ± 0.01), corresponding to approximately 100 mL of concentrated microalgal solution per 300 L tank.

The third dietary treatment (SD) was based on *Posidonia oceanica* seagrass detritus. Dead leaves were collected during winter from extensive shore banquettes in Civitavecchia (Central Tyrrhenian Sea; 42°08'58" N, 11°44'36" E), periodically removed from the shoreline as waste. The Mediterranean banquettes are monospecific and, and the dead material was identified by research experts. Leaves were oven-dried at 65°C until reaching constant weight and subsequently ground twice using a blender. The resulting material was sieved through a 500 µm stainless steel mesh to standardize particle size; larger fragments were reprocessed until all particles were < 500 µm (Fig. 2a, b). Dry detritus was then suspended in seawater at a concentration of 5×10^{-2} g/mL in 10 L round-bottom flask bioreactors equipped with aeration to maintain particles in suspension (Fig. 2c). Bioreactors were filled with seawater filtered at 5 µm to remove larger particles while retaining bacteria. To promote the development of the heterotrophic bacterial community, additional 3.5 g of urea was supplied to increase a C:N ratio ranging between 14–20, as recommended for bio-floc aquaculture production (Panigrahi et al., 2018). Seagrass detritus culture were maintained for 15 days to allow conditioning of the seagrass detritus through heterotrophic bacterial colonization prior to feeding. Each day, 10% of the culture volume was replaced with freshly prepared detritus suspension, and urea was proportionally supplemented to maintain the target C:N ratio. This procedure restored the initial volume and supported sustained microbial activity throughout the conditioning period. Daily feeding volumes were adjusted to reach the same target optical density (OD₇₂₀ = 0.05 ± 0.01), corresponding to a conditioned culture containing approximately 10 g (dry weight) of seagrass detritus per feeding event. Samples of dried leaves have been preserved as reference material at the University of Rome Tor Vergata.

This optical density was selected to standardize feeding conditions across the three heterogeneous diets based on preliminary rearing trials, which showed that specimens were unable to completely filter the available food within an 8 h period, thereby ensuring ad libitum feeding conditions (Fig. 3a, b, c).

Prior to feeding, rearing tanks were isolated from the recirculating aquaculture system (RAS) by closing the recirculation valves, and internal circulation pumps (NWA 10700) were activated to maintain food particles in suspension. Tanks remained isolated for 8 h during each feeding period, after which water circulation was restored.

Data collection

The experiment lasted three months, during which specimens were monitored daily to assess reactivity and overall vitality. Biometric measurements were collected monthly. Shell width was measured at the widest point using a caliper. To avoid stress and potential damage to the byssal threads, specimens were not extracted from the substrate during the experimental period. Consequently, shell length was measured as the unburied portion of the shell, using a ruler from the substrate surface.

Total shell length (TL) was estimated by summing the measured unburied length and the buried length (BL). Buried length was calculated from shell width at the substrate level (Sw) according to the following equation:

$$BL = 1.79 \times Sw \text{ (García-March et al., 2002).}$$

At the end of the experiment, all specimens were carefully removed from the substrate and fully measured to obtain total shell length (Tf), without detaching the byssal connections to the lapillus. Following the experimental trial, organisms were returned to rearing conditions within the RAS and maintained under a single feeding regime based on seagrass detritus and Shellfish Diet®.

Statistical analysis

Monthly growth rates (mm/month) were calculated for each individual and analyzed separately for the two size classes (Small and Large). The effects of Diet and Size class, and their interaction, on growth rate were first evaluated within a two-way ANOVA approach. Normality and homogeneity of variances were assessed on model residuals using the Shapiro–Wilk and Levene tests, respectively (Whitlock and Schluter, 2010). Because residuals deviated from normality, a non-parametric factorial approach based on the Aligned Rank Transform (ART) was applied to test the main effects of Diet and Size and their interaction. Following the detection of a significant interaction, growth rates were further analyzed within each size class. Differences among dietary treatments were assessed using the Kruskal–Wallis test, followed by Dunn’s post hoc comparisons with Bonferroni correction when appropriate. The significance level was set at $p < 0.05$. All statistical analyses were conducted in R version 4.1.3.

Results

Survivorship

After 20 days of rearing under the live microalgae diet, a generalized debilitation of specimens was observed, characterized by reduced reactivity and very low growth rates. During this phase, mortality occurred in three specimens, including one individual in the small size class and two individuals in the large size class. Based on these observations, the live microalgae diet was interrupted, and all remaining specimens were reassigned to the maintenance diet consisting of seagrass detritus and the Shellfish Diet® 1800 following biometric measurements; only data collected prior to this change were considered for the experimental analyses. Furthermore, although the LM treatment was interrupted after 20 days, growth rates were standardized per month, allowing comparison across treatments.

In the other experimental groups, no signs of debilitation or reduced reactivity were observed throughout the rearing period, and survivorship was 100%. No additional mortality occurred during the subsequent breeding period.

