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Effects of Rearing Density, Substrate Height, and Feeding Frequency on Growth and Biomass Production of *Hediste diversicolor*

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Abstract: The polychaete *Hediste diversicolor* is a suitable species for industrial aquaculture; however, cost-effective culture techniques need to be developed for its intensive production. The aim of this study is to evaluate the effects of worm density and substrate height and their interaction, as well as feeding frequency, on the rearing performance of *H. diversicolor*. Two trials were conducted. In trial 1, the effects of two substrate heights—6 and 12 cm—and two rearing densities—1000 and 4000 individuals m^{-2}—were assessed in terms of worm growth and biomass production. Worm initial wet weight was 48 mg, and specimens were fed with commercial fish feed during a 70-day assay. The results show no interaction between rearing density and substrate height, and confirm density as a key factor in growth; however, a density of 4000 individuals m^{-2} results in a significant increase in production (final biomass three times higher for the highest rearing density) without affecting survival. In trial 2, the effect of three levels of feeding frequency—seven days a week; three times a week; and once a week—on growth in individuals of three weight classes—small (25–50 mg); medium (100–150 mg); large (250–350 mg)—was evaluated in a 15-day growing assay. Feeding frequency showed a major influence on the smallest size class, with the best growth indicators obtained at the highest feeding frequency. This study shows that *Hediste diversicolor* can be reared at a high stocking density to obtain a higher biomass production, and that feeding frequency must be considered as an important factor and adapted to the culture phase.

Keywords: *Hediste diversicolor*; density; substrate height; feeding frequency

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1. Introduction

The polychaete *Hediste diversicolor*, commonly known as ragworm, is a suitable species for industrial aquaculture due to its adaptability to wide environmental conditions, its feeding flexibility, and its high growth rate [1]. Even though ragworms can be used in a traditional manner as fishing bait [2,3], they have also been used as feed supplements for some farmed fish and crustaceans, promoting gonad maturation and spawning [1,4]. This species has attracted a great deal of interest in recent years as an alternative feed ingredient for the aqua-feed industry due to its high content of unsaturated fatty acids—such as eicosapentaenoic acid (EPA: 20:5n-3), docosahexaenoic acid (DHA: 22:6n-3), and arachidonic acid (ARA: 20.4n-6)—proteins, phospholipids, and hormones [5]. Its desaturation capacity is of particular relevance, allowing *H. diversicolor* to produce *de novo* polyunsaturated fatty acids (PUFA), including EPA and DHA [6]. *H. diversicolor* is also a candidate species for land-based integrated multitrophic aquaculture (IMTA) systems, with high potential for biomitigation of superintensive fish farm effluents [7]. Some important concerns regarding this species are the negative environmental impact of its collection from the wild [1] and the need to produce pathogen-free polychaetes in aquaculture, especially in shrimp broodstock feed [8].

The biology and ecology of *H. diversicolor* have been reviewed by Scaps [2]. Briefly, this infaunal species inhabits the shallow marine and brackish waters in the north temperate zone of the Atlantic, with a great tolerance to variations in environmental conditions (temperature, salinity, and hypoxia); this is a gonochoristic and monotelic species that dies after reproduction. *H. diversicolor* may behave as a deposit-feeder which consumes organic matter and plant detritus from the sediment surface, but carnivorous behaviors have also been reported.

Different aspects of *H. diversicolor* aquaculture have been reviewed in Pombo et al. [1]. These authors suggest that, despite advances over the last decades in knowledge of ragworm biology and cultivation, for its intensive production, growth promotion, biomass increase, and maturation delay are necessary. Considering their recommendation on the development of cost-effective aquaculture techniques for marine annelid worms, we study, in the present work, aspects related to intraspecific density, substrate height, and feeding to optimize worm farming.

The relationship between growth rate and density is well known for *H. diversicolor* and other polychaete species, and adverse effects of high rearing density on growth have been described [9,10]. For *H. diversicolor*, Nesto et al. [9] obtained an greater growth rate and similar biomass production with a density of 1000 individuals m^{-2} than that obtained with a density of 3000 individuals m^{-2}; however, production with such low density is scarce, and intensification of cultivation is necessary to achieve industrial profitability.

H. diversicolor lives buried in the substrate, where it builds U- or Y-shaped burrows; burrow depth increases with size [11]. Shape, size, and number of burrows are variable, and territorial worms can minimize contact with their neighbors by restricting the burrow to a vertical shaft [12]. Miron et al. [13] documented *Nereis virens* building a U-shaped burrow when population density was low, but an I-, L-, or Y-shaped burrow at higher densities. Similarly, the polychaete species *Perinereis cultrifera* can increase the vertical component of its burrow when surface is limited due to high population density [14]. A behavioral study of *N. virens* revealed that individuals compete for burrow space rather than food [15]. Therefore, an increase in substrate height could result in increased space for growing worms, and we hypothesize a potential interaction between density and substrate height.

The influence of the type of sediment on the development of *H. diversicolor* has been studied by different authors [16,17], and substrate heights from 5 to 15 cm are normally used under experimental conditions [7,9,15,18–22]. However, no specific experimentation has been performed on the effect of substrate height on growth or biomass production, or its interaction with density.

Other husbandry issues are also relevant to optimizing production; feeding frequency is one aspect not yet well defined for *H. diversicolor* cultivation. Determining the optimal feeding frequency is important to enhance growth performance and survival, ensure maximal feed conversion ratios, reduce feed wastage, and improve animal welfare [23,24]. Feeding frequency modulates aggression and cannibalism in aquatic animals [25]. The optimal feeding frequency may vary according to age, size, feed quality, water temperature, and culture system [23,26]. Different feeding frequencies have been applied in *H. diversicolor* [4,9,16,18,19] but not experimentally investigated.

The aim of the present study is to evaluate the effects of worm density and substrate height and their interaction, as well as feeding frequency, on the rearing performance of *H. diversicolor* considering worm size.

2. Materials and Methods

2.1. Experimental Animals

Juveniles of *H. diversicolor* were obtained from a captive stock cultivated for several generations in stocking tanks (height 0.5 m; area 1 m^2) at the "El Bocal" Marine Aquaculture Plant (Cantabria, N Spain). The base of each tank was covered with three filter layers: (1) a bottom layer (pool tiles), (2) a middle layer (geotextile fabric), and (3) a top layer (1000-μm plastic mesh). A 20 cm high sand column kept the worms permanently submerged below a 10–15 cm water column. Water entered the tanks at the surface and drained through the sediment column and filter layers, evacuating from the bottom of the tank. Spontaneous reproduction events occurred while feeding the worms with fish feed under said conditions, and new generations of worms were repeatedly recruited. By sieving the sand, individuals of a wide range of body weights and ages were obtained.

2.2. Experimental Cultivation System Design and Experimental Conditions

Trials were performed in specially designed experimental units (EUs), which consisted of cylindrical PVC frames (trial 1: height 0.2 m, area 0.01 m^2; trial 2: height 0.2 m, area 3.25×10^{-3} m^2) closed at the base and side by 335-µm mesh (Figure 1). EUs were filled with quarry sand (grain size: 0.25–1.0 mm). Groups of four (trial 1) or nine (trial 2) EUs were placed inside polycarbonate trays (width: 0.35 m; height: 0.30 m; length: 0.54 m) connected to a recirculating aquaculture system (RAS) that included biological and mechanical filtration, skimming, UV sterilization, and temperature control. Each tray was provided with a microbubble aeration bar. The recirculating flow rate in each tray was 1.5 L min^{-1}. The EUs inside the trays remained partially submerged so that the sediment columns were always immersed but the upper part of the EUs were out of the water, meaning that both the ragworms and the feed supplied were prevented from escaping the experimental units, while allowing water exchange [27].

Figure 1. (**A**) Experimental units (EUs) for rearing polychaetes in trials 1 and 2. (**B**) Polycarbonate trays in which experimental units were placed.

Temperature, salinity, and photoperiod were set at 20 °C, 36‰, and 16/8 h light/dark, respectively. Dissolved oxygen (DO), pH, temperature, and salinity were recorded daily, and ammonia and phosphates once a week, using colorimetric tests (SERA GmbH aquarium Test).

2.3. Experimental Design and Statistical Procedures

2.3.1. Trial 1: Rearing Density and Substrate Height

The effect of two substrate heights—low height: 6 cm; high height: 12 cm—and two rearing densities—low density: 1000 individuals m^{-2}; high density: 4000 individuals m^{-2}—was assessed in terms of worm growth. The number of individuals per EU was 10 in the low-density treatment and 40 in the high-density ones. Twelve EUs were distributed in three polycarbonate trays (four per tray) following a randomized block design with two fixed factors, so that one replicate of each treatment was present in each tray. Juveniles of *H. diversicolor* with wet body weight (BW) of 48 ± 19 mg, (mean ± standard deviation (SD)) were used in this trial. The duration of the trial was set at 70 days. Every two weeks, the sand in the EUs was gently sieved (1000 µ), and the worms were counted and weighed after wiping on tissue paper. This means that four intermediate samplings, as well as a final one, were performed; therefore, the experimental design also includes sampling times as a repeated measures factor.

Polychaetes were fed with sole (*Solea senegalensis*) weaning feed pellets (0.35–0.50 mm in size; Skretting GEMA NEO: protein 60%, fat 11%) in a total amount equivalent to 4% of the wet biomass, incremented daily at 4% and distributed three times per week. The presence of feed pellets on the surface of the sand every day ensured an ad libitum feeding manner. After each sampling, the feed ration for the next period was recalculated according to the biomass recorded.

Sources of variability in the experimental design were mean BW, absolute growth rate (AGR), specific growth rate (SGR), and survival rate (SR). AGR and SGR were calculated for each intersampling period and for the total period as follows:

$$\mathrm{AGR} = (\mathrm{BW_f} - \mathrm{BW_i})/\mathrm{t}$$

$$\mathrm{SGR} = 100 \times (\mathrm{Ln}(\mathrm{BW_f}) - \mathrm{Ln}(\mathrm{BW_i}))/\mathrm{t}$$

where $\mathrm{BW_i}$ and $\mathrm{BW_f}$ are initial and final wet body weight in mg and t is time in days.

SR was calculated after each rearing period as the percentage of live worms in relation to the initial number.

The null hypothesis is that the interaction of density with height shows no significant effect on the sources of variability under consideration, and neither do either of them separately. Hypothesis testing was performed using a randomized block design analysis of variance (ANOVA) with two fixed factors (height and density) and a repeated measures factor (sampling time). Shapiro–Wilk and Levene's tests were used to determine the normality and equality of error variance. Post hoc comparisons of significant interactions between factors were analyzed by pairwise multiple comparisons adjusted by Bonferroni correction. SGR and AGR for the total period were analyzed using a univariate ANOVA with two factors (height and density). The statistical significance level was set to $p < 0.05$. Statistical analyses were performed using SPSS software version 25. The results are shown as mean $\pm$ SD.

Total biomass production (g m^{-2}) was calculated as the product of density by mean BW at every sampling point as a proxy for productivity. In the two last samplings, the number of individuals with a size greater than 0.5 g was also estimated.

