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An experimental approach to the ecophysiology of the interstitial polychaete *Polygordius eschaturus* (Annelida: Polygordiidae)

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Summary: Specimens of *Polygordius eschaturus* from a reflective sandy beach were tested in the laboratory for tolerance to salinity and temperature and for their response to light, gravity and oxygen tension. After 192 h at 10°C, almost all specimens died at salinities of 10, 20 and 50 psu and were slow moving from 30 to 40. At 20°C and 30°C, all specimens died when salinity was 10; few showed abnormal behaviour at 50 and all presented normal behaviour from 20 to 40. At 40°C, all specimens died within 24 h. In our experimental conditions, the observed migration patterns corresponded to photonegative geopositive behaviours, but also indicated a positive response to a higher oxygen tension. These patterns, together with euryhalinity and eurythermy, are in accordance with the typical habitat of the species, from 10 to 20 cm depth in coarse sand above the swash zone. In the intertidal zone of the tropical beach where the worms were originally found, the combined behaviour leading to an attraction to well-oxygenated, dark subsurface sediment layers results in successful protection from the strong changes in salinity and temperature of the more superficial layers.

Keywords: meiofauna; sandy beach; Vermelha Beach; tropical.

Un ensayo experimental sobre la ecofisiología del poliqueto intersticial *Polygordius eschaturus* (Annelida: Polygordiidae)

Resumen: El presente estudio se centra en el análisis de la tolerancia a los cambios de salinidad y temperatura, así como las respuestas a la intensidad de la luz, la gravedad y la tensión de oxígeno de individuos de *Polygordius eschaturus* recogidos en una playa reflectante con sedimentos arenosos. La exposición a salinidades de 10, 20 y 50 psu durante 192 h a 10°C, causó la muerte de casi todos los individuos, mientras que los expuestos a 30 y 40 psu se movían lentamente. A 20-30°C todos los individuos sometidos a 10 psu murieron, mientras que a 50 psu algunos se comportaron anormalmente, entre 20 y 40 psu todos se comportaron con normalidad y a 40°C todos murieron al cabo de 24 h. El modelo de migración observado en nuestros experimentos se explica por la existencia de conductas fotonegativas y geopositivas, pero también por una respuesta positiva a gradientes positivos de tensión de oxígeno. Estas pautas de comportamiento, junto con el carácter eurihalino y euritermo de la especie, coinciden con las características típicas de su hábitat, situado a entre 10 a 20 cm de profundidad en arenas gruesas por encima de la zona de salpicaduras. En la zona intermareal de la playa tropical donde se recogieron los ejemplares estudiados, un comportamiento combinado que genere una atracción por las capas subsuperficiales, más oscuras aunque aún bien oxigenadas, supone una exitosa adaptación ya que resulta en una adecuada protección frente los fuertes cambios de temperatura y salinidad de las capas más superficiales.

Palabras clave: meiofauna; playa de arena; Playa Vermelha, tropical.

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INTRODUCTION

The family Polygordiidae Czerniavsky, 1881 includes monotypic interstitial polychaetes usually inhabiting highly energetic marine coasts with coarse sands worldwide. However, this family has been poorly recorded due to difficulties of recognition. Except for a pair of minute, stiff palps, the Polygordiidae resemble nematodes due to their smooth body surface, predominant longitudinal musculature and lack of external segmentation, parapodia and chaetae (Lehmacher et al. 2016).

Polygordius eschaturus Marcus, 1948 was described from the intertidal of a medium to coarse sandy beach at São Sebastião Island (state of São Paulo, Brazil). It measures 40 mm long, 0.21 mm in body diameter and 0.3 mm in caudal bulb (or pygidium) diameter, and has two caudal appendages 0.05 to 0.07 mm long. It has pygidial adhesive glands that allow it to remain attached to the sand and caudal appendages that are robust taxonomic characters and twist up into a spiral knot when touched (Marcus 1948).