Growth

The degrees of freedom used in statistical analyses reflect individual-level measurements rather than tank-level replication, and tank-specific effects cannot be fully disentangled from dietary effects. This limitation is unavoidable given the constraints of the experimental setup, and nonetheless provide indicative results.

Growth of *Pinna nobilis* specimens was evaluated by comparing biometric measurements collected at the beginning of the experiment with those obtained at the end of the rearing period. After three months of breeding, all groups, except those fed live microalgae, showed a significant increase in shell length and width relative to T0 (Table 2). Growth rate was significantly influenced by dietary treatment and size class, with a significant Diet × Size interaction indicating that the effect of diet varied between small and large juveniles. The aligned rank transform (ART) two-way ANOVA (Table 1) confirmed this pattern, revealing a significant interaction ($F_{2,81} = 9.16$, $p = 2.60 \times 10^{-4}$), together with highly significant main effects of Diet ($F_{2,81} = 79.50$, $p < 2.22 \times 10^{-16}$) and Size ($F_{1,81} = 42.81$, $p = 5.06 \times 10^{-9}$).

Overall, monthly growth rates were higher in the small size class (150–190 mm) than in the large size class (190–220 mm) (Fig. 4). Consistent with the significant interaction, growth rates differed significantly among experimental diets in both size classes (KW, $p < 0.001$). In the small size class, specimens fed the LM diet exhibited the lowest mean monthly growth rate (1.59 ± 0.92 mm), which was significantly lower than those observed under both the CM (6.67 ± 1.89 mm; Dunn test with Bonferroni correction, $p < 0.001$) and SD diets (8.00 ± 1.74 mm; $p < 0.001$). No significant difference in growth rate was detected between the CM and SD diets ($p > 0.05$), although the SD diet showed higher mean values.

In the large size class, significant differences among diets were also detected (KW, $p < 0.001$). Specimens fed the SD diet showed the highest mean monthly growth rate (6.01 ± 3.73 mm), which was significantly higher than that observed under both the LM (0.98 ± 0.67 mm; Dunn test with Bonferroni correction, $p < 0.001$) and CM diets (2.44 ± 2.15 mm; $p < 0.05$). No significant difference was found between the LM and CM diets ($p > 0.05$).

Table 1
Table X. Results of aligned rank transform (ART) two-way ANOVA testing the effects of Diet, Size class, and their interaction on juvenile growth rate. Significant effects are indicated as *** ($p < 0.001$)

Effect	Df	Df.res	F value	p-value	Significance
Diet	2	81	79.503	$< 2.22 \times 10^{-16}$	***
Size	1	81	42.813	5.06×10^{-9}	***
Diet × Size	2	81	9.158	2.60×10^{-4}	***

Discussions

The trophic ecology of *Pinna nobilis* has long been assumed to be primarily based on phytoplankton and microzooplankton consumption, as is typical for suspension-feeding bivalves (Alomar et al., 2015). However, this assumption alone fails to explain the persistence of dense populations in highly oligotrophic environments, particularly within *Posidonia oceanica* meadows, where planktonic primary production is often insufficient to sustain large filter feeders. Previous observations of gut contents and particle retention efficiency have already indicated that detrital material may represent a substantial fraction of the ingested particles, reaching up to 95% of total intake (Davenport et al., 2011).

Building on these observations, large-scale spatial analyses suggested that seagrass detritus could represent a primary food source for *P. nobilis*, especially in oligotrophic coastal systems where hydrodynamic conditions favor detritus resuspension and transport (Pensa et al., 2022). The present study provides the first experimental evidence supporting this hypothesis, demonstrating that seagrass detritus, when conditioned and enriched by heterotrophic bacteria, sustains juvenile growth rates even better than those achieved with optimized phytoplankton-based diets commonly used in bivalve aquaculture.

The role of seagrass detritus in coastal food webs is well documented, but its nutritional value is largely mediated by microbial processing. Detrital particles derived from *P. oceanica* leaves undergo progressive fragmentation during transport and resuspension, increasing their surface area and exposure to microbial colonization (Danovaro, 1996; Danovaro & Fabiano, 1997). This microbial conditioning results in a marked enrichment in protein content and bioavailable nutrients, effectively converting refractory plant material into secondary microbial production (Peduzzi & Herndl, 1991). In this context, *P. nobilis* likely derives a substantial proportion of its energy intake from heterotrophic bacteria associated with detrital particles rather than from the plant material itself.

Pinna nobilis growth was found to fit the von Bertalanffy growth model, with the highest growth rate in juveniles decreasing with age, with a consistent reduction (~50%) from the first to the second year (Hendriks et al., 2012). Indeed, in natural populations around Mallorca, shell growth of 8–9 mm/month was recorded during the first year (shell length from 0–100/150 mm) and 4–5 mm/month during the second year (shell length from 100/150 to 200/250 mm). However, in more eutrophic ecosystems, such as the Gulf of Trieste, high growth rates have been reported also for juveniles in their second year of age, with growth reaching approximately 6 mm per month (Pitacco et al. 2025a).

