VOL IV

Estudos em Ciências Agrárias e Ambientais

Eduardo Spers
(Organizador)

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APRESENTAÇÃO

É com grande satisfação que apresentamos o volume IV da coletânea **Estudos em Ciências Agrárias e Ambientais**, resultado do esforço colaborativo de pesquisadores de diferentes regiões e instituições, que compartilham aqui reflexões, dados e contribuições relevantes para o avanço do conhecimento técnico-científico em suas áreas de atuação.

Este volume reúne 13 trabalhos organizados em cinco eixos temáticos que refletem a diversidade e complexidade do campo agrário e ambiental contemporâneo: Sistemas de Produção Aquático e Animal; Sustentabilidade Ambiental e Conservação de Recursos Naturais; Sistemas de Produção Vegetal e Agricultura de Precisão e Educação e Inovação no Meio Agrário.

Os temas abordados vão desde o manejo sustentável de recursos naturais, passando por inovações tecnológicas na agricultura e aquicultura, até discussões sobre formação profissional e segurança sanitária nas cadeias produtivas. Essa pluralidade é o reflexo da crescente interdisciplinaridade que caracteriza os estudos agrários e ambientais hoje – exigindo diálogos entre a ciência, a tecnologia, a educação, a economia e a sociedade.

Além da qualidade dos estudos apresentados, destacamos o compromisso dos autores com a pesquisa aplicada, a sustentabilidade e a busca por soluções adaptadas às realidades locais, muitas vezes desafiadoras. A presença de autores da América Latina e Europa também fortalece o caráter internacional da obra, fomentando o intercâmbio de experiências e metodologias.

Agradecemos a todos os autores pela confiança em compartilhar seus trabalhos conosco. Que esta publicação possa inspirar novas pesquisas, colaborações e, acima de tudo, práticas que contribuam com a construção de sistemas agrários e ambientais mais resilientes, justos e inovadores.

Desejamos a todos uma excelente leitura!

Eduardo Eugênio Spers

SUMÁRIO

CAPÍTULO 2

INTRASPECIFIC DENSITY EFFECT ON GROWTH OF *Marphysa* "SP". JUVENILES[1]

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ABSTRACT: There is growing demand for the territorial tube-building genus *Marphysa* (Eunicidae: Polychaete), commonly known in Portugal as "goose", for use as fishing bait, and it is being harvested all around the world for that purpose. Effects of intraspecific density on juvenile growth were studied over a four-month period in laboratory facilities. Three polychaete densities (low, 50 worms; medium, 150 worms; and high, 250 worms) were used in a 0.25 m^2 aquarium containing sandy sediment and recirculating water. Total length, dry weight and number of segments were recorded for 60% of the initial population. All polychaetes were also counted to determine mortality rate and territorial behaviour through the existence of body lesions and broken and regenerating posterior segments. The results obtained in this study showed that density had a significant effect ($p<0.001$) on growth rates in any of the densities studied and that growth was significantly higher at lower densities ($p<0.001$). For all density levels, estimated daily growth was higher in the first month, decreasing progressively over time. The high aggressiveness and territorial behaviour of *Marphysa* "sp." juveniles, well evidenced by the highest mortality (35%) under high density and by the presence of worms with lesions under low density (30%) observed in the first month, suggests that territoriality is probably the main factor involved in the organization and spatial arrangements of individuals within a population. Marphysa juveniles probably compete for burrow space. The results reveal that Marphysa juveniles have a very territorial and aggressive behaviour that should be considered if the species is used for

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aquaculture production. Additional studies are required to determine the density effects for different developmental stages.
KEYWORDS: aquaculture; density; growth; *Marphysa*; polychaetes.