Previous studies on horizontal and vertical (surface to 30 cm depth) field distribution of the interstitial meiofauna from Vermelha Beach showed a highly diverse community, with Copepoda being the most abundant group, as expected due to the coarse sand (i.e. between 500 and 1000 µm, Silva et al. 1991). *Polygordius eschaturus* was very abundant 12 m above the swash zone midline, from 10 to 20 cm depth into the sediment (Santos and Silva, unpublished data). However, no attempts have been made to correlate its distribution with the existing ecological gradients.

Polygordius was reported on one (swash zone of Estaleiro Beach) of six sandy beaches in the states of Santa Catarina and Paraná, Brazil (Di Domenico et al. 2009). Conversely, the European and North American species inhabit sublittoral coarse sands or gravel zones (Villora-Moreno 1997, Ramey 2008, Martins et al. 2013), while one species even occurred in muddy bottoms of the Adriatic Sea (Rota and Carchini 1999).

Most published studies address the systematics and morphology of this genus (see Lehmacher et al. 2016 and references therein). Its physiology has received little attention but some data on life cycles are available (Ramey 2008).

Temperature, salinity, light, gravity and oxygen tension gradients are known to play an essential role in conditioning the spatial distribution of intertidal species on sandy beaches (Jansson 1968a, Giere 2009, Maria et al. 2016). Therefore, the aim of the present study was to test the effect of these factors on the survival and vertical distribution of *P. eschaturus* in laboratory conditions.

MATERIALS AND METHODS

Vermelha Beach (Fig. 1) is a reflective beach with moderate morphodynamic variability, showing a maximum tidal range of 1.7 m and high wave energy (waves up to 1.5 m high under stormy conditions) (Silva et al. 1991). It has coarse sand (mean grain size 0.875 mm), a low organic matter content (1 to 4%), a seawater temperature ranging from 19 to 29°C and a salinity ranging from 29.1 to 35.5 psu (Albuquerque and Genofre 1999). Sand temperature (at a depth of 5 cm) at the sampling site ranged from 22.2 to 26.2°C in July and December (Silva et al. 1991). The annual underground water temperature ranged from 19 to 29°C and salinity from 27.3 to 36 (Albuquerque and Genofre 1999).

Specimens of *P. eschaturus* were collected at the intertidal Vermelha Beach between July and November 1987 from the lower level of the retention zone, just 1 m above the upper part of the swash zone. The first 10 cm of dry sand was removed to leave the deeper wet sand open. Wet sand was collected, placed

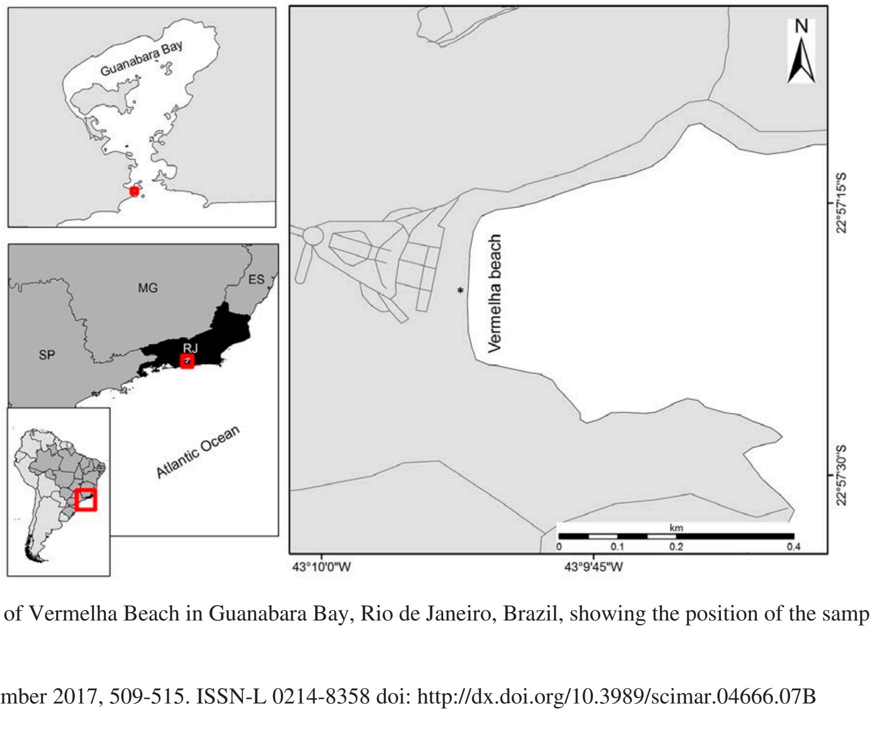

Fig. 1. – Location of Vermelha Beach in Guanabara Bay, Rio de Janeiro, Brazil, showing the position of the sampling station (*).