Previous artificial diets tested, especially those based on microalgae (Prado et al., 2021), revealed to be inadequate evidencing growth rates generally lower than wild growth performances (Hendriks et al. 2012; Hernandis et al., 2023; Prado et al., 2020). Hendriks et al. (2012) demonstrated that growth in juveniles (initial length 14–56 mm) is greater during the early stages when fed a mix of aquaculture algae (*Rhinomonas* and *Tetraselmis* spp.) but remains much lower compared to organisms monitored in the wild (5.4 mm/month vs. 8.4 mm/month). A similar result was found by Prado et al. (2020), among artificial diets, the best performing diet resulted in the control one, based on microalgae and sediment rich in organic matter. However, even in this case, juveniles' (69–137 mm) growth rate was 30–40% lower than the one observed in the wild, leading the authors to conclude that these differences are linked to nutritional deficiencies. Along with the findings of Hendriks et al. (2021) on other pinnids, it is hypothesized that food availability is a key factor influencing shell growth rates, with strong seasonality observed (Katsanevakis 2007).

The present study reveals that juvenile *P. nobilis* exhibit significantly higher monthly growth rates even than those observed in other studies in smaller naturally fed juveniles (Acarli et al., 2011; Hendriks et al., 2012; Kozul et al., 2012; Prado et al., 2020; Theodorou et al., 2024; Trigos et al., 2015). The highest growth rate was observed in small size class (150–190 mm) in seagrass detritus diet treatment, reaching values of 8.00 ± 1.74 mm/month. For the first time, these results experimentally confirm that seagrass detritus enriched by heterotrophic bacteria serves as a food source driving *P. nobilis* growth, an hypothesis previously inferred from trophic behavior and spatial association of the species with seagrass meadows (Najdek et al., 2013; Pensa et al., 2022; Trigos et al., 2014). Furthermore, when comparing growth rates to specimens fed with nutritionally optimized phytoplankton mix for bivalve aquaculture (Shellfish Diet®), those raised on seagrass detritus performed better. This proves that seagrass detritus is an effective and optimal performing food source for captive breeding of *P. nobilis*.

Environmental implications

In natural environments, mortality in *P. nobilis* is often observed during the summer, with factors like increased temperature, salinity, and reduced phytoplankton availability have been suggested as main contributing factors (García-March et al., 2020; Lattos et al., 2023). However, the presence of residual populations in estuarine environments with extensive seagrass meadows reinforces the seagrass detritus role in supporting population density. Moreover, the survival of *P. nobilis* in estuaries influenced by freshwater inputs suggests that during the oligotrophic late summer period, nutrient-rich freshwater may support phytoplankton growth, thus aiding population survival in seagrass detritus scarcity season (Rakaj et al., under review). Indeed, higher growth rates are reported in estuarine systems compared to coastal environments (García-March et al., 2020). This study demonstrates that while seagrass detritus is expected to

be the primary food source for *P. nobilis*, phytoplankton also contributes to growth, though with lower mean performances. These results suggest that during summer, when detritus availability is reduced, due to either scarcity related to the generative period of seagrass meadows or reduced hydrodynamics, phytoplankton, generated by freshwater nutrient inputs, may act as an alternative food source, helping populations endure periods of nutritional scarcity (Rakaj et al., under review). In this context, the interaction between food scarcity and debilitation deriving from *P. nobilis* infection can fully support the survivance observed in eutrophic systems, such as estuaries and lagoons.

The findings of the presents study highlight the crucial role of seagrass detritus transport and accumulation in oligotrophic environments for sustaining *P. nobilis* populations. These results have broader ecological implications, especially considering the massive decline of *P. nobilis* following the Mass Mortality Event (MME), which not only threatens biodiversity but also eliminates a key species for nutrient transformation and energy flow in the *P. oceanica* detritus food chain.

Due to the species risk of extinction, the captive breeding of *P. nobilis* is an emerging field, and research on feeding requirements for its growth and survival remains critical. We offer practical guidelines that can be directly applied to develop ex situ rearing of this species in captivity (Supplementary Guidelines, Rakaj et al., under review). This study demonstrates that juveniles collected as spat and artificially reared on a diet tailored to meet the species' nutritional requirements can serve as an indispensable resource for ex-situ conservation and broodstock development, supporting future restorative aquaculture efforts.