EFEITO DA DENSIDADE INTRAESPECÍFICA NO CRESCIMIENTO DE *Marphysa* "SP". JUVENIS

RESUMO: O poliqueta Marphysa "sp." (Eunicidae), vulgarmente conhecido em Portugal por "ganso", é amplamente utilizado em todo o mundo como isco para a pesca desportiva e profissional. Os efeitos da densidade intraespecífica no crescimento juvenil foram estudados em laboratorio durante um período de quatro meses. Foram utilizadas três densidades: baixa (50 poliquetas), média (150 poliquetas) e alta (250 poliquetas) em aquários de 0,25 m^2, com areia e água recirculada. O comprimento total (cm), o peso seco (g) e o número de segmentos foram registados em 60% da população inicial. Foram também contados todos os poliquetas para determinar a taxa de mortalidade e o comportamento territorial através da presença de lesões corporais, segmentos posteriores ausentes ou em regeneração. Os resultados obtidos mostraram que o crescimento foi significativamente maior nas densidades mais baixas ($p<0{,}001$). Para todos os níveis de densidade, o crescimento diário estimado foi maior no primeiro mês, diminuindo progressivamente ao longo do tempo. A elevada agressividade e o comportamento territorial dos juvenis de Marphysa "sp", claramente evidenciados no primeiro mês, pela maior mortalidade (35%) verificada em alta densidade e pela presença de indivíduos com lesões em baixa densidade (30%), sugerem que a territorialidade é provavelmente o principal factor regulador da organização e distribuição espacial dos indivíduos dentro de uma população. Em conclusão, os resultados obtidos revelam que os juvenis de Marphysa "sp." têm um comportamento muito territorial e agressivo, o que deve ser considerado se se pretender utilizar a especie para produção aquícola. São necessários estudos adicionais para determinar os efeitos da densidade em diferentes fases de desenvolvimento.
PALAVRAS CHAVE: aquicultura; densidade; crescimento; *Marphysa*; poliquetas.

1. INTRODUCTION

The iteroparous and long-lived polychaete Marphysa "sp." used in this study, and originally described from the south coast of England, has been recorded from many localities around the world, often without an exhaustive description or deposition of voucher material (Hutchings and Karageorgopoulos 2003). This genus has a global distribution and is of considerable value in the bait industry worldwide. Recently, there has been some debate on whether this genus may in fact encompass a series of cryptic species from different geographical regions that are morphologically difficult to distinguish (Lewis and Karageorgopoulos 2008, Asmaa et al. 2018, Lavesque et al. 2019). Therefore, we prefer to use the genus until we have evidence that this species is *Marphysa sanguinea* (Montagu 1813).

Competition for space and food are important structuring agents of populations of sediment-dwelling species and are therefore key factors in species distribution and community organization (Rosenberg et al. 1997). In areas where food is abundant, space can become a factor limiting population expansion. Several studies of infaunal marine soft-bottom species showed that individual growth, larval recruitment and survivorship are negatively affected by an increase in density (Reise et al. 2001, Safarik et al. 2006 and references therein). In the laboratory it has been shown that an increase in population density is usually associated with a decrease in fertility (Zajac 1996) and in territorial and food competition (Scaps et al. 1998, Grelon et al. 2006). However, intraspecific relations between benthic individuals, particularly among polychaetes, are more complex, with several factors interacting simultaneously. According to Lemieux et al. (1997), the ability to exploit a habitat depends on the ability to defend a burrow, and this ability is related to aerobic capacity. In addition to the intrinsic interest in annelid polychaetes as ubiquitous and highly abundant members of all marine benthic ecosystems, there is a growing demand for these organisms as fishing bait, so they are being commercially exploited all around the world (Watson et al. 2016, Cole et al. 2018), including Europe (Gambi et al. 1994, Olive 1999, Lavesque et al. 2017). They have also been introduced in integrated polycultures to help manage organic matter and waste produced by bivalves and fish (Batista et al. 2003). They are a natural source of proteins and omega-3 fatty acids, so they can be used as a nutritional resource and maturation diet in crustacean and fish aquaculture (Noda et al. 1994, Watson et al. 2016, Glasby et al. 2019). Therefore, in addition to the impacts of traditional bait harvesting (Carvalho et al. 2013), new mechanized methods of collection are being developed to increase efficiency, productivity and revenue (Beukema 1995, Birchenough 2013). The traditional bait digging exploitation continues to occur, presenting an ill-defined boundary between anglers collecting strictly for their own use and those collecting for profit, who represent a form of parallel economy in conflict with a controlled, sustainable use (Olive 1993; Cabral et al. 2019, Pombo et al. 2018).

1.1. POTENTIAL USES

Marphysa “sp.”, commonly known by the name of “goose”, is one of the most appreciated bait species among those collected and commercialized in Portugal because of its high resistance in the hook and its reputed ability to attract valuable fish species such as the seabreams *Sparus aurata* and *Diplodus sargus*. Though it is difficult to value this resource because the sales are not declared (Castro 1993), harvesting is one of the most important socio-economic resources for local fishermen, and sales of €500000 per year are estimated (unpublished data) (Garcês and Pereira 2010).

