Living on the edge: single-species dominance at the Pakistan oxygen minimum zone boundary

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ABSTRACT: Oxygen minimum zones (OMZs) are naturally occurring, low-oxygen water masses that create hypoxic conditions where they impinge on the seafloor. Their lower boundaries are characterised by elevated densities of hypoxia-tolerant fauna and an abundant food supply. The polychaete *Linopherus* sp. nov. (Amphinomidae) is the dominant taxon at the Pakistan margin (PM) lower OMZ, at near suboxic dissolved oxygen (DO) concentrations of 5 to 8 μM . We explored the response of *Linopherus* sp. nov. to gradients in oxygen and organic matter (OM) availability from depths of 700 to 1100 m during the inter- and late monsoon periods. *Linopherus* sp. nov. was present from 800 to 1000 m, and highest densities were found at 850 m. Population size structuring was evident and smaller individuals were present at depths of lowest DO concentrations. *Linopherus* sp. nov. showed morphological adaptation to low DO, and respiratory surface areas were significantly larger in worms at sites of lowest DO concentrations. Stable carbon isotopes ($\delta^{13}\text{C}$) revealed that *Linopherus* sp. nov. feeds mainly on sedimentary OM while enriched $\delta^{15}\text{N}$ values suggest that *Linopherus* sp. nov. also utilises predation as a foraging strategy. Lipid biomarkers indicate an omnivorous lifestyle, in which *Linopherus* sp. nov. uses phytodetrital, bacterial and invertebrate/carrion food sources. Pulse-chase experiments demonstrated that *Linopherus* sp. nov. consumes phytodetritus and contributes significantly to OM processing, potentially altering OM quality and thus the availability of food resources to the benthic community. Severe oxygen stress leads to single-species dominance, which in turn simplifies macrofaunal ecosystems and thus reduces trophic complexity.

KEY WORDS: Oxygen minimum zone · Hypoxia · Single-species dominance · Organic matter processing · *Linopherus* · Stable isotopes · Lipid biomarkers

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INTRODUCTION

Mid-water oxygen minimum zones (OMZs), water masses where dissolved oxygen (DO) concentrations fall below 22 μM , are widespread hydrographic features in the world's oceans (Kamykowski & Zentara 1990, Helly & Levin 2004). Oxygen depletion results

from a complex set of interactions that starts with nutrient injection via upwelling and intense surface water production. This is exacerbated by limited ventilation within the water column owing to stratification and isolation of older water masses (Sarmiento et al. 1988). The interception of mid-water OMZs onto continental margins results in benthic hypoxia and

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currently affects over 1 million km² (approximately 2%) of margin seafloor (Helly & Levin 2004). Climate models and observational data show an expansion of mid-water OMZs in the world's oceans, resulting from global environmental change, e.g. temperature-driven changes (i.e. declining oxygen solubility), altered ocean circulation patterns, increased stratification, changes in biological production, variations in biological oxygen demand and altered remineralization rates (Bopp et al. 2002, Goes et al. 2005, Stramma et al. 2010). Concurrently, the extent and frequency of coastal hypoxia is also increasing as a result of anthropogenic forcing (Diaz & Rosenberg 2008, Diaz et al. 2009, Rabalais et al. 2009).

Field programs have been carried out to survey benthic communities inhabiting OMZs throughout the world's continental margins, e.g. West Africa (Zettler et al. 2009), Oman (Gooday et al. 2000, Levin et al. 2000), Pakistan (Gooday et al. 2009, Hughes et al. 2009), India (Ingole et al. 2010, Hunter et al. 2011), Peru (Levin et al. 2002) and Chile (Gallardo et al. 2004, Palma et al. 2005). The majority of these investigations are based on transects where stations are spaced fairly widely apart (e.g. Hughes et al. 2009). Such studies, when they include an experimental component, have provided insight into ecosystem processes and function, e.g. Woulds et al. (2007, 2009), Andersson et al. (2008). From these field programs the lower boundaries of OMZs have been identified as particularly dynamic settings where animal densities can be remarkably high, but diversity is often low (Levin 2003). This is true of both the water column (Wishner et al. 1995, 1998, 2000) and the seabed (Levin et al. 1991, Wishner et al. 1995, Gooday et al. 2009).

A number of taxa have been identified as being particularly tolerant to permanent hypoxia in the deep sea. These include some spionid, cirratulid and cossurid polychaetes (Levin & Edesa 1997, Lamont & Gage 2000), glochinemid nematodes (Neira et al. 2001, 2005), ampeliscid amphipods (Levin 2003) and some oligochaetes (Levin et al. 2003). However, sampling designs have yielded few autecological studies that investigate in detail the adaptations or population biology of species within OMZs. Exceptions are the detailed studies of the pelagic copepod *Lucicutia grandis* by Wishner et al. (2000), the Humboldt squid *Dosidicus gigas* by Rosa & Seibel (2010), the benthic mud-ball forming cirratulid *Monticellina* sp. by Levin & Edesa (1997) and the spionid *Paraprionospio pinnata* by González & Quiñones (2000). Responses to the physiological constraints of OMZs have also been documented in several species of calanoid copepods

via ontogenetic zonation (Wishner et al. 2000, 2008, Loick et al. 2005, Criales-Hernandez et al. 2008).

Recent studies across the Pakistan margin (PM) OMZ have revealed the rarity of infaunal macrofauna within the OMZ core (300 to 600 m, Hughes et al. 2009). Polychaetes dominate in the lower OMZ transition zone (750 to 1100 m) and account for 61 to 100% of the macrofauna. One undescribed, new species of amphinomid polychaete, *Linopherus* sp. nov., constitutes over 80% of total macrofaunal abundance at some lower OMZ sites (Levin et al. 2009b). From high-resolution sampling across sharp oxygen gradients in different seasons it should be possible to infer the ways in which oxygen and food supply affect population and community structure at the PM.

The present study focuses on the autecology of *Linopherus* sp. nov., the dominant infaunal macrofaunal organism within the lower OMZ on the PM (700 to 1100 m, Levin et al. 2009b) and uses it as a model for exploring larger fundamental questions regarding ecosystem functioning within OMZs. Because this species exists over a gradient of critical oxygen thresholds (5 to 8 µM) and ranges from being almost the only macrofaunal species present at its lower oxygen limits to being rare among many others at its lower depth limit, we can explore how oxygen influences ecosystem processes via species activities. We ask: (1) How does the lower OMZ environment (i.e. oxygen and organic matter [OM] quantity and its associated biotic thresholds) influence the distribution and size structure of *Linopherus* sp. nov.? (2) Does *Linopherus* sp. nov. exhibit morphological or ecological adaptations that allow it to be so successful in low oxygen environments? (3) How does *Linopherus* sp. nov. affect biodiversity and ecosystem functioning, defined here as OM processing, within the lower OMZ transition zone?

In order to address these questions, we present both new data and synthesize previously published data to provide an integrated view of how *Linopherus* sp. nov. functions across the lower OMZ. The new data presented here for *Linopherus* sp. nov. are (1) detailed information on the size distribution including biomass (wet weights of individuals) and size (length estimated from number of chaetigers), (2) detailed analyses of respiratory structures, (3) video footage of the species in its natural habitat and (4) stable isotope (carbon and nitrogen) and lipid data to elucidate the trophic ecology. These new data are set in the context of, and interpreted using, previously published information regarding the abundance and distribution of both the macrofauna and *Linopherus* sp. nov., the environmental conditions at the lower

Fig. 1. (A) Location of the Pakistan margin working area and (B) detail of the lower oxygen minimum zone transect. Grey circles indicate depths sampled by megacorer and black circles indicate depths where *in situ* oxygen measurements were obtained in addition to megacores

OMZ boundary (i.e. DO, organic carbon and nitrogen, total phytopigments and grain size) (Levin et al. 2009b), sediment structure and burrow activity (Levin et al. 2009b), and OM processing by *Linopherus* sp. nov. determined from shipboard and *in situ* pulse-chase experiments using ^{13}C -labeled diatoms (Woulds et al. 2007).

MATERIALS AND METHODS

Study area

The study area was located north of the Indus canyon, at the PM, Arabian Sea, around $23^{\circ}33'$ to $22^{\circ}66'$ N, $65^{\circ}83'$ to $66^{\circ}83'$ E (Fig. 1A). The Arabian Sea is characterized by steep gradients in bottom-water oxygen and organic carbon concentrations (Cowie 2005). Seasonally reversing monsoons lead to upwelling of nutrients and high primary production; ~5% of the total global primary production occurs in the Arabian Sea (Qasim 1982, Pfannkuche & Lochte 2000). This elevated primary production leads to

some of the highest recorded particle fluxes to the deep ocean (~3000 m), ranging from 0.1 to 190 $\text{mg m}^{-2} \text{d}^{-1}$ (Haake et al. 1993, 1996, Honjo et al. 1999). Detailed information on the hydrography and sediment geochemistry of the study area is given in Cowie & Levin (2009) and references therein. Both oxygen concentration and food availability (sedimentary organic carbon) are known to affect the abundance and diversity of foraminifera, macrofauna and megafauna at the PM (Hughes et al. 2009, Larkin & Gooday 2009, Levin et al. 2009b, Murty et al. 2009). In turn, the abundance and biomass of fauna at the PM influence sedimentary OM quality through their feeding processes (Jeffreys et al. 2009a). These effects are more pronounced at the lower boundary of the PM OMZ (1000 to 1300 m), where there is a balance between a rich food supply and relief from oxygen limitation, resulting in high densities of deposit- and filter-feeding fauna removing labile OM, i.e. polyunsaturated fatty acids from the water column and sediments by molecular filtration (Jeffreys et al. 2009a, Levin et al. 2009b, Murty et al. 2009). The dominant macrofaunal organism at the PM lower OMZ transition zone/boundary (700 to 1100 m) is *Linopherus* sp. nov. (Levin et al. 2009b), the subject of this study.

Field sampling

Sampling was carried out on board RRS 'Charles Darwin' during 2 periods: March–April 2003, a period of presumed low particulate flux (~60 $\text{mg m}^{-2} \text{d}^{-1}$, Haake et al. 1993), termed the 'inter-monsoon period' (IMP); and in September–October 2003 following high particulate flux at the end of the SW monsoon (~125 $\text{mg m}^{-2} \text{d}^{-1}$, Haake et al. 1993), termed the 'late monsoon period' (LMP). Sampling was conducted at the sea floor every 50 m water depth from 700 to 1100 m (Fig. 1B). These sites were defined by their depth, bottom-water oxygen concentrations and biogeochemical properties (see Tables 1 & 2). Bottom-water oxygen data were obtained from 22 CTD casts in total deployed during the IMP and LMP between bottom-water depths of 100 to 1850 m. The CTD was deployed as close to the seafloor as was possible (~5 m above bottom). The CTD (Seabird 911) was fitted with an SBE 43 dissolved oxygen polarographic probe (Brand & Griffiths 2009) and was calibrated using micro-Winkler titration (Hansen 1999). The micro-Winkler titration returned a correlation coefficient of 0.997 for 33 water samples measured during 'Charles Darwin'

Cruise 145 (CD145). This calibration was used on CD146, CD150 and CD151 because oxygen concentrations within the OMZ were very low during these cruises and produced negative calculated concentrations from the micro-Winkler titration (Brand & Griffiths 2009). Oxygen data from all casts were amalgamated and averaged in 5 m depth intervals to produce summary profiles for each season (Bett et al. 2004a,b). Data were extracted from 50 m depth intervals from 700 to 1100 m for this study, as shown in Levin et al. (2009b).

Bottom-water oxygen concentrations were also obtained *in situ* (millimetres above the sediment-water interface) with a microelectrode profiler (Profilur; Gundersen & Jorgensen 1990) at water depths of 850, 950 and 1000 m. For exact details see Breuer et al. (2009). Briefly, the Profilur autonomous lander consisted of 5 polarographic oxygen microelectrodes with a built-in reference and internal cathode guard and one resistivity sensor (Unisense®). The microelectrodes were calibrated on the ship before and after each deployment. Bottom-water oxygen concentrations were calculated from linear 2-point calibrations of the microelectrodes achieved *in situ*. The lander was deployed to the seafloor and allowed to settle for at least 1 h while continuously recording bottom-water oxygen. The second electrode value for calibration (zero) was recorded when the electrode penetrated the anoxic zone of the sediment, defined as the depth where the oxygen microelectrode signal reached zero current.

Sediments were sampled using a megacorer that contained 8 core tubes with an inner diameter of 9.6 cm. During each sampling season 2 cores per drop of the megacorer were processed for infaunal macrofauna (>0.3 mm), except at 950 m where 7 cores were processed. During each sampling season 5 cores from 950 m (from the study of Hughes et al. 2009) were sectioned at intervals of 0 to 10 and 10 to 20 cm. In addition, 2 cores from each depth (700 to 1100 m, from the study of Levin et al. 2009b) were sectioned at intervals of 0 to 1, 1 to 2, 2 to 5 and 5 to 10 cm, in order to study the vertical distribution of *Linopherus* sp. nov. All cores were preserved in 8% buffered formalin. Organisms were sieved on a 0.3 mm screen and sorted at 12× magnification. *Linopherus* sp. nov. were counted and measured (biomass and length via number of chaetigers). Morphological adaptation to hypoxia was ascertained by examining branchial structure. The branchiae are respiratory structures occurring in pairs and usually found starting on the third or fourth chaetiger (Fig. 2C shows one half of a branchial pair). The number of branchial

pairs per specimen were counted. Approximate respiratory area was estimated by normalizing the number of branchial pairs of a specimen to its wet weight in milligrams.

A separate set of megacores from 800, 850, 900 and 950 m were sectioned at 0 to 1, 1 to 2, 2 to 5 and 5 to 10 cm and were sieved on a 0.3 mm screen for biochemical analyses only. From these cores, individuals of *Linopherus* sp. nov. and large visible filamentous bacteria were isolated from the sediment using a binocular microscope, rinsed in filtered seawater and frozen at –20°C; this was done when cores were retrieved on board the ship for stable isotope and lipid analyses on return to the laboratory.

Sediment samples collected for geochemical analyses (n = 1 per depth and n = 3 at 950 m during each season) were sectioned and frozen. The surface layer, i.e. 0 to 0.5 cm of each core, was analyzed for the following: grain size and total phytopigments as described in Levin et al. (2009b); organic carbon content (%C_{org}), total nitrogen (%TN) and stable carbon (δ¹³C) and nitrogen isotopes (δ¹⁵N), as described in Cowie et al. (2009); and lipids (from 950 m) as described in Jeffreys et al. (2009a). Separate megacore tubes were sampled for burrow morphology using X-radiography as described in Levin et al. (2009b). The CTD was equipped with a 24 bottle (10 l) GOFLO Rