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Density-dependent growth of the polychaete *Diopatra aciculata*

MILADA SAFARIK, ANNA M. REDDEN and MARIA J. SCHREIDER

School of Applied Sciences, Ourimbah Campus, University of Newcastle, PO Box 127 Ourimbah NSW 2258 Australia.
E-mail: anna.redden@newcastle.edu.au

SUMMARY: Effects of intraspecific density on growth of the tube-building polychaete *Diopatra aciculata* (Onuphidae) were examined over a three-month period within a marine worm aquaculture facility. Three polychaete densities (500, 1000 and 2000 worms/m^2) were represented within triplicate 0.30 m^2 boxes containing late juvenile *D. aciculata,* sandy sediment and recirculating seawater. Daily food ration per worm was held constant across all density levels. Total length, weight and number of segments were recorded for 20 polychaetes randomly removed from each of nine treatment boxes at weeks 1, 7 and 14. Mean daily growth was higher during weeks 1-7 than during weeks 7-14 for all growth variables at each density level. Polychaetes at the highest density level exhibited lower rates of growth and more broken and/or regenerating posterior segments than those at low density. High *D. aciculata* density was also associated with reduced dissolved oxygen concentrations and high polychaete mortality (20%). At medium polychaete density (1000/m^2), *D. aciculata* exhibited low levels of apparent stress and high biomass return per unit area, both of which are important considerations in the aquaculture rearing of this species. We suggest that further studies focus on age- and size-related factors contributing to density effects on polychaete growth.

Keywords: *Diopatra aciculata*, Onuphidae, tubeworm, polychaete growth, density-dependent growth, intraspecific competition, aquaculture.

RESUMEN: CRECIMIENTO EN FUNCIÓN DE LA DENSIDAD DE POLIQUETOS *DIOPATRA ACICULATA*. – Durante un período de más de tres meses se examinaron en instalaciones de acuicultura de gusanos marinos los efectos de la densidad interespecífica en el crecimiento del poliqueto tubícola *Diopatra aciculata* (Onuphidae). Se utilizaron tres niveles de densidad de poliquetos (aproximadamente 500, 1000 y 2000 gusanos/m^2) en cajas experimentales de 0,30 m^2 replicadas, conteniendo ejemplares juveniles avanzados de *D. aciculata* y sedimento arenoso, todo ello con recirculación de agua marina. La ración diaria de alimento por gusano se mantuvo constante en todos los niveles de densidad. Se registró la longitud total, el peso, y el número de segmentos en 20 poliquetos extraídos aleatoriamente para cada tratamiento durante las semanas 1, 7, y 14. Para cada uno de los niveles de densidad estudiados, el crecimiento diario promedio durante el período entre la semana 1 y la 7 fue mayor que durante el período entre la semana 7 y la 14 en todas las variables de crecimiento. Los poliquetos cultivados a mayores densidades poblacionales exhibieron menores tasas de crecimiento y un mayor número de segmentos posteriores fragmentados y/o regenerados que aquellos cultivados a menores densidades de población. Asimismo, la alta densidad de *D. aciculata* provocó menores concentraciones de oxígeno disuelto y un mayor índice de mortalidad de poliquetos (20% promedio). Al nivel medio de densidad de poliquetos (1000/m^2), los ejemplares de *D. aciculata* presentaron un bajo nivel de estrés aparente y una elevada producción de biomasa por unidad de área, dos factores importantes en la crianza mediante técnicas de acuicultura de esta especie. Para posteriores estudios, se propone concentrarse en los efectos de la densidad en el comportamiento interactivo y en la competencia por el alimento.

Palabras clave: Diopatra aciculata, Onuphidae, gusanos constructores de tubos, crianza de poliquetos, efectos de la densidad, competición, acuacultivo.

INTRODUCTION

Density-dependent factors, including competition for space and food, are important structuring agents for populations of a number of sediment-dwelling polychaete species (Levin, 1982; Pesch *et al*, 1987; Trueblood, 1991; Lemieux *et al.,* 1997; Scaps *et al*., 1998; Buekema *et al*., 2000; Omena and Zacagnini, 2000; Reise *et al.*, 2001). An increase in density has been linked to reduced reproduction and juvenile recruitment in the polychaetes *Ceratonereis pseudoerythraeensis* (Kent and Day, 1983) and *Polydora ligni* (Zajac, 1986). *Neanthes virens* has been shown to spend more time prospecting as polychaete density increases, with dispersion patterns indicating competition for burrow space rather than for food (Miron *et al.,* 1991). 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). Scaps *et al*. (1993) recorded a reduction in growth and survival of lab-reared juveniles of *Hediste diversicolor* at high densities (3000/m^2), even in the presence of abundant food.