The long-term exploitation of this genus requires a good knowledge of the biological and ecological characteristics of natural populations in order to develop rearing techniques for intensive production with the aim of increasing supply, reducing harvesting and fostering sustainable use.

With this purpose in mind, a small-scale experiment was conducted in order to address the way in which intraspecific density can influence somatic growth in juveniles of *Marphysa* “sp.” when food and sediment are not limiting factors. These effects were studied over a four-month period in laboratory facilities.

Further development of polychaete culture is considered of economic importance beyond that associated with the bait supply industry which acted as the initial catalyst for this research.

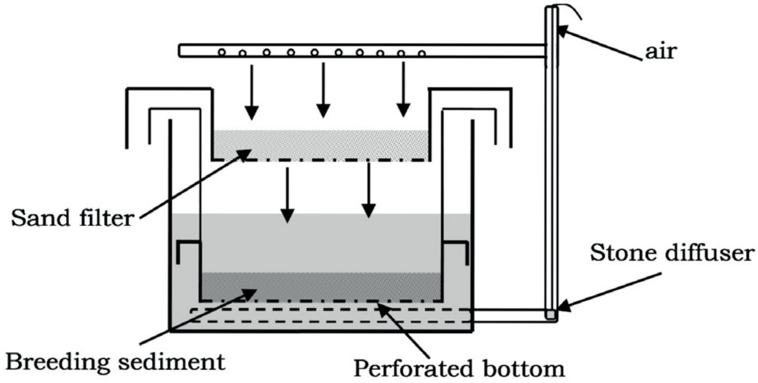
2. MATERIALS AND METHODS

A four-month on-site controlled experiment was conducted with *Marphysa* “sp.” juveniles reared in the laboratory (25 to 30 setigerous segments corresponding to a total length of 0.7±0.05 cm to 0.9±0.08 cm, respectively). The juveniles were placed in glass aquaria (0.5 x 0.5 x 0.5 m) (Fig. 1). Each aquarium was filled with a 6 cm layer of beach sand (>95% fine grain <50 µm). The sand was previously screened to remove macroalgae and any associated macrofauna, sun dried and then rinsed several times with freshwater and seawater alternately. It was further heated and sterilized in an autoclave.

Before being added to aquaria that were randomly placed in the laboratory to reduce any position effects, the water was filtered through a biological filter and a cartridge series and maintained under constant oxygenation. Each aquarium was connected to a recirculation system (RAS) to allow continuous seawater flow and continuous aeration (see Figure 1).

Three different densities were used and replicated three times: high density (250 juveniles corresponding to 1000 juveniles/m^2), medium density (150 juveniles corresponding to 500 juveniles/m^2) and low density (50 juveniles corresponding to 200 juveniles/m^2). The worms were placed inside each aquarium in a suspended tray (see Fig. 1) fitted with a perforated bottom. The water was circulated by the action of an air stone placed inside the circulation tube. The biological filter, composed of beach sand of different grain sizes, was also placed inside a tray at the top of the structure.

Figure. 1: - Schematic aquarium design used in the assays.



During the first 48 h of acclimatization to the laboratory conditions, the juvenile worms were able to settle (construct and establish burrows). The glass walls of the aquariums allowed the polychaetes inside them to be observed regarding their feeding and territorial behaviour. Water temperature, salinity, dissolved oxygen, pH, ammonium (NH_4^+) and nitrites (NO_2^-) were measured three times per week: temperature, dissolved oxygen and pH were measured by a multiparameter Hanna instruments model HI9829 water quality meter, salinity by a Hanna instruments HI83306 refractometer and ammonia and nitrites by two Hanna instruments kits, HI93700 and HI93708, respectively. In each aquarium, a mixture of crushed cockles (20%), mussels (60%) and shrimp (20%) were provided daily, fresh and ad libitum, five times a week, representing an amount equivalent to 3% wet body weight. They were fed daily to avoid cannibalism. Excess food was removed three hours after being supplied. The diet was adjusted to account for increases in mean polychaete weight through time. Growth data were collected monthly for a sub-sample of 60% of the initial polychaete numbers for each density and aquarium after the beginning of the study. Morphological variables were only considered for whole individuals that did showed no signs of regeneration, broken segments or injuries. All polychaetes were also counted to determine mortality rate, and confrontation and territorial behaviour was observed through the existence of body lesions and broken and regenerating posterior segments.