2.3.2. Trial 2: Feeding Frequency

In this trial, the effect of feeding frequency on growth was evaluated in individuals of three weight classes: small (25–50 mg), medium (100–150 mg), and large (250–350 mg). Ragworms were fed with the sole weaning feed specified in trial 1 at a daily rate of 4% of the wet biomass. Three levels of feeding frequency were assessed: daily (D: seven days a week), three times a week (3T), or once a week (1T). In 3T and 1T, the total feed for seven days was distributed over three and one feedings per week, respectively; therefore, for every weight class, the total amount of feed supplied was the same, regardless of feeding frequency. Worms in the EUs were reared at a density of 4000 individuals m^{-2} and a substrate height of 12 cm, and triplicate EUs for each feeding frequency were placed in a polycarbonate tray (three trays in total). The experiment lasted 15 days; at the end of the experiment, all polychaetes in the EUs were gently sieved, counted, and individually weighed.

Sources of variability under consideration were mean BW, AGR, SGR, SR, and the coefficient of variation (CV) of BW. The null hypothesis is that neither the interaction of size with feeding frequency, nor either factor separately, show any significant effect on the sources of variability under consideration. Given that the homoscedasticity and normality criteria were not met even when transforming the data, we performed a nonparametric permutational analysis of variance [28] based on Bray–Curtis dissimilarities of untransformed mean BW, AGR, SGR, SR, and CV data using 4999 residuals under a reducer model.

As in the experiment described above, the statistical significance level was set to $p < 0.05$, and data were analyzed using PRIMER 6 and PERMANOVA + software.

3. Results

3.1. Trial 1: Rearing Density and Substrate Height

Temperature and salinity stayed in the preset order (18.9 ± 1.0 °C and 36.1 ± 0.9‰, respectively). DO saturation was always above 90%, pH was 8.04 ± 0.1, ammonia was below 0.025 mg L^{-1}, and phosphate was below 0.25 mg L^{-1} during the experimental period (70 days).

No significant block effect was observed. For AGR and SGR, the analysis showed no significant interactions between time and height, time and density, or density and height; for BW, a significant interaction was found between time and density. When considering the factors separately, a significant effect of time and density (not of height) was observed for all three variables (AGR, SGR, and BW; Table 1). In relation to time, BW increased throughout the culture for both rearing densities; SGR decreased and AGR increased, except during the last period (57–70 days). All three variables showed higher values at low densities (Figure 2, Table 2).

Table 1. ANOVA results for the effects of time, substrate height and density.

	AGR	SGR	BW	Biomass	Survival
Time	***	***	***	***	***
Time × height	n.s.	n.s.	n.s.	*	n.s.
Time × density	n.s.	n.s.	***	***	n.s.
Time × height × density	n.s.	n.s.	n.s.	n.s.	n.s.
Density × height	n.s.	n.s.	n.s.	n.s.	n.s.
Density	**	**	**	***	n.s.
Height	n.s	n.s	n.s.	n.s.	n.s.

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; n.s. nonsignificant.

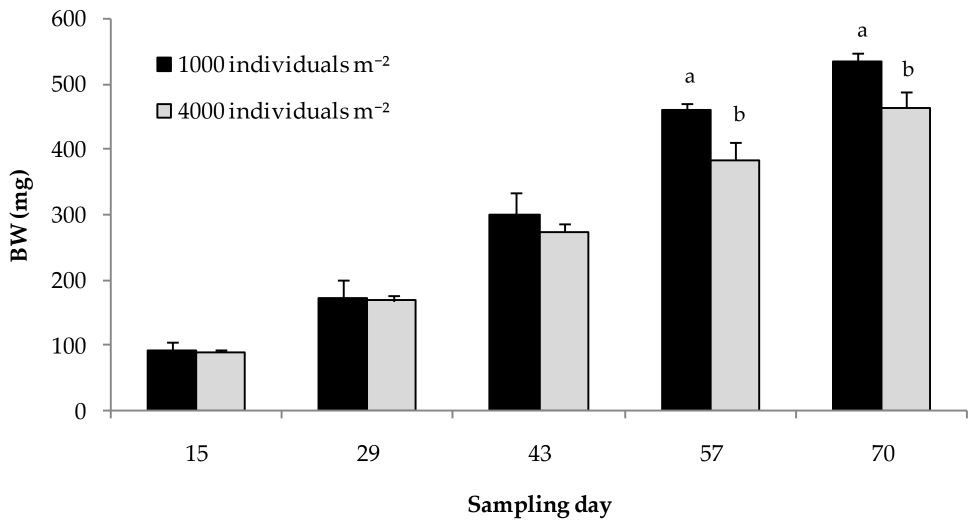

Figure 2. Wet body weight (BW) of *H. diversicolor* reared at two densities at the different sampling points. Different letters indicate significant differences between 1000 individuals m^{-2} and 4000 individuals m^{-2} ($p < 0.05$).

Post hoc comparisons showed that the effect of rearing density on mean BW and AGR were not significant until day 57, and no difference was observed in SGR. When considering the complete period of time studied (from day 1 to day 70), significant differences were found in SGR and AGR (Figure 2; Table 2).

Table 2. Growth indicators of *H. diversicolor* reared at two densities (1000 and 4000 individuals m^{-2}) for 70 days at different time periods. Results are presented as mean ± SD. Different superscript letters indicate significant differences between rearing densities ($p < 0.05$).

	Time Period:	1–15	15–29	29–43	43–57	57–70	1–70
AGR	1000	3.19 ± 0.84	5.79 ± 1.15	9.04 ± 0.93	11.35 ± 3.46 ^{a}	5.46 ± 3.00	7.07 ± 0.50 ^{a}
	4000	2.99 ± 0.21	5.26 ± 0.47	7.90 ± 0.99	7.87 ± 1.34 ^{b}	5.63 ± 1.01	6.01 ± 0.37 ^{b}
SGR	1000	4.65 ± 0.90	4.45 ± 0.42	3.92 ± 0.42	3.03 ± 0.83	1.22 ± 0.72	3.47 ± 0.08 ^{a}
	4000	4.47 ± 0.27	4.27 ± 0.24	3.7 ± 0.45	2.40 ± 0.30	1.45 ± 0.29	3.28 ± 0.10 ^{b}

SR was affected by time but not by substrate height or rearing density (Table 1); survival was higher than 97 ± 2% until day 43, then decreased to 91 ± 5% by day 57, and, by day 70, to 74 ± 12%. Although no statistical significance was found for substrate height, a tendency to obtain a higher final SR with greater height was observed (Table 3).

Table 3. Survival rate of *H. diversicolor* at day 70 reared at two densities (1000 and 4000 individuals m^{-2}) and two substrate height (6 and 12 cm). Results are presented as mean ± SD.

Substrate Height	Density	SR (%)
6 cm	1000 individuals m^{-2}	77 ± 8
6 cm	4000 individuals m^{-2}	60 ± 14
12 cm	1000 individuals m^{-2}	80 ± 10
12 cm	4000 individuals m^{-2}	80 ± 0

Regarding biomass production, the analysis showed a significant interaction between time and density, and an interaction between time and height. When considering the factors separately, a significant effect of time and density (not of height) was observed on biomass production (Table 1). Total biomass at day 70 was 412 ± 30 g m^{-2} and 1301 ± 287 g m^{-2} for low and high densities, respectively (i.e., about three times higher when an initial stocking density four times higher was used; Figure 3). Note that the maximum biomass production was reached on day 57, coinciding with the onset of the mortality increase. Post hoc pairwise multiple comparisons showed significant differences for biomass production at day 70 between 6 and 12 cm when individuals were reared at high density (Figure 4).

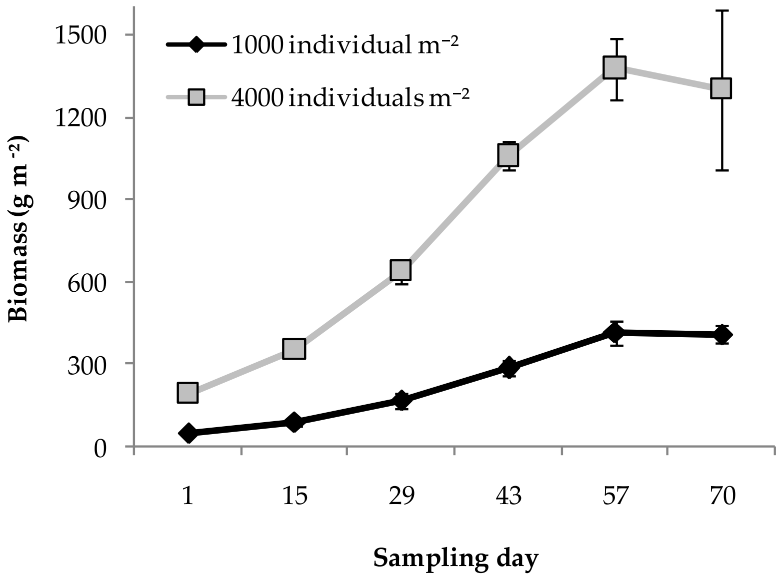

Figure 3. Biomass production (g m^{-2}) for different rearing densities (mean ± SD).

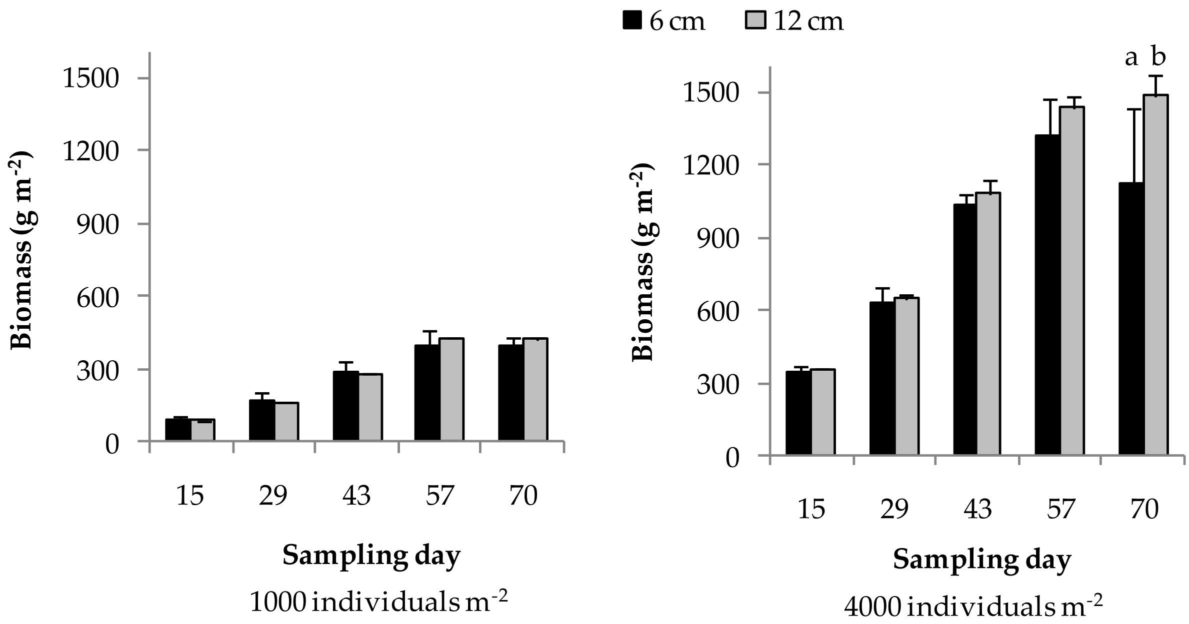

Figure 4. Biomass production (g m^{-2}) at each sampling point and each rearing density regarding substrate height. Different superscripts indicate a significant difference ($p < 0.05$).

At day 57, 39 ± 12% of the individuals reared at low density and 27 ± 6% of those reared at high density reached a size that can be sold as bait (size greater than 0.5 g). When considering survival on this day, the number of individuals that can be sold as bait was 2.7 times higher with high density (950 ± 217 individuals m^{-2}) than with low density (358 ± 107 individuals m^{-2}). Likewise, at day 70, 53 ± 8% of the individuals reared at low density and 37 ± 4% at high density reached a size greater than 0.5 g; the number of bait size individuals was 2.6 times higher with high density (1050 ± 28 individuals m^{-2}) than with low density (408 ± 58 individuals m^{-2}).

3.2. Trial 2: Feeding Frequency

Temperature and salinity stayed in the preset order (20.2 ± 0.3 °C and 35.6 ± 0.9‰, respectively). DO saturation was always above 90%, pH was 7.78 ± 0.08, and ammonia and phosphate were undetectable during the experiment.

The nonparametric permutational analysis of variance showed a significant effect of the interaction between feeding frequency and size and a significant effect of both of them separately on growth indicators (Table 4). The effect of feeding frequency was different among the different size classes: (1) for small worms highest growth indicators were obtained with the highest feeding frequency (D) and decreased significantly as feeding frequency decreased; (2) for medium worms, no differences were appreciated regarding feeding frequency; (3) for large worm, growth rates for the 1T group were significantly lower than those for the D group (Table 5).

Table 4. Nonparametric permutational analysis of variance results for the effects of size and feeding frequency.

	BW	SGR	AGR	Survival	CV
Size	***	***	***	**	*
Feeding frequency	***	***	***	n.s.	n.s.
Feeding frequency × size	***	*	**	n.s.	n.s.

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; n.s. nonsignificant.

Table 5. Growth for daily (D), three times a week (3T), and once a week (1T) feeding frequency in small (25–50 mg), medium (100–150 mg), and large (250–350 mg) worms. Results are presented as mean ± SD. Different superscript letters in the row indicate significant differences ($p < 0.05$).

		D	3T	1T
Small (25–50 mg)	SGR	7.4 ± 0.1 ^{a}	6.4 ± 0.1 ^{b}	5.3 ± 0.1 ^{c}
	AGR	4.7 ± 0.2 ^{a}	3.9 ± 0.2 ^{b}	2.9 ± 0.0 ^{c}
	BW	103.0 ± 5.3 ^{a}	93.3 ± 5.5 ^{a}	77.7 ± 1.2 ^{c}
Medium (100–150 mg)	SGR	5.3 ± 0.4 ^{a}	4.9 ± 0.1 ^{a}	4.3 ± 0.5 ^{a}
	AGR	9.9 ± 0.8 ^{a}	8.7 ± 0.4 ^{a}	7.3 ± 1.3 ^{a}
	BW	266.3 ± 9.5 ^{a}	247.3 ± 10.1 ^{a}	228.7 ± 20.2 ^{a}
Large (250–350 mg)	SGR	3.7 ± 0.5 ^{a}	3.3 ± 0.3 ^{a,b}	2.5 ± 0.5 ^{b}
	AGR	14.5 ± 2.5 ^{a}	12.1 ± 1.5 ^{a,b}	8.9 ± 1.8 ^{b}
	BW	496.0 ± 31.5 ^{a}	459.0 ± 18.5 ^{a,b}	422.3 ± 23.1 ^{b}

Size showed a significant effect on both CV and survival; feeding frequency did not show any significant effect (Table 4). CV increased in all size classes after a 15-day culture period. Initial and final CV were 20 ± 2% and 30 ± 7%, respectively, for small worms, 12 ± 2% and 22 ± 6%, for medium worms, and 10 ± 1% and 20 ± 4% for large worms. Significant differences in SR were found between large worms and the other two size classes. SR was high in all cases:100%, 99 ± 3%, and 94 ± 5%, for small, medium, and large, respectively.

4. Discussion

The development of cost-effective aquaculture techniques for marine annelid worms is essential [1]. To obtain maximum production at the lowest possible cost, zootechnical aspects must be improved, to optimize cultivation space and feed management. One of the most important management strategies producers can handle and control is the stocking density of each culture cycle [29]. Feeding frequency is also important to maximize growth, reduce feed waste, and optimize the cost of labor. In two different experiments in the present work, we studied (1) the effects of worm density and substrate height and their interaction, and (2) the effects of feeding frequency depending on the size of the individuals, on the growth of *H. diversicolor*. The results show no interaction between rearing density and substrate height and confirm density as a key factor in growth. Substrate height did not show any effects on growth; however, it showed an effect on biomass production. A density of 4000 individuals m^{-2} resulted in a significant increase in biomass production without affecting survival. In addition, in smaller sizes, a daily feeding frequency was more advantageous.