Table 2
Literature and present study data on *P. nobilis* growth experiments

Sampling Area	Feeding	Number of specimens	Average initial length (mm)	Duration	Survivance (%)	Growth rate (mm/month)	Reference
North Adriatic Sea	Artificial (<i>Isochrysis</i> , <i>Phaeodactylum</i> , and <i>Tetraselmis</i>)	14	173.1 ± 10.8	20 days	92.86%	1.59 ± 0.92	Present Study
North Adriatic Sea	Artificial (<i>Isochrysis</i> , <i>Phaeodactylum</i> , and <i>Tetraselmis</i>)	15	201.7 ± 7.8	20 days	86.67%	0.98 ± 0.67	Present Study
North Adriatic Sea	Artificial (Shellfish Diet 1800)	14	173.5 ± 9.60	90 days	100	6.67 ± 1.89	Present Study
North Adriatic Sea	Artificial (Shellfish Diet 1800)	15	201.9 ± 7.6	90 days	100	2.44 ± 2.15	Present Study
North Adriatic Sea	Artificial (Seagrass detritus)	15	172.5 ± 11.9	90 days	100	8.00 ± 1.74	Present Study
North Adriatic Sea	Artificial (Seagrass detritus)	14	201.5 ± 8.0	90 days	100	6.01 ± 3.73	Present Study
Balearic Islands (Balearic Sea)	Artificial (<i>Rhinomonas</i> and <i>Tetraselmis</i>)	63	14.1–55.7	13 days	n.r.	5.4	Hendriks et al., 2012
Ebro delta (Balearic Sea)	Artificial (<i>Isochrysis</i> , <i>Tetraselmis</i> , and <i>Chaetoceros</i> + riverine detritus)	8	69–137	5 months	63	6.43 ± 0.92	Prado et al., 2020
Ebro delta (Balearic Sea)	Artificial (<i>Isochrysis</i> , <i>Tetraselmis</i> , and <i>Phaeodactylum</i>)	8	69–137	5 months	0	0.93 ± 0.31	Prado et al., 2020
Ebro delta (Balearic Sea)	Artificial (riverine detritus)	8	69–137	3 months	0	0.93 ± 0.31	Prado et al., 2020
Ebro delta (Balearic Sea)	Artificial (<i>Tetraselmis</i> + riverine detritus)	8	69–137	5 months	13	0.93 ± 0.31	Prado et al., 2020
Ebro delta (Balearic Sea)	Artificial (<i>Tetraselmis</i>)	8	69–137	5 months	0	0.93 ± 0.31	Prado et al., 2020
n.a. = not assessed; n.r. = not reported; * = estimated data							

Sampling Area	Feeding	Number of specimens	Average initial length (mm)	Duration	Survivance (%)	Growth rate (mm/month)	Reference
Ebro delta (Balearic Sea)	Artificial (<i>Tetraselmis</i> , and <i>Isochrysis</i>)	8	69–137	5 months	0	0.93 ± 0.31	Prado et al., 2020
Ebro delta (Balearic Sea)	Artificial (<i>Tisochrysis</i> and <i>Chaetoceros</i>)	8	294 ± 22	233 days	n.r.	n.a.	Hernandis et al., 2023
Ebro delta (Balearic Sea)	Artificial (<i>Tisochrysis</i> and <i>Chaetoceros</i>)	6	428 ± 33	201 days	n.r.	n.a.	Hernandis et al., 2023
Embiez Island (Ligurian Sea)	Artificial (<i>Isochrysis</i> , <i>Pavlova</i> , <i>Chaetoceros</i>)	19	31.3 ± 17.9	9 months	100	7.8	Trigos et al., 2015 (conference poster)
Embiez Island (Ligurian Sea)	Artificial (<i>Isochrysis</i> , <i>Pavlova</i> , <i>Chaetoceros</i>)	18	23.7 ± 15.0; 26.3 ± 3.1; 24.9 ± 9.2	9 months	n.r.	15.8 ± 4.9; 12.8 ± 4.8; 13.8 ± 4.0	Martinović et al., 2021
Venice lagoon (Adriatic Sea)	Artificial (<i>Isochrysis</i> , <i>Diacronema</i> , <i>Chaetoceros</i> + Easy Reef)	11	280–435	6 months	36.4	n.a.	Ferranti et al., 2024
Gulf of Trieste (Adriatic Sea)	Commercial food (no detail)	13	5–24	3 months	77	1–4	Pitacco et al., 2025a
Gulf of Trieste (Adriatic Sea)	Commercial food (no detail)	39	6–80	3 months	54	1–10	Pitacco et al., 2025a
Gulf of Trieste (Adriatic Sea)	Easy Reefs	n.r.	32 (10–80) *	8 months	0	3.5 (0.0–11) *	Pitacco et al., 2025b
Gulf of Trieste (Adriatic Sea)	Artificial (<i>Isochrysis</i> , <i>Chaetoceros</i> and <i>Diacronema</i>)	n.r.	36 (20–50) *	8 months	0	0.6 (0.2–8.5) *	Pitacco et al., 2025b
Ambracian Gulf (Ionian Sea)	Wild	12	54.3 ± 12.0	152 days	58.33	3.2	Theodorou et al., 2024
Balearic Islands (Balearic Sea)	Wild	12	n.r.	3 months	n.r.	8.4	Hendriks et al., 2012
n.a. = not assessed; n.r. = not reported; * = estimated data							