In this work, total length (TL) and total dry weight (TDW) (Fig. 2) were adopted as biometric measures according to the following expressions (Castro 1993):

(1) $TL=3.9303 \times WBS^{1.3307}$ $r=0.9770$; $n=192$

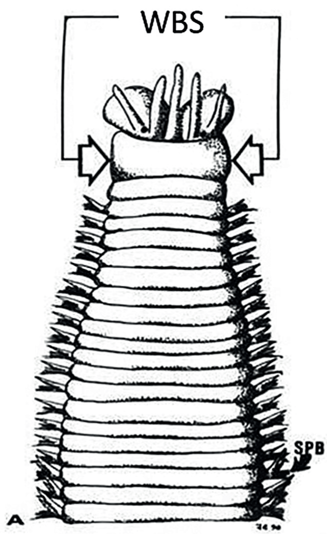
(2) $TDW=0.0026 \times WBS^{3.6894}$ $r=0.9845$; $n=356$

where WBS (width of buccal segment) = LSB (put on legend).

Length was always determined from the number of setigers formed (SPB in Figure 2). Segments were counted under a stereomicroscope equipped with a micrometric ocular at 20x magnification. The linear measurements of WBS obtained were taken under a stereomicroscope equipped with a micrometric ocular at 2x magnification.

The dimensions obtained were processed by the Qwin Lite 2.4 image-processing software system (Leica Microsystems Imaging Acquisition). Statistical analysis was performed using SPSS software (version 18; SPSS inc, Chicago, Il). The relationships between density and time and polychaete growth and survival were analysed# by two-factor ANOVA followed by a Tukey test when significant differences were found.

Figure. 2: Dorsal view of the anterior region of *Marphysa.* sp., adapted from Garcês and Pereira (2010). B WBS = Width of buccal segment.

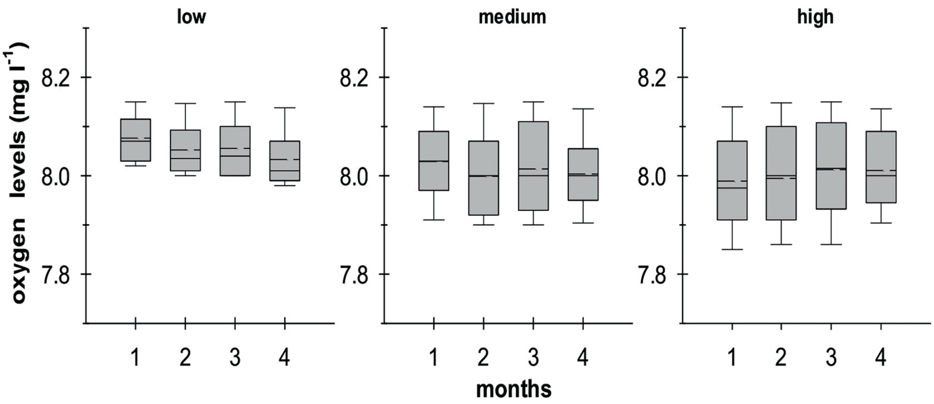


3. RESULTS

3.1. WATER PARAMETERS

During the study, the physico-chemical water parameters remained approximately constant. There were no significant differences between replicates ($p \geq 0.05$) for the parameters under study. However, over the 4 months gradual and slight variations in water temperature (19ºC-25ºC), salinity (30-33), pH (7.6-8.1) ammonium (0.4-0.0) and nitrites (0.2-0.03) were nonetheless observed. The slight increase in ammonia and nitrite values during the experiment may be correlated with the confrontations and the associated mortality, but they did not reach high values that could have influenced this mortality. The control of these parameters reveals a perfect functioning of the biological filter. There was a higher dispersion value in dissolved oxygen concentrations according to the density, which means an increased consumption by worms. However, there were no significant differences (*p*≥0.05) between the replicates or in each density over time (Figure 3).

Figure 3: Dissolved oxygen concentrations (ppm) during the study at the three densities tested (low, medium and high).



3.2. FEEDING BEHAVIOUR

During this study, juveniles of *Marphysa* “sp.” were observed to capture and ingest food particles on the sediment surface using their jaws, which are located ventrally as the worm moves over the substratum. The feed intake was observed to vary with density. At low density, the food particles were almost immediately consumed by the worms, which sometimes emerged completely from the gallery, getting fully exposed in the sediment surface. At medium and high densities, feeding was mainly at night, and the worms never left the gallery, collecting food in a circular area and fleeing immediately into the gallery at the slightest sign of danger. At high density, the food was not entirely consumed during the first two months. However, it was not possible to determine the frequency with which searching activities resulted in particle consumption. Worms were observed seizing particles with their forceps jaws on many occasions, on both the substratum and over their tube or over the tube of a neighbour without confrontation.