In the experiment on rearing density and substrate height, our results show that SGR decreases progressively over time in accordance with Nesto et al. [9]. The highest SGR for the 70-day culture period was 3.47% for 1000 individuals m^{-2}, lower than that obtained by some authors [4,9,19] but similar to that obtained by others [5,10,21], which may be due to differences in experimental conditions (e.g., salinity, density, feed type, feeding frequency, initial weight); possible genetic differences between populations should not be excluded either [2].

Final biomass was three times higher for the highest rearing density than for the lowest. This result is consistent with Scaps et al. [30]; in their study, *H. diversicolor* rearing densities were 1400 or 3000 individuals m^{-2}, and the final biomass was approximately three times higher for higher densities. Our result is also consistent with that of Safarik et al. [31], who found that, although daily growth was higher at a low density, at medium density, *Diopatra aciculata* showed low levels of apparent stress and high biomass return per unit area. Nevertheless, in *H. diversicolor*, Nesto et al. [9] found similar values of final biomass and a negative effect on survival when stocking density was 1000 or 3000 individuals m^{-2}. High survival is of paramount importance to obtain high biomass production. In the present work, the similar survival rate between the two rearing densities tested could have counterbalanced the lower growth. The experimental units used by other authors are, in most cases, containers closed at the base and sides and submerged in water tanks [9,30]; in those conditions, oxygen supply and waste removal would be compromised. Our high survival result obtained with the higher density could be related to the suitability of the experimental units used, which allowed an adequate water exchange through the whole sediment column. The water renewal system could have important implications for industrial polychaete production and must be further investigated.

High rearing densities resulted in a lower mean BW and AGR, but this difference between 1000 and 4000 individuals m^{-2} only occurred from day 57 onwards; this result suggests that split tanks could be used as a management strategy when a certain biomass is reached. Survival was high and constant for both densities and both heights tested until day 57 and then decreased, in agreement with data reported by Scaps et al. [30] when they reared *H. diversicolor* at 3000, 9000 and 18,000 individuals m^{-2}. Consequently, maximum biomass was reached at day 57 and then decreased, which may be due to reproductive events causing the death of mature worms, which would equally affect both densities since, according to Nesto et al. [9], the stocking density of juveniles does not influence the onset of gametogenesis nor the attainment of the sexual maturity stage. We did not assess reproductive events as no larvae was detected (a possible explanation is the net walls of the culture units might have allowed larvae to escape); however, at day 70, we inspected 33% of the survived worms and found the diameter of the oocytes of surviving females to be 129 ± 39 μ, an advanced stage of maturity according to the literature [9,32]. Our results suggest that time of harvesting is a key factor to attain high production; this represents a compromise between maximizing growth and controlling reproduction, both of which depend on environmental factors, mainly temperature [27] and type of food [9,19].

Mean final BW was higher with a low density than with a high density. Depending on the commercial end use, production objectives may be to obtain large individuals for use as bait or to produce biomass for use as new ingredients in aquaculture feeds. As reported by Marques et al. [7], the biomass of small, medium, or large cultured polychaetes shows an equal nutritional value for use as a premium ingredient in the diets of cultured organisms, in particular in the fatty acid profile; therefore, obtaining high biomasses is of paramount importance, even if the animals are small. Despite the fact that, for high density, individuals did not achieve the average weight of 0.5 g indicated by Nesto et al. [9] as a commercial size to be sold as bait, at the end of our experiment, the final number of bait-size worms was 2.6 times higher for the high density than for the low density, demonstrating that high rearing densities can allow to obtain a high number of worms for bait that have a high economic value.

Few specific investigations have been conducted on the effect of substrate height on worm production. In *Nereis virens*, Herwati et al. [33] showed substrate height to have a significant effect on growth, feed conversion ratio, survival rate, and also on protein, fat, methionine, and EPA contents. In our study the substrate heights tested did not affect growth significantly, although a tendency to obtain higher biomass production with higher substrate heights was observed, especially at higher densities. The cultivation temperature may have influenced the results; *H. diversicolor* burrow depth is estimated at 10.7 cm and varies inversely with temperature: at 20 °C, the temperature in our experiment, depth is approximately 6 cm; at 10 °C, 14 cm [34]. Burrow depth increases with body size and, in nature, burrows as deep as 24–29 cm have been found [11]; consequently, substrate height could be of greater importance for large worms at high densities. The optimal substrate height needs to be defined for *H. diversicolor* cultivation, especially with the aim of increasing space efficiency in indoor facilities, where a three-dimensional utilization of space has been proposed, using cultivation units one above the other [35].

Feeding practices and feed management present numerous and critical implications for the success of an aquaculture operation. Our results indicate a tendency to obtain better growth indicators as feeding frequency increases, especially significant for the smaller size class. While a daily feeding frequency is advisable in small worms. a clear pattern was not found for medium and large worms; in any case, the frequency of three times per week could be used to optimize labor without major effects on growth or survival. To date, this aspect has not been studied in worms, but some works have investigated this in other invertebrates, such as reef squid juveniles [26] or sea cucumber juveniles [24], for which feeding frequency affects growth and coefficients of variation. We found no differences in CV, which may be due to the short cultivation period. Feeding is labor intensive, and feeding frequency can have significant implications on the manner aquaculture in which facilities are managed. Time and labor typically spent on feeding can be optimized by considering the best feeding frequency in relation to worm size.

In conclusion, *Hediste diversicolor* can be reared at a high stocking density to obtain higher biomass production. Feeding frequency also needs to be considered as an important factor and adapted to the culture phase. Further research on zootechnical aspects is needed to develop husbandry protocols that will allow *H. diversicolor* to be a commercial aquaculture species, especially the use of low-cost feed (e.g., waste of fish aquaculture or food industry by-products).