Sampling Area	Feeding	Number of specimens	Average initial length (mm)	Duration	Survivance (%)	Growth rate (mm/month)	Reference
Ebro delta (Balearic Sea)	Wild	8	69–137	5 months	100	8.9	Prado et al., 2020
Izmir Bay (Aegen Sea)	Wild	30	32	16 months	93	7.2	Acarli et al., 2011
Izmir Bay (Aegen Sea)	Wild	30	73	16 months	100	5.39	Acarli et al., 2011
Kotor Bay (Adriatic Sea)	Wild	29	39.9 ± 16.3; 41.1 ± 8.8	12 months	79	12.7 ± 2.2; 13.4 ± 5.6	Martinović et al., 2021
Mali Ston Bay (Adriatic Sea)	Wild	354	29 ± 12.1	330 days	n.d.	10.56 (1° year)	Kozul et al., 2011a
Mali Ston Bay (Adriatic Sea)	Wild	120	145.23	400 days (1° year)	90–95	8.23	Kozul et al., 2012
Mali Ston Bay (Adriatic Sea)	Wild	120	240 *	400 days (2° year)	90–95	7.09	Kozul et al., 2012
Mali Ston Bay (Adriatic Sea)	Wild	163	35.2 ± 14.3	24 months	n.r.	n.a.	Kozul et al., 2024
Gulf of Trieste (Adriatic Sea)	Wild	11	180–420 *	360 days	n.r.	0.73–10.75	Pitacco et al., 2025a
Gulf of Trieste (Adriatic Sea)	Wild	6	150–248 *	7 months	n.r.	3–10	Pitacco et al., 2025a
Balearic Islands (Balearic Sea)	Wild - eutrophic	n.r.	n.r.	749 days	27.8 *	5.48 *	Alomar et al., 2015
Balearic Islands (Balearic Sea)	Wild - oligotrophic	n.r.	n.r.	749 days	88.5 *	3.35 *	Alomar et al., 2018
n.a. = not assessed; n.r. = not reported; * = estimated data							

Conclusion

This study provides experimental evidence that seagrass detritus, enriched by heterotrophic bacteria, represents a primary food source for *Pinna nobilis*, supporting juvenile growth even in oligotrophic environments. The higher growth rates observed in individuals fed detritus compared to phytoplankton-based diets highlight the nutritional importance of detritus-mediated trophic pathways for this species. Seasonal fluctuations in detritus availability may therefore influence population dynamics, with phytoplankton potentially acting as a complementary resource during periods of reduced detrital input.

These findings have important conservation implications, particularly for ex-situ rearing and broodstock development. Improved understanding of the trophic requirements of *P. nobilis* can inform the design of effective restorative aquaculture strategies, enhancing growth performance under controlled conditions while reducing dependence on wild populations. Deeper understanding of the species ecology, especially the dependence on detritus to support historically high population densities observed in oligotrophic environments, guides the goals for species restoration in the wild.

Overall, this study bridges ecological theory and applied conservation, demonstrating that replicating natural detritus-based trophic pathways in captivity can substantially improve rearing outcomes and contribute to the long-term recovery of *Pinna nobilis* populations in the Mediterranean Sea.

Declarations

The authors declare no competing interests.

The collection of biological samples used in this study complied with the national and international legislation, and IUCN guidelines for protected species, both plants and animals.

The collection of juvenile *Pinna nobilis* from mussel farms was carried out under authorization from the Italian Ministry of Environment; authorization reference: 0066959/2023.

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Author Contribution

A.R.: conceptualization, methodology, investigation, resources, project administration, validation, and supervision, writing—review and editing D.P.: investigation, formal analysis, writing—original draft G.P.: formal analysis, writing—review and editing L.G.: investigation, writing—review and editing; A.F.: investigation, writing—review and editing; J.D.S.: writing—review and editing; S.C.: supervision, writing—review and editing

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Data Availability

The data are available on request from the corresponding author, A.R.