in Petri dishes with a dark bottom and examined to detect twisted worms. Once detected, the samples were immediately transported to the laboratory, where the specimens were sorted under a stereomicroscope using pincers to collect the sand grain to which they were attached. The worms were then placed in Petri dishes with a fine layer of native sand (about 5 mm deep) and unfiltered native seawater (salinity 33) and maintained at 20°C with a natural photoperiod (11 h of dark and 13 h of light). The Petri dishes were closed in plastic trays with distilled water at the bottom to avoid evaporation. The animals in Petri dishes were directly observed under the stereomicroscope and showed no external indications of sex or developmental stage. When collected, all lacked the pygidial appendages, which were regenerated after a few days in laboratory conditions, allowing the species identification to be confirmed. Some specimens also lost their pygidium during the experiments, but they were regenerated after ca. 10 days. However, the last segments became adhesive to replace it. Therefore, we assumed that this could not interfere with the expected results, so the absence of pygidium was not considered during the experiments.

Salinity and temperature tolerance

The worms were directly transferred from maintenance to test conditions and seawater was not replaced during the incubations. Survival rates were estimated after 192 h (eight days, with observations every 24 h). Immobile specimens after stimulation were considered dead, while slow-movements indicated abnormal behaviour.

A pilot experiment was carried out to test the most appropriate range of salinities for the main experimental design, in which the worms were placed at salinities ranging from 0 to 70 at 20°C. As a result, 20 combinations of salinity (10, 20, 30, 33, 40 and 50) and temperature (10, 20, 30 and 40°C) were tested using two groups of ten specimens.

A non-parametric Friedman ANOVA followed by a Dunn test was used to compare means among treatments as the data were not normally distributed.

Phototaxis

Phototaxis (reaction to light) was assessed by allowing the animals to choose between light and dark conditions. Four groups of 50 animals were placed in the centre of rectangular recipients with a fine native sand layer (ca. 5 mm deep) and native seawater. Transparent and black plastic covers provided natural light and blocked light, respectively. The experiments were run in four recipients, two half-illuminated/half-dark, one fully illuminated (control 1) and one fully dark (control 2). Control recipients were used to assess any gregarious behaviour not related to the light preferences. After 12 h, the light and dark halves were separated to count the respective number of worms. A chi-square (χ^2) test was used to compare the results between test and control recipients.

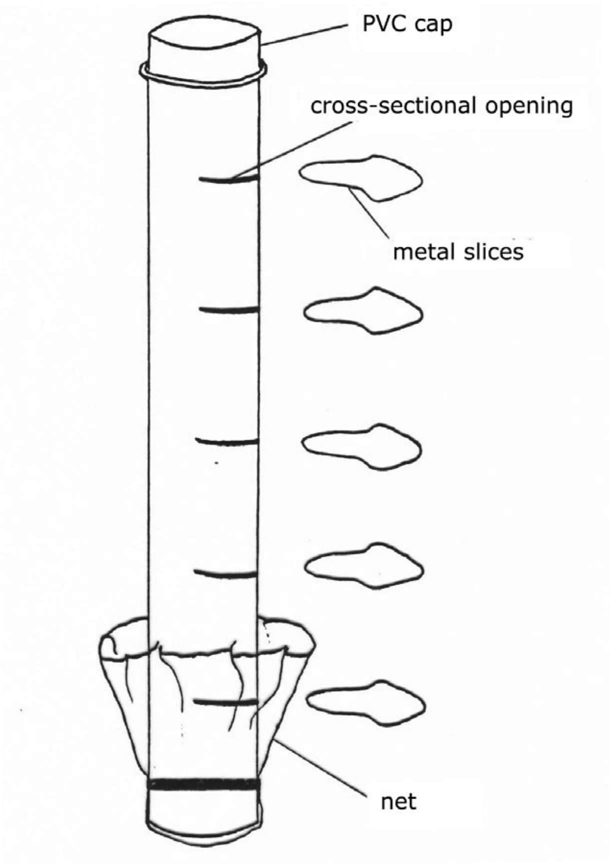

Fig. 2. – Illustration of Core 4 at the start of the experiment of geotaxis and effect of oxygen tension, showing the position of the cross-sectional openings.