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References

1. Pombo, A.; Baptista, T.; Granada, L.; Ferreira, S.M.F.; Gonçalves, S.C.; Anjos, C.; Sá, E.; Chainho, P.; Cancela da Fonseca, L.; Fidalgo e Costa, P.; et al. Insight into aquaculture's potential of marine annelid worms and ecological concerns: A review. *Rev. Aquac.* **2018**, *12*, 107–121. [CrossRef]
2. Scaps, P. A review of the biology, ecology and potential use of the common ragworm *Hediste diversicolor* (O. F. Müller) (Annelida: Polychaeta). *Hydrobiologia* **2002**, *470*, 203–218. [CrossRef]
3. Cole, V.J.; Chick, R.C.; Hutchings, P.A. A review of global fisheries for polychaete worms as a resource for recreational fishers: Diversity, sustainability and research needs. *Rev. Fish Biol. Fish.* **2018**, *28*, 543–565. [CrossRef]
4. Batista, F.M.; Fidalgo e Costa, P.; Ramos, A.; Passos, A.M.; Pousao Ferreira, P.; Cancela da Fonseca, L. Production of the ragworm *Nereis diversicolor* (O. F. Müller, 1776), fed with a diet for gilthead seabream *Sparus auratus* L., 1758: Survival, growth, feed utilization and oogenesis. *Bol. Inst. Esp. Oceanogr.* **2003**, *19*, 447–451.
5. Pajand, Z.O.; Haddadi Moghaddam, K.; Chubian, F.; Farzaneh, E.; Hosseinnia, E. Effects of dietary various oil sources on zootechnical performance, sexual maturation, whole-body proximate composition and fatty acid profile of *Hediste diversicolor*. *Aquac. Res.* **2022**, *53*, 3078–3089. [CrossRef]
6. Kabeya, N.; Gür, I.; Oboh, A.; Evjemo, J.O.; Malzahn, A.M.; Hontoria, F.; Navarro, J.C.; Monroig, Ó. Unique fatty acid desaturase capacities uncovered in *Hediste diversicolor* illustrate the roles of aquatic invertebrates in trophic upgrading. *Phil. Trans. R. Soc. B* **2020**, *375*, 20190654. [CrossRef]
7. Marques, B.; Lillebø, A.I.; Ricardo, F.; Nunes, C.; Coimbra, M.A.; Calado, R. Adding value to ragworms (*Hediste diversicolor*) through the bioremediation of a super-intensive marine fish farm. *Aquac. Environ. Interact.* **2018**, *10*, 79–88. [CrossRef]
8. Mandario, M.A.E. Addressing gaps in the culture of pathogen–free polychaetes as feed in shrimp hatcheries. *Fish People* **2018**, *16*, 19–23.