3.3. SOMATIC GROWTH

No significant differences were found in growth between replicates of each treatment during the four months of the study, although differences tended to be greater between replicates in the high-density treatment. A two-factor ANOVA showed significant density and time effects ($p<0.001$) and a significant interaction between density and time ($p>0.001$) for all the growth variables in every month.

Means of total length, weight and number of segments were inversely related to density. In all treatments, juveniles grew steadily during the four months of the study (Figure 4), but a comparison of the monthly average increase shows that the three parameters under study evolved differently for each density treatment.

At low and medium density levels, the estimated monthly increase in the number of segments was higher in the first month and decreased progressively over time. Under high density, however, there was a progressive increase in the number of segments from the second month. Only increase in weight was, in general, continuous at all densities.

After four months, at the lowest density the polychaetes formed 61.5±4.2 segments, gained 0.1±0.01g in dry weight and grew 4±0.2 cm, at average density they formed 51.8±4 segments (0.08±0.008 g, 3±0.2 cm) and at high-density they formed 43±6 segments (0.05±0.01g, 2.4±0.3 cm) (see Fig. 4). It is noteworthy that at low density a significant decrease in the three parameters was observed in the last month (p>0.0001).

Figure: 4. - Monthly Growth of *Marphysa "sp."* at each density level (low, medium and high). T_0 – beginning of study; T_1 – period at month 1 and so on (T_2, T_3 and T_4); monthly growth (T_0-T_{n-1}); specific growth rate (T_0- T_{n-1})/30 days.

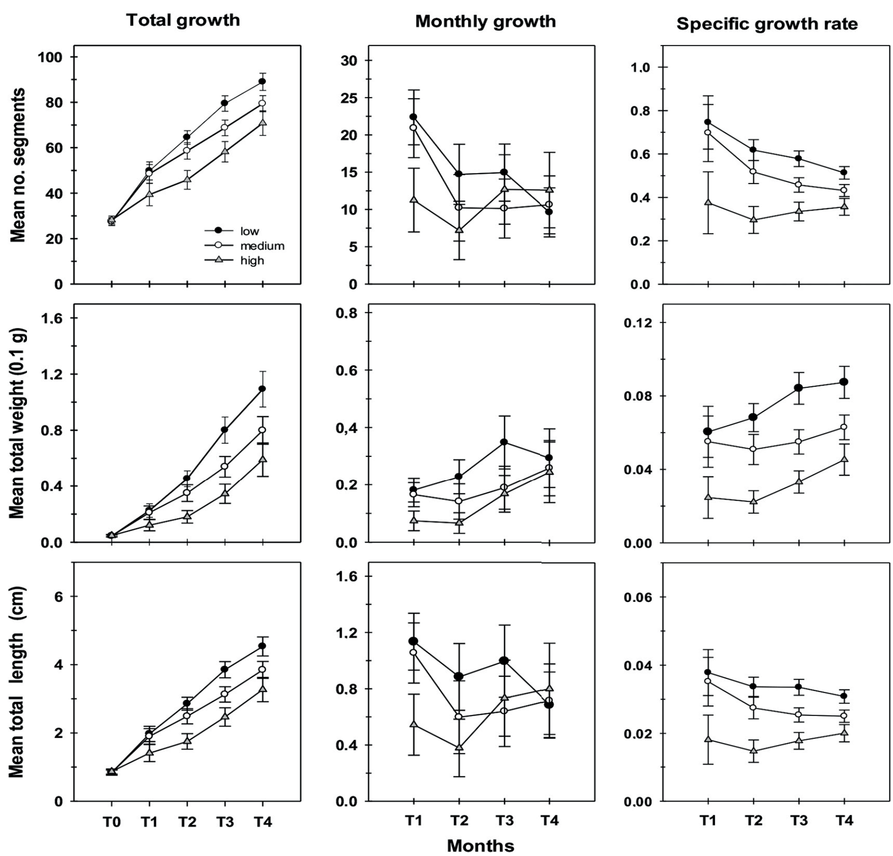
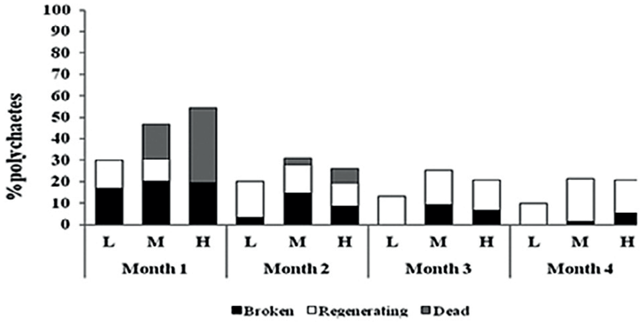


Figure 5. - Monthly percentage occurrence of *Marphysa* *“sp.”* Mortality and broken and regenerating posterior segments observed at each density level.

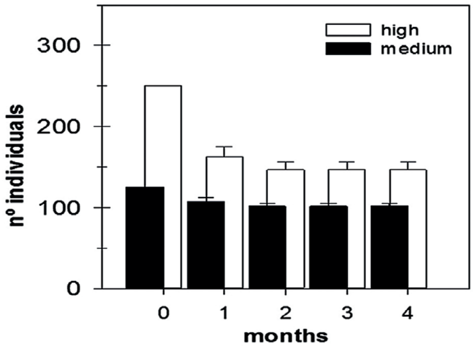


In this study, this high aggressiveness and territorial behaviour of *Marphysa* “sp.” is well evidenced by the higher mortality (35%) and greater incidence of broken and regenerating posterior segments (19.3%) observed under high density in the first month and by the presence of worms with lesions under low density (30%) (Fig. 5).

#Although the incidence of broken posterior segments was overall slightly higher (16%) at medium density, mortality was much lower# (15%). The sharp decline both in mortality and in the incidence of broken posterior segments that occurred in the following months reveals that the worms tended towards an equilibrium density. However, confrontation never ceased, as shown by 5.2% and 1.3% of individuals with lesions in high and medium densities respectively in the fourth month of the study (Fig. 5). Average estimated mortality after four months was 44.9%, 18.9%, and 0% at high, medium and low-density levels, respectively.

Despite this very high mortality, the average evolution of the existing population (Figure 6) shows that significant differences in the number of individuals (p>0.001) continued to exist between the high and medium densities throughout the study, though it did not call into questiohn the existence of three densities.

Figure: 6. - *Marphysa* *“sp.”* population evolution at high and medium densities for four months.



The existence of confrontations in the low-density tanks (with 16.7% and 13.3% of broken and regenerating individuals, respectively) confirms the high aggressiveness and territoriality evidenced by this species and suggests that, up to a certain point, the density can be higher than the optimum without necessarily resulting in death.

4. DISCUSSION

4.1. WATER PARAMETERS

Most infaunal animals irrigate their burrows with oxygen-rich overlying water. They are active 20% to 50% of the time, alternating between active and quiescent periods in a rhythmic fashion, according to species (Garcês and Costa 2009, Galaktionov et al. 2004, Kristensen 2001). *Marphysa* “sp.” dwells in L-shaped burrows and display vigorous bio-irrigation activity by pumping oxygenated water via peristaltic motions. According to Webber 1978, this genus has a high affinity to oxygen and high sensitivity to changes in pH, suggesting a permanent life in galleries subject to low oxygen concentrations.

According to (Lemieux et al. 1997), the ability to explore a site largely depends on the ability to defend the gallery with a minimum of injuries. These authors have found a significant correlation between aerobic metabolism and the duration of the fight in *Nereis virens*. They argue that short-term confrontations are advantageous because they increase survival while still limiting the mobilization of anaerobic metabolism. On the other hand, high aerobic activity may be related to high metabolic rates, so the benefit of fights may be somewhat offset by a high metabolic costs for the antagonists, decreasing the energy available for growth and reproduction.

4.2. FEEDING BEHAVIOUR

Effects of increased density were also observed to lead to modifications in burrow shape. The absence of connections between the burrows as the density increased suggests that territoriality is certainly the main factor involved. A preference for the centre of the aquarium was observed during this study, i.e. colonization was made from the centre to the periphery of the aquaria at all densities. Scaps et al. (1998), in a study of *Perinereis cultrifera,* found that the area around the burrow opening is not defended and therefore does not constitute a territory, and they postulate that *P. cultrifera* probably competes for burrow space but may share food#. By contrast, Levin (1982) and Taghon (1992) showed that spacing between neighbours in Spionid species can be affected by the particle supply mechanism and the source of food particles. In *Paralvinella sulfincola*, a polychaete worm living near deep-sea hydrothermal vents, Grelon et al. (2006) showed that the size of the territory and the control exerted over intruders depends on worm-density and individual body weight, reinforcing the importance of size and aggressiveness in the defence of the foraging area. The method of feeding may also partly explain the observed behaviour. The use of sand and the type of diet, which was deposited directly onto the substrate surface, forced the worms to use their jaws to capture the food particles, therefore exposing their anterior region.

4.3. SOMATIC GROWTH

The results obtained suggest that confrontation may have significantly ($p<0.001$) reduced growth, particularly at medium and high densities and in the first month (Figs 4, 5). The rising growth, which occurred at high density (Fig. 4) in the last two months, can be explained by the sharply declining number of individuals and therefore a decrease in confrontation with the concomitant establishment of undisputed territories. The same pattern was observed for the increased frequency of individuals injured, regenerated and killed. The negative density-dependent effects on growth of *Marphysa* “sp.” juveniles are similar to those reported for several other polychaete species (Grelon et al. 2006, Safarik 2006, Nesto et al. 2012). The negative density-dependent effects on growth of juvenile and late juvenile *M. sanguinea* obtained by Parandavar et al. (2015) are similar to those obtained in our study. According to these authors, *Marphysa sanguinea* juveniles possess the ability to grow better at low density, and survival is negatively influenced by high rearing density. In the present study, survival was negatively influenced by high rearing density, as has also been already reported for *N. arenaceodentata* (Dillon et al., 1993), *D. aciculata* (Safarik et al. 2006) and *Hediste diversicolor* (Nesto et al. 2012). The decreasing survival percentages are likely to be related to the increasing intraspecific competition. Rearing density—related patterns in growth and mortality provide evidence for negative density-dependent interactions with neighbouring worms. According to Safarik et al. (2006), in *Diopatra aciculate* the negative effects of density on growth are due to intraspecific interactions, including competition for resources other than food. Territoriality can be fundamental for the survival of this species because the individual territory serves primarily for defence and refuge from predators, but also appears to be the primary foraging ground.

The existence of confrontations in the low-density tanks (with 16.7% and 13.3% of broken and regenerating individuals, respectively) confirms the high aggressiveness and territoriality evidenced by this species and suggests that, up to a certain point, the density can be higher than the optimum without necessarily resulting in death. However, in this study the worms were limited to a rigid physical space, and as they grow, they increase not only the body volume but also the territorial surface, obviously increasing the likelihood for encounters. Competition for space has also been shown for *Neanthes arenaceodentata,* which exhibit aggressive behaviour at high density, as demonstrated by an increase in the frequency of polychaetes regenerating posterior segments (Bridges et al. 1996).

Studies undertaken by (Esnault et al*.* 1990, Scaps et al*.* 1993) showed a similar reduction in growth, biomass and survival at high densities, even in the presence of abundant food. Stress associated with frequent handling of *Hediste diversicolor* and changes in water temperature and salinity have also been found to induce negative effects on growth (Scaps et al. 1993, Garcês and Pereira 2010). The decreasing survival percentages are likely to be related to the increasing intraspecific competition. Rearing density – related patterns in growth and mortality provide evidence for negative density-dependent interactions with neighbouring worms.

5. CONCLUSIONS

The results have shown that space occupation by *Marphysa* "sp." juveniles depends primarily on aggressive interaction. However, these observations may not apply to the entire life cycle of the species, which is known to be under the control of a greater range and complexity of movements.

The feeding method may also partly explain the observed behaviour. The use of sand and the type of diet, which was directly deposited onto the substrate surface, forced the worms to use their jaws to capture the food particles, therefore exposing their anterior region.

The results obtained in this study reveal that density has a significant effect ($p<0.001$) on somatic growth of *Marphysa sp* juveniles, and that densities higher than 1000 juveniles per m^2 cause high mortality (45%). Finally, it is important to highlight that the very territorial and aggressive behaviour evidenced by *Marphysa* "sp." juveniles should be considered for aquaculture production. Further studies are required to determine the density effects for different developmental stages.

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ÍNDICE REMISSIVO

Símbolos

A

B

C

D

O

P

R

S

T

V


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2025