Geotaxis and oxygen tension

The combined effects of oxygen tension and geotaxis (reaction to the force of gravity) was investigated using polyvinyl chloride (PVC) cores (30 cm long, 4 cm in diameter) with cross-sectional openings every 5 cm to allow the different sand layers to be sampled (Fig. 2). The cores were suspended vertically in an aquarium with aerated seawater. The cross-sectional openings were closed with adhesive tape during the experiments.

Four types of cores were tested: 1) sealed at both ends with PVC caps to prevent oxygen entering from the surrounding water; 2) closed at both ends with nets (64 µm pore size) to allow oxygen diffusion from the surrounding seawater from both core ends; 3) closed with a net at the top and a PVC cap at the bottom to allow oxygen diffusion only from the surrounding seawater to the upper core end; and 4) closed with a PVC cap at the top and a screen at the bottom to allow oxygen diffusion only from the surrounding seawater to the bottom core end. Oxygen gradients in Cores 1 and 2 were expected to be homogeneous, while those in Cores 3 and 4 were expected to be oriented top-down and bottom-up, respectively

Each core was first half-filled with sand. Then, 50 worms were placed inside and covered by sand to the top of the core. After 24 h in complete darkness, metal slices were introduced through each cross-sectional

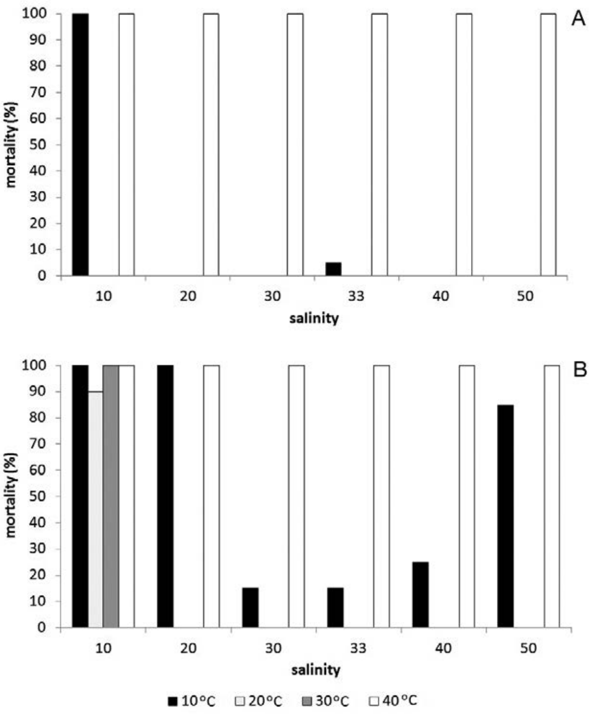

Fig. 3. – Salinity and temperature (°C) tolerance of *Polygordius eschaturus* after 24 h (A) and 192 h (B) of exposure.

opening to isolate six 5-cm sediment layers, which were then sampled and labelled from surface to bottom according to the depth range in cm. A χ^2 test was used to compare the distribution of animals in Cores 1 and 2 with the expected homogeneous distribution and the cores with each other.

RESULTS

Salinity and temperature tolerance

Virtually all specimens kept at salinities of 0, 60 or 70 died 8h after the pilot experiment started and the results did not change at the end, 192 h later.

Mortality was significantly affected by temperature (Friedman=20.28, p=0.0001) after 24 h and the Dunn test ($p<0.05$) indicated that 40°C was different from all other temperatures (Fig. 3A). Salinity did not significantly affect mortality (Friedman=1.25, p=0.94), although at 10°C and 10 salinity mortality was 100%.

After 192 h, mortality was also significantly affected by temperature (Friedman=26.2, $p<0.0001$) (Fig. 3B), and the Dunn test distinguished between temperatures of 20 and 30°C, at which less mortality occurred, and 10 and 40°C, at which greater mortality occurred. Again, salinity did not significantly affect mortality (Friedman=9.9, p=0.08), although at 10°C and salinities 10 and 20 mortality was 100%.

At 10°C, most specimens died when salinity was 10, 20 and 50 and they showed an abnormal behaviour at all observation times when salinity ranged from 30 to 40. At 20 and 30°C, all specimens died when salinity was 10 and a few showed an abnormal behaviour at 50. However, the behaviour was normal between 20 and 40. At 40°C, all specimens died within 24 h.

Table 1. – Phototaxis responses of *Polygordius eschaturus* (as number of individuals on each half of four experimental recipients). χ^2 results comparing test vs controls. * $p<0.05$.

Recipient	Light conditions			
	Test 1 vs Control 1		Test 2 vs Control 2	
Tests	Light 0	Dark 50	Light 0	Dark 50
Controls	Light 9	Light 41	Dark 12	Dark 38
χ^2	10.98*		15.79*	

Phototaxis

The worms significantly ($p<0.05$) preferred the dark side of the test recipients (Table 1), while they were more evenly distributed in both light and dark controls.

Geotaxis and effect of oxygen tension

Core 1 and Core 2 results differed from an expected homogeneous distribution, as the animals were significantly more concentrated from 20 to 30 cm depth (d.f.=5, χ^2=40.5, $p<0.0001$) and from 10 to 30 cm depth (d.f.=4, χ^2=12, p=0.017), respectively (Table 2). Moreover, Core 1 significantly differed from Core 2 (d.f.=4, χ^2=18, p=0.011), as the animals were concentrated deeper into the sediments in the lower oxygen tension conditions of the former. In turn, Core 2 significantly differed from Core 3 (d.f.=4, χ^2=245, $p<0.0001$), as more than half the animals were concentrated in the two surface layers in the latter. There were no significant differences between Cores 2 and 4 (d.f.=4, χ^2=0.8, p=0.94), as all worms were concentrated in the four deepest layers in both cases. Cores 3 and 4 significantly differed from each other (d.f.=4, χ^2=266, $p<0.0001$) as in Core 3 more than half the animals were concentrated in the two surface layers in the former and in the latter they were concentrated in the four deepest layers.

DISCUSSION

Marine sandy beaches are characterized by a network of horizontal (at right angles to the water line) and vertical (from the sand surface into the vertical sediment profile) gradients of ecological factors. Changes

Table 2. – Geotaxis and oxygen tension responses of *Polygordius eschaturus*, as number of worms in the different sediment layers at the four experimental corers. WA, without aeration; A, aerated.

Layers (cm) surface	Core 1 WA	Core 2 A	Core 3 A	Core 4 WA
0-5	0	1	16	1
5-10	3	5	12	3
10-15	6	12	4	12
15-20	6	11	2	11
20-25	19		2	4
25-30	19	22	14	18
bottom	WA	A	WA	A

occur as a function of wave intensity, tidal regime and seasonal variations. This complex network of interactions often makes it impossible to determine the causes of a given interstitial meiofauna distribution based on field studies. Laboratory tolerance and preference experiments are therefore a valuable tool for identifying the role of isolated or combined ecological factors, thus allowing field distributions to be interpreted (Jansson 1967c, 1968a, Giere 2009).

Many interstitial polychaetes show a distinct zonation on beaches, exhibiting clear preferences in the existing sediment structure and organic content gradients (Villora-Moreno 1997), and *Polygordius* seems to be no exception. Among six Brazilian sandy beaches, it was only present in the swash zone of Estaleiro Beach, showing higher abundances in winter and a strong positive correlation with both greater wave height and greater swash zone declivity (Di Domenico et al. 2009).

Vermelha Beach showed more marine than estuarine conditions, despite being located near the opening of Guanabara Bay (Fig. 1). However, the salinity of the groundwater in the area where the specimens of *P. eschaturus* were collected was highly variable (from 27.3 in February to 36 in August), probably due to a combination of evaporation during low tide exposure and dilution from rain or groundwater. In the laboratory, this species was considerably tolerant to changes in salinity, as it survived from values of 10 to 50 (although an abnormal behaviour was observed at both extremes, probably to save energy). Therefore, we expect *P. eschaturus* to not exhibit mortality or a reduction in mobility promoted by salinity changes during most of the year at Vermelha Beach. Its preference for the intertidal zone (Santos and Silva, unpublished data), which has a high water percolation rate that results in considerable variation in interstitial water content, could help to explain this tolerance to a large salinity range. Intertidal surface sediments have lower water contents and are more affected by evaporation and dilution by rainfall or groundwater, thus influencing the associated organisms (Jansson 1967b, c, 1968a).

In fact, the euryhaline character of *P. eschaturus* is also observed in other beach meiofaunal organisms. Some species may even have a greater physiological tolerance to salinity, with ranges of more than 15 psu (Jansson 1968a). These include the mystacocarids *Derocheilocaris remanei* Delamare Deboutteville and Chappuis, 1951 (Jansson 1966) and *D. typica* Pennak and Zinn, 1943 (Kraus and Found 1975), the turbellarian *Corohelmis lutheri* Ax, 1951, the copepods *Schizopera baltica* Lang, 1965, *Nitochra fallaciosa* Klie, 1937 and *Nitocra fallaciosa f. baltica* Lang, 1965 (Jansson 1968b), and the oligochaetes *Marionina southerni* (Černosvitov, 1937) (Jansson 1968b) and *Aktedrilus monospermatecus* Knöllner, 1935 (Jansson 1962). Other species have a more restricted range (less than 7.5 psu). These include the turbellarian *Haplovejdovskya subterranean* Ax, 1954, the oligochaetes *Marionina subterranea* (Knöllner, 1935) (Jansson 1968b) and *M. preclitellochaeta* Nielsen and Christensen, 1963 (Jansson 1962), and the copepods *Paraleptastacus spinicauda* (Scott and Scott, 1895) and *Parastenocaris vicesima* Klie, 1935. In some cases, however, these narrow ranges do not always correspond to the native habitat salinities, which suggest that some acclimatization must occur (Jansson 1968a). Genetic adaptation to different salinities has also been described for three populations of the turbellarian *Gyratrix hermaphroditus* Ehrenberg, 1831 (Jansson 1968a).

There are very few data on the ecophysiology of tropical species of meiofauna. The combined effects of temperature and salinity on the tolerance of the interstitial isopod *Coxicerberus ramosae* Albuquerque, 1978 from Vermelha Beach revealed that 35°C was lethal, and that salinity tolerance was narrow at 5°C. However, the salinity tolerance of this species is higher than that of *P. eschaturus*, as it survived to salinities of up to 70 at 20°C (Albuquerque et al. 2009).

Polygordius eschaturus tolerated temperatures ranging from 10 to 30°C, although low activity was found at 10°C. This upper limit is higher than that of typical temperate species, which have low tolerance to 30°C (mean lethal time from 5 h to 5 days), but the lower limit is also higher (Jansson 1968a, b), as expected for a tropical species. Typical temperate animals easily endure 5°C and may even survive under frozen sand, although with low activity (Jansson 1968a, b). The tropical isopod *C. ramosae* seems to be more tolerant (5 to 30°C) than *P. eschaturus*, although it shows a narrow salinity tolerance after 24 h at 5°C (Albuquerque et al. 2009). Although we did not test 5°C, all worms exhibited a low activity in all the 10°C experiments, allowing us to assume an even lower tolerance at 5°C. Our experiments also reveal that the temperature tolerance was significantly modified by its interaction with salinity. For instance, the worms survived better at 10°C with normal salinity (30 to 40) than at low salinity (10) after 24 h or than at extreme salinities (10, 20 and 50) after 192 h. This agrees with the postulated increasingly dramatic effects of two interacting factors vs. each one working separately, which results in nearing the tolerance limits (Vernberg and Coull 1975).