Our study examined the effects of intraspecific density on a variety of growth variables (length, weight and number of segments), posterior segment condition and mortality of the tube-building marine polychaete *Diopatra aciculata* Knox and Cameron (Onuphidae). This species is commonly found in both intertidal and subtidal sandy sediments in Australia (Paxton, 1993) and appears to have a lifespan of approximately five years (pers. obs.). *D. aciculata* is an important aquaculture species within Australia and is currently cultivated for use as recreational fishing bait and as food in the conditioning of prawn broodstock, particularly *Penaeus monodon*. This study was initiated by the need to determine the effects of *D. aciculata* stock density on polychaete growth and survival in an aquaculture facility. It was anticipated that, over a three-month incubation period, juvenile *D. aciculata* would exhibit negative density-dependent growth at densities ranging from 500 - 2000 worms/m^2 and evidence of physical stress at high density.

METHODS

A 14-week on-site controlled experiment was conducted with late stage juvenile (4-month old) *D. aciculata* that were reared in the Aquabait Pty Ltd aquaculture facility at Dora Creek, NSW, Australia. As density levels in the grow-out ponds at the Aquabait facility were naturally variable and peaked at about 2000 worms/m^2, three worm densities were selected as low, medium and high density treatments: 500, 1000 and 2000 worms/m^2. Anaesthetised polychaetes were transplanted from the grow-out ponds to replicate treatment boxes (0.30 m^2), measuring 60 cm long x 50 cm wide x 40 cm deep. Disclosure of the details of the anaesthetic solution, which has been used in local polychaete aquaculture (Aquabait Pty Ltd) for many years, is protected by an intellectual property agreement. The boxes were half-filled with local beach sand (>95% medium grain; 212-500 micron), which was sun dried and then rinsed several times with seawater. The boxes were randomly positioned in a room with temperature control and a light regime of 12 h light and 12 h dark. All nine boxes were connected to a seawater recirculation system to allow flushing with filtered seawater, sourced from a local estuary (Lake Macquarie). Water quality (temperature, salinity and dissolved oxygen) of the inflowing seawater, and oxygen concentration within each treatment box, were measured daily.

D. aciculata in each treatment box were supplied with commercially purchased high protein food pellets, representing an amount equivalent to 3% body weight per day. The ration was adjusted to account for increases in mean polychaete weight through time. At t weeks 1, 7, and 14, water flow was temporarily stopped and was followed by application of the anaesthetic solution; relaxed polychaetes emerged from their tubes and laid on the sediment surface. While in relaxed mode, 20 individuals were randomly removed from each of the nine treatment boxes. After the anaesthetic solution was flushed from each box, seawater recirculation was re-established. The remaining *D. aciculata* returned to regular activity within 30 minutes. There were no signs of any negative impact on remaining polychaetes as a result of the anaesthetic solution and handling procedures.

Polychaetes removed from the treatment boxes were measured (total length, mm), weighed (nearest 0.1 g) and the total number of segments counted. The condition of the posterior segments (broken or regenerating) was also noted. Length and weight measurements were conducted on anaesthetised *D. aciculata*. Segments were counted following preservation in 70% ethanol. Mortality of polychaetes was assessed at the completion of the 14-week study by conducting polychaete tube counts and by assuming one tube per polychaete.

The effect of density and time on polychaete growth was analysed by two-factor ANOVA, followed by Student-Newman-Keuls test, using GMAV5 for Windows (University of Sydney, Australia). The assumption of homogeneity of variances was tested using Cochran's *C* test, and where significant, data were transformed to sqrt (x + 1). Individuals with posterior segment loss were excluded from the analyses of differences among density treatments.

RESULTS

During the three-month study, there was little variation in water temperature (23-26°C) or salinity (35-38 ppt) in the treatment boxes. Dissolved oxygen concentrations within the treatment boxes ranged from 5.5-6.1 mg/l (Fig. 1), which were lower than levels in the incoming seawater (6.2 mg/l), and varied significantly with density (L>M>H, $P<0.001$). Although oxygen levels in the replicate treatment boxes with medium polychaete density were significantly different (1<3=2, $P<0.01$), there was no effect on polychaete length or weight ($P>0.05$).