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Figures

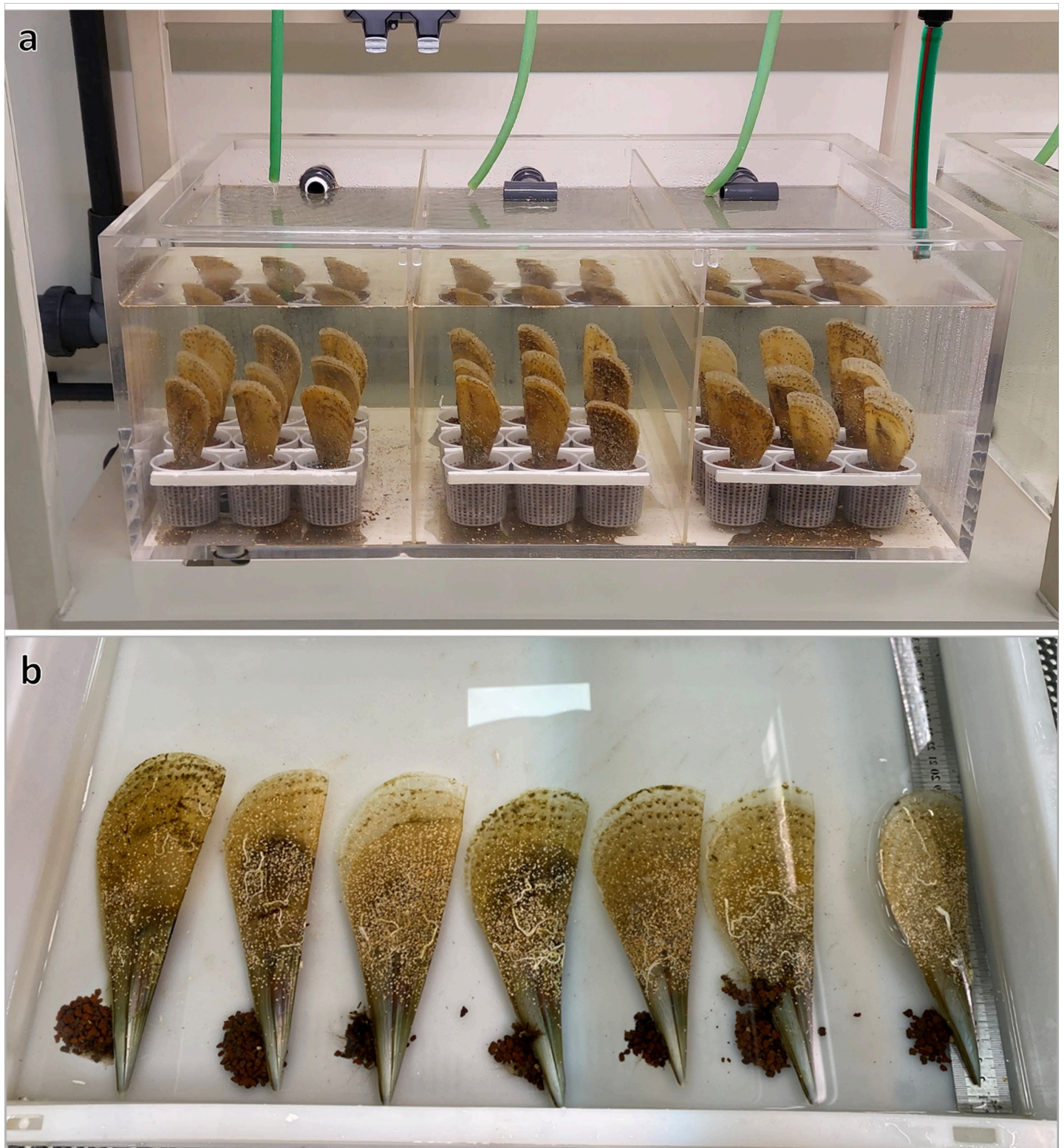


Figure 1

a) Juveniles first maintenance; b) Collection of biometric data before the beginning of the feeding experiment

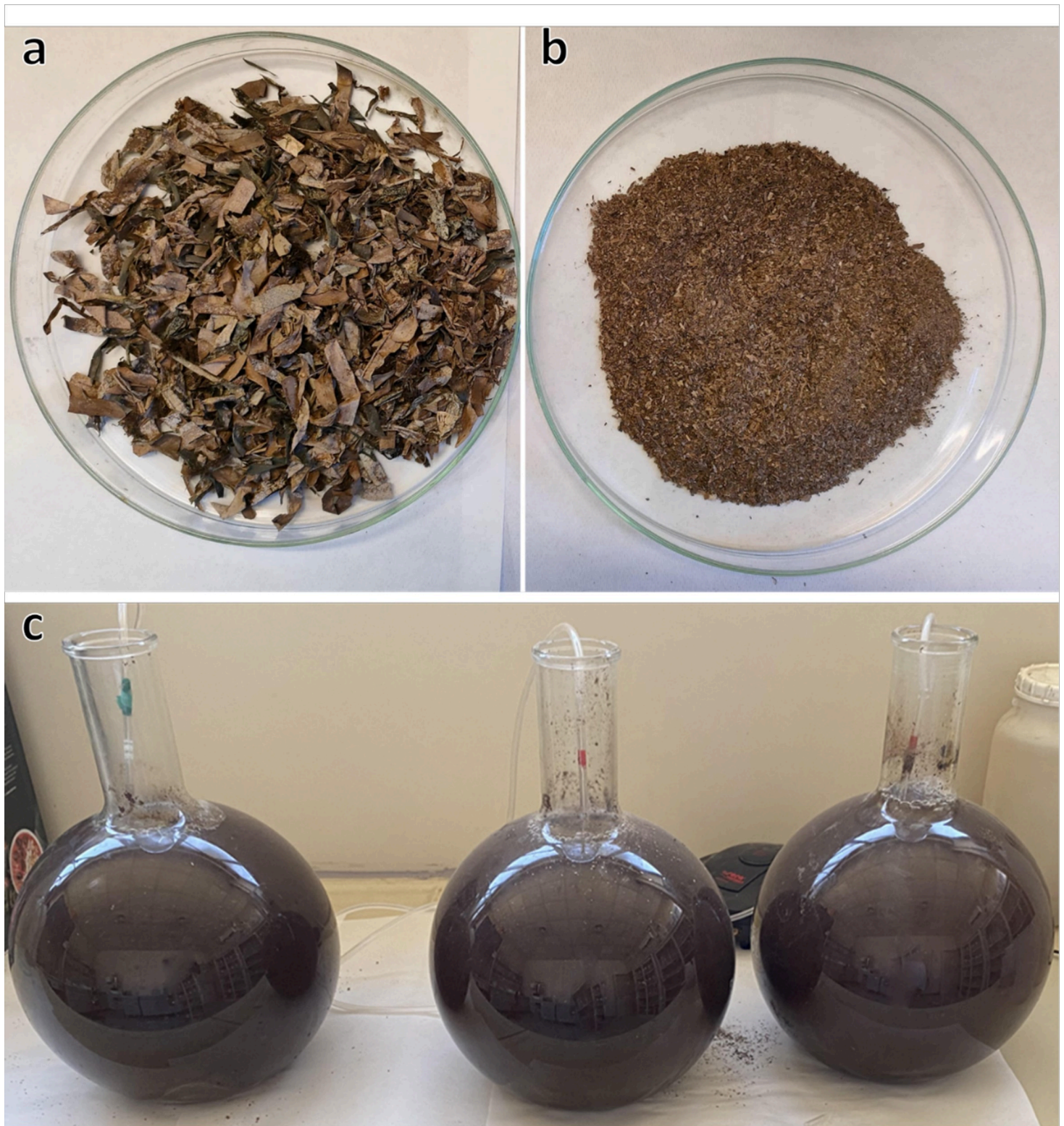


Figure 2

Seagrass detritus preparation: a) dry *Posidonia oceanica*'s leaves; b) mashed and sieved *P. oceanica*'s leaves; c) 10L round-bottom flasks bioreactors for conditioning the detritus

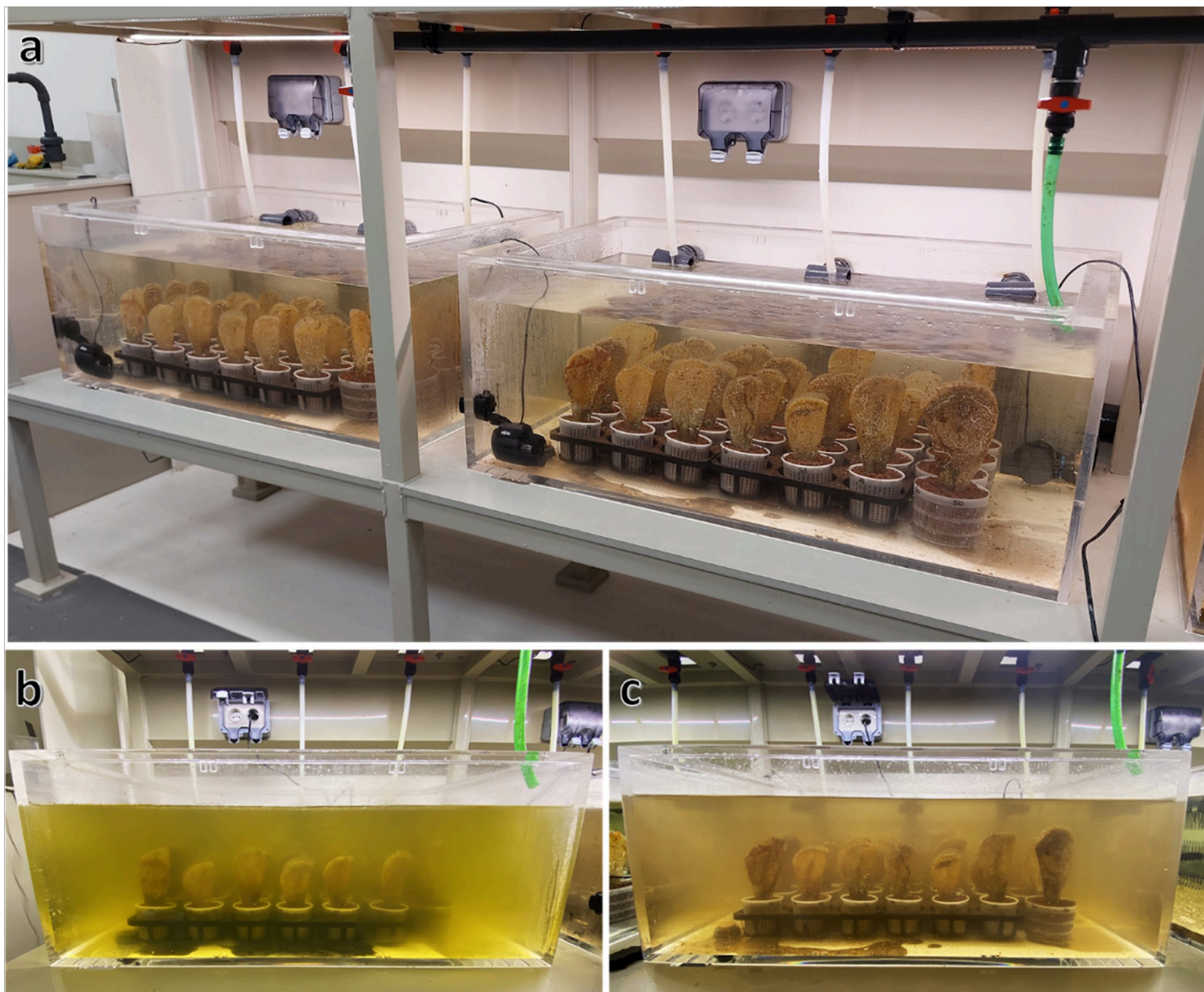


Figure 3

a) experimental setting; b) Shellfish Diet and c) seagrass detritus diet

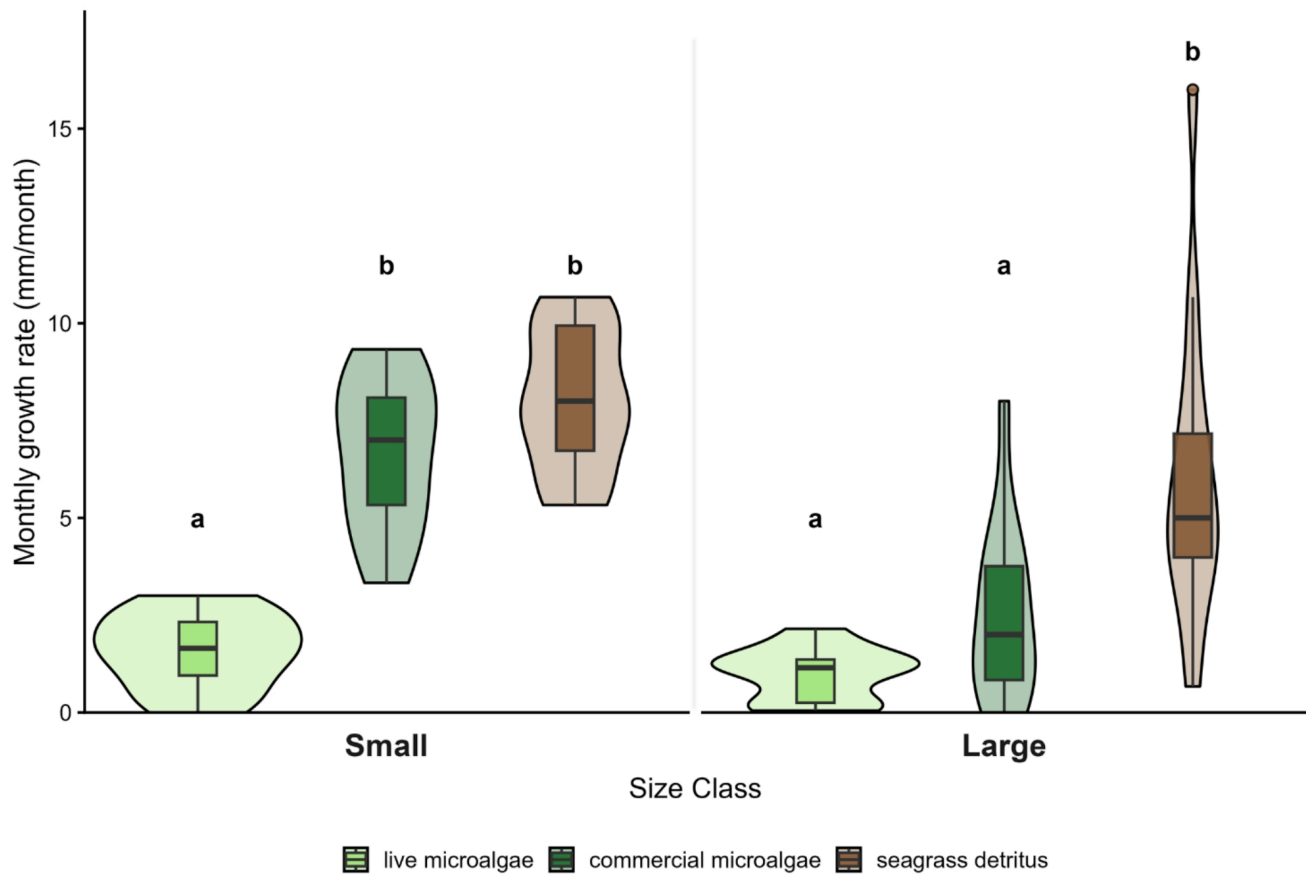


Figure 4

Monthly growth rate (mm/month) of *Pinna nobilis* juveniles in two size classes (Small and Large) exposed to three diet treatments: live microalgae, commercial microalgae, and seagrass detritus. Violin plots show the distribution of individual growth values, with overlaid boxplots representing medians and interquartile ranges. Different letters above the boxplots indicate statistically significant differences within the same size group ($p < 0.05$)

Supplementary Files

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