Polygordius eschaturus also exhibited photonegative behaviour, a common feature of other interstitial organisms (Boaden 1963, Gray 1966, 1967, 1968) that is probably related to its habitual presence in deep sediment layers. Some Polygordiidae have photoreceptor-like sense organs in the frontal part of the brain, as recently described for *Polygordius appendiculatus* Fraipont, 1887 using transmission electron microscopy (Wilkens and Purschke 2009). As these sense organs may be associated with (or responsible for) phototaxis responses, the photonegative behaviour strongly suggests the presence of such photoreceptor-like organs in *P. eschaturus*.

The vertical distribution of *P. eschaturus* within the experimental cores seemed also to respond to a geopositive behaviour, combined with positive migrations toward the zones with higher oxygen tension. Accordingly, the effect of lower oxygen percolation in Core 1 (Table 1) resulted in a greater concentration of worms at the 20- and 30-cm-deep sediment layer than in Core 2, with higher oxygen tension and worms distributed from 10 to 30 cm. These results showed that at lower oxygen tension worms migrate deeper in the sediment profile. Although this could be seen as an incongruent behaviour in sediments with subsurface reduced oxygen layers, the coarse grain size and high wave energy at Vermelha Beach intertidal promote deeper oxygenation, so the reduced oxygen layer occurs at more than 30 cm deep into the sediment, with minima of 4.7 mg L^{-1} in the interstitial water between 20 and 70 cm depth (Albuquerque and Genofre 1999). Oxygen availability is strongly governed by sediment physicochemical characteristics, such as porosity, permeability and air volume, while maximum oxygen availability depths in interstitial waters are greatly determined by wave action (Jansson 1967a). Therefore, our experimental results, combined with the particular characteristics of the habitat of *P. eschaturus* at Vermelha Beach, strongly support the hypothesis that oxygen is not playing a key role as limiting factor.

Previous studies using similar methods to address the influence of oxygen on the vertical distribution of interstitial animals showed that *Protodriloides symbioticus* (Giard, 1904) (reported as *Protodrilus symbioticus*) did not respond to gravity and migrated toward the zones with greatest oxygen tension (Gray 1966). In turn, *Lindrilus rubropharyngeus* (Jägersten, 1940) (reported as *Protodrilus rubropharyngeus*) exhibited geonegative behaviour and also migrated toward the greatest oxygen tension (Gray 1967), while the copepod *Leptastacus constrictus* Lang, 1965 exhibited geonegative behaviour independently of oxygen tension gradients (Gray 1968). Therefore, the response to gravity and oxygen tension gradients of *P. eschaturus* differs from those of these previously studied meiofaunal species. A broad set of studies will be required to assess whether these responses are species-specific.

Saccocirrus pussicus Marcus, 1948 is another interstitial polychaete living in the high hydrodynamic swash and surf zones of Brazilian sandy beaches. However, it is more abundant at a sediment depth of 0 to 20 cm, which is more prone to sediment transportation (Di Domenico et al. 2014). Most meiofaunal taxa, however, respond to sediment disturbance by migrating downward or living in deeper layers (Giere 2009, Di Domenico et al. 2014), which seems to be the case of *P. eschaturus* (Santos and Silva, unpublished data). The migration of this species down to 10 or 20 cm deep into the sediment may also be caused by the low water content of surface sediments in the retention zone, the intertidal zone above the swash, and the resurgence zone (Jansson 1967c). Therefore, this overall geopositive behaviour keeps *P. eschaturus* in more humid, oxygenated and protected sediment layers.

In conclusion, the intertidal surface sediments at Vermelha Beach appear to be hot and dry and suffer from frequent rainfall, so drying, together with changes in salinity and temperature, are probably much greater than the tolerance range for *P. eschaturus*. This species is euryhaline and eurytherm, exhibits photonegative and geopositive behaviour and tends to migrate toward layers of greater oxygen, which explains why it mainly inhabits the most protected sediments above the swash zone 10 to 20 cm down into the sediment. In turn, the high wave action and coarse sand typical of Vermelha Beach would favour high oxygen availability in these deep sediment layers, thus helping to increase the survivorship of *P. eschaturus*.

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