Growth measurements recorded after the first week showed no significant effect of density on *D. aciculata* total length, wet weight or number of segments ($P>0.05$). Density effects on growth, however, were apparent in the longer term. As expected, means of total length, weight and number of segments were highest at low density and lowest at high density, following both 7 and 14 weeks of incubation (Fig. 2a-c). Two-factor ANOVA showed significant density and time effects, and a significant density x time interaction, for all growth variables (Table 1). At all density levels, estimated daily growth was higher during weeks 1-7 than during weeks 7-14 (Table 2). After the first seven weeks, average biomass was 3.5-4 times greater than polychaete weight following one week of incubation. Thereafter, declines in growth rate were observed in all treatments but were most marked for polychaetes at high density, which showed little further increase in weight or segment number.

Density-related competition for space was demonstrated by an increase in the frequency of *D. aciculata* tail damage, with the highest percentage of polychaetes with broken and regenerating poste-

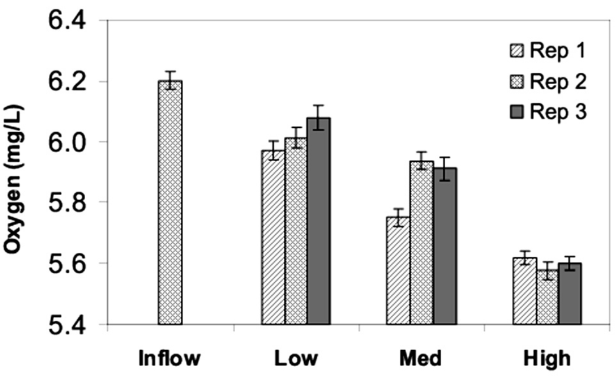

FIG. 1. – Mean dissolved oxygen concentrations (mg/l, ±SE) in three replicate treatment boxes, measured daily over the 14-week experimental period (n = 94 days).

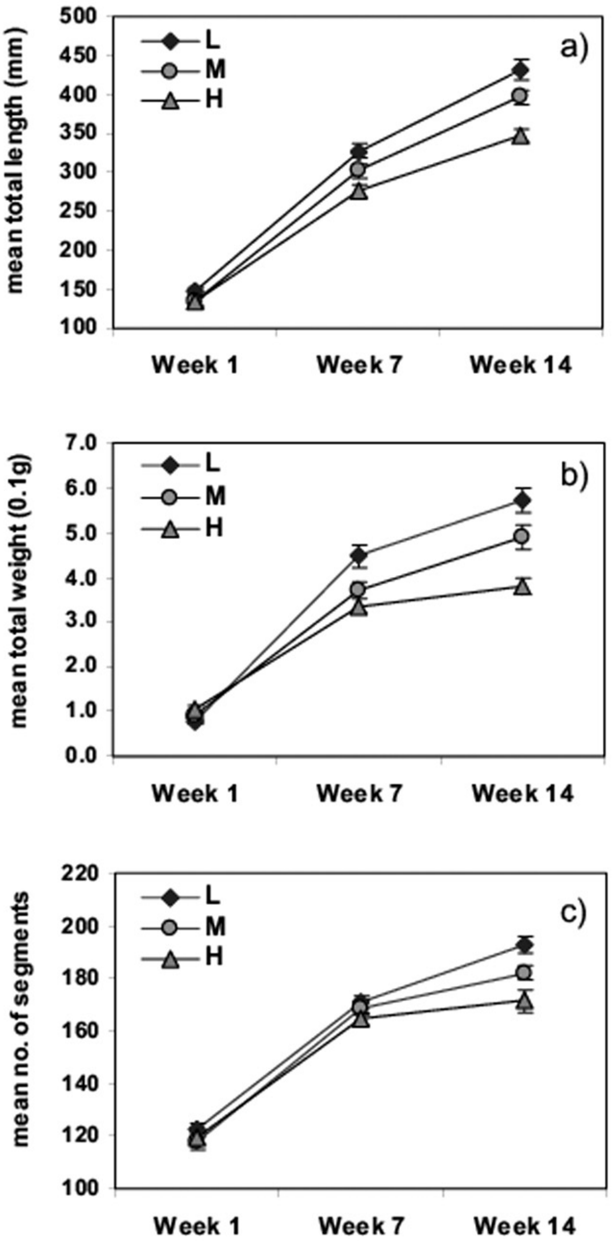

FIG. 2. – Growth measurements of *Diopatra aciculata* (n = 60; ±SE) taken at each density level (low, medium and high) at weeks 1, 7 and 14: a) total length (mm), b) total weight (0.1 g) and c) number of segments.

TABLE 1. – Summary of two-factor ANOVA for total length, total weight and total number of segments in *D. aciculata*.

		Length		Weight		Number of segments	
Source of variation	*df*	MS	*F*	MS	*F*	MS	F
Density	2	148191.77	33.55***	36.90	591.32***	15.58	582.89***
Time	2	5379.77	37.52***	1.86	29.88***	0.31	11.93***
Density x Time	4	1047.38	7.30**	0.95	15.23***	0.17	6.41**
Residuals	18	143.38		0.06		0.02	

** (P<0.01), *** (P<0.001)

TABLE 2. – Estimated daily growth in *D. aciculata:* total length, total weight and number of segments at low, medium and high density, during each time interval and overall.

Incubation period	Length gain mm/day			Weight gain g/day			Segment number segments/day		
	L	M	H	L	M	H	L	M	H
Wk 1-7	3.17	2.94	2.46	0.06	0.05	0.04	0.86	0.89	0.80
Wk 7-14	2.81	2.56	1.94	0.03	0.03	0.01	0.59	0.36	0.18
Wk 1-14	3.03	2.79	2.26	0.05	0.04	0.03	0.75	0.68	0.55

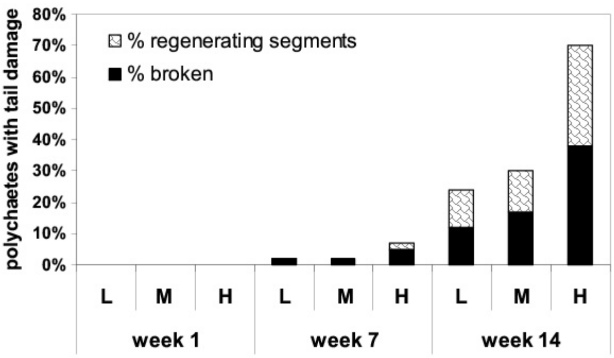

FIG. 3. – Percentage occurrence of broken and regenerating posterior segments observed at each density level at weeks 1, 7 and 14.

rior segments observed in the high density treatment (2000 polychaetes/m^2) at week 14 (Fig. 3). Mortality estimates after 14 weeks, based on tube counts, indicated 3%, 9% and 20% mortality at low, medium and high density levels, respectively.

DISCUSSION

In this study, the estimate of daily growth of *D. aciculata* after 14 weeks of incubation was lower at high density (2.3 mm/day) when compared to medium (2.8 mm/day) and low-density treatments (3.0 mm/day). Patterns in growth, and the increase in frequency of tail damage/regeneration and mortality, as density increased, provide evidence for negative density-dependent interactions with neighbouring worms. Reduced oxygen levels, particularly in the high-density treatment, may have caused additional stress.

The observed negative density-dependent effects on growth of late juvenile to early adult *D. aciculata* are similar to those reported for a number of other polychaete species (Zajac, 1986; Pesch *et al.,* 1987; Esnault *et al.,* 1990; Miron *et al.,* 1991; Scaps *et al.,* 1998). There has been only one report of a positive growth effect with increasing polychaete density (Bridges *et al.,* 1996), but this study was short in comparison (4 weeks) and was conducted on *Neanthes arenaceodentata* emergent juveniles (three weeks old) contained in 1000 ml beakers with 2-3 cm of fine sand and artificial seawater. Although growth appeared to be enhanced at high density, the authors observed an increase in the frequency of regenerating posterior segments at high densities, indicating density-related aggressive encounters between polychaetes. Interestingly, an earlier study by Pesch *et al.* (1987) showed negative density-dependent effects on the growth, reproduction and survival of *N. arenaceodentata* in a laboratory experiment that did not use sediment. The presence of sediment has been highlighted as being important in the determination of density effects for this species (Bridges *et al.,* 1996).

In this study, it is highly likely that the negative effects of *D. aciculata* density on growth are due to intraspecific interactions, including competition for resources other than food. Studies undertaken by Esnault *et al.* (1990) and 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 were also found to induce negative effects on growth (Scaps *et al*., 1993).

This study provides the first known account of the growth rates of juvenile *D. aciculata* and demonstrates the effect of density on growth rates, posterior segment condition and mortality when food is not limiting. Additional study is required to determine the density effects for different developmental stages of *D. aciculata*, as size (i.e. biomass) and age (i.e. feeding history and reproductive maturity) are likely to be important factors affecting intraspecific behaviour related to competition for space and food.

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