9. Nesto, N.; Simonini, R.; Prevedelli, D.; Da Ros, L. Effects of diet and density on growth, survival and gametogenesis of *Hediste diversicolor* (O. F. Müller, 1776) (Nereididae, Polychaeta). *Aquaculture* **2012**, *362–363*, 1–9. [CrossRef]
10. Garcês, J.P.; Pousao-Ferreira, P. Intraspecific density effect on growth of *Marphysa* "sp." Juveniles. *Sci. Mar.* **2021**, *85*, 137–144. [CrossRef]
11. Esselink, P.; Zwarts, L. Seasonal trend in burrow depth and tidal variation in feeding activity of *Nereis diversicolor*. *Mar. Ecol. Prog. Ser.* **1989**, *56*, 243–254. [CrossRef]
12. Davey, J.T. The architecture of the burrow of *Nereis diversicolor* and its quantification in relation to sediment-water exchange. *J. Exp. Mar. Biol. Ecol.* **1994**, *179*, 115–129. [CrossRef]
13. Miron, G.; Desrosiers, G.; Retière, C.; Lambert, R. Évolution spatio-temporelle du réseau de galeries chez le polychète *Nereis virens* (Sars) en relation avec la densité. *Can. J. Zool.* **1991**, *69*, 39–42. [CrossRef]
14. Scaps, P.; Brenot, S.; Retière, C.; Desrosiers, G. Space occupation by the polychaetous annelid *Perinereis cultrifera*: Influence of substratum heterogeneity and intraspecific interactions on burrow structure. *J. Mar. Biol. Assoc. UK* **1998**, *78*, 435–449. [CrossRef]
15. Miron, G.; Desrosiers, G.; Retière, C.; Lambert, R. Dispersion and prospecting behavior of the polychaete *Nereis virens* (Sars) as a function of density. *J. Exp. Mar. Biol. Ecol.* **1991**, *145*, 65–77. [CrossRef]
16. Fidalgo e Costa, P. Reproduction and growth in captivity of the polychaete *Nereis diversicolor* O. F. Müller, 1776, using two different kinds of sediment: Preliminary assays. *Bol. Inst. Esp. Oceanogr.* **1999**, *15*, 351–355.
17. Wang, H.; Hagemann, A.; Reitan, K.I.; Handå, A.; Uhre, M.; Malzahn, A.M. Embryonic and larval development in the semelparous Nereid polychaete *Hediste diversicolor* (OF Müller, 1776) in Norway: Challenges and perspectives. *Aquac. Res.* **2020**, *51*, 4135–4151. [CrossRef]
18. Fidalgo e Costa, P.; Narciso, L.; Cancela da Fonseca, L. Growth, survival and fatty acid profile of *Nereis diversicolor* (O. F. Müller, 1776) fed on six different diets. *Bull. Mar. Sci.* **2000**, *67*, 337–343.
19. Santos, A.; Granada, L.; Baptista, T.; Anjos, C.; Simões, T.; Tecelão, C.; Fidalgo e Costa, P.; Costa, J.L.; Pombo, A. Effect of three diets on the growth and fatty acid profile of the common ragworm *Hediste diversicolor* (O. F. Müller, 1776). *Aquaculture* **2016**, *465*, 37–42. [CrossRef]
20. Yousefi-Garakouei, M.; Kamali, A.; Soltani, M. Effects of rearing density on growth, fatty acid profile and bioremediation ability of polychaete *Nereis diversicolor* in an integrated aquaculture system with rainbow trout (*Oncorhynchus mykiss*). *Aquac. Res.* **2019**, *50*, 725–735. [CrossRef]
21. Wang, H.; Seekamp, I.; Malzahn, A.; Hagemann, A.; Carvajal, A.K.; Slizyte, R.; Standal, I.B.; Handå, A.; Reitan, K.I. Growth and nutritional composition of the polychaete *Hediste diversicolor* (OF Múller, 1776) cultivated on waste from land based salmon smolt. *Aquaculture* **2019**, *502*, 232–241. [CrossRef]
22. Jerónimo, D.; Lillebø, A.I.; Rey, F.; Ii, H.K.; Domingues, M.R.M.; Calado, R. Optimizing the Timeframe to Produce Polychaetes (*Hediste diversicolor*) Enriched with Essential Fatty Acids Under Different Combinations of Temperature and Salinity. *Front. Mar. Sci.* **2021**, *8*, 671545. [CrossRef]
23. De Silva, S.; Turchini, G.; Francis, D. Nutrition. In *Aquaculture: Farming Aquatic Animals and Plants*, 2nd ed.; Lucas, J.S., Southgate, P.C., Eds.; Wiley-Blackwell: Oxford, UK, 2012; pp. 164–187.
24. Xia, B.; Ren, Y.; Wang, J.; Sun, Y.; Zhang, Z. Effects of feeding frequency and density on growth, energy budget and physiological performance of sea cucumber *Apostichopus japonicus* (Selenka). *Aquaculture* **2017**, *466*, 26–32. [CrossRef]
25. Manley, C.B.; Rakocinski, C.F.; Lee, P.G.; Blaylock, R.B. Feeding frequency mediates aggression and cannibalism in larval hatchery-reared spotted seatrout (*Cynoscion nebulosus*). *Aquaculture* **2015**, *437*, 155–160. [CrossRef]
26. Satjarak, J.; Thongprajukaew, K.; Kaewtapee, C.; Suanyuk, N.; Klomklao, S.; Preedaphol, K. Optimal feeding frequency for bigfin reef squid (*Sepioteuthis lessoniana*). *Aquac. Res.* **2021**, *52*, 2740–2750. [CrossRef]
27. Aguado-Giménez, F.; García-García, B.; Martín, I.E.; Rasines, I. Influence of Body Weight And Water Temperature on Growth in Ragworm *Hediste diversicolor*. *Aquac. J.* **2023**, *3*, 19–31. [CrossRef]
28. Anderson, M.J. A new method for non-parametric multivariate analysis of variance. *Austral. Ecol.* **2001**, *26*, 32–46.
29. Mistiaen, J.A.; Strand, I. Optimal feeding and harvest time for fish with weight-dependent prices. *Mar. Resour. Econ.* **1999**, *13*, 231–246. [CrossRef]
30. Scaps, P.; Retière, C.; Desrosiers, G.; Miron, G. Effects de la ration alimentaire, de la densité intraspécifique et des relations entre individus sur la croissance des juvéniles de l'espèce *Nereis diversicolor* (Annelida: Polychaeta). *Can. J. Zool.* **1993**, *71*, 424–430. [CrossRef]
31. Safarik, M.; Redden, A.M.; Schreider, M.J. Density-dependent growth of the polychaete *Diopatra aciculata*. *Sci. Mar.* **2006**, *70*, 337–341.
32. Olive, P.J.W.; Garwood, P.R. Gametogenic cycle and population structure of *Nereis (Hediste) diversicolor* and *Nereis (Nereis) pelagica* from North-East England. *J. Mar. Biol. Assoc. UK* **1981**, *61*, 193–213. [CrossRef]
33. Herwati, V.E.; Elfitasari, T.; Rismaningsih, N.; Riyadi, P.H.; Tarangkoon, W.; Radjasa, O.K.; Windarto, S. Analysis of growth and nutritional quality of sea worm (*Nereis virens*) as a mass cultured natural feed on different substrate media thicknesses. *Biodiversitas* **2021**, *22*, 3299–3305. [CrossRef]

34. Gilbert, F.; Kristensen, E.; Aller, R.C.; Banta, G.T.; Archambault, F.; Belley, R.; Bellucci, L.G.; Calder, L.; Cuny, P.; de Montaudouin, X.; et al. Sediment reworking by the burrowing polychaete *Hediste diversicolor* modulated by environmental and biological factors across the temperate North Atlantic. A tribute to Gaston Desrosiers. *J. Exp. Mar. Biol. Ecol.* **2021**, *541*, 151588. [CrossRef]
35. Kvaestad, B.; Solvang, T.; Hagemann, A.; Wang, H.; Reitan, K.I.; Handa, A.; Malzahn, A.M. Advances in polychaete cultivation technology—An indoor space efficient cultivation system. In Proceedings of the Aquaculture Europe, Berlin, Germany, 7–8 October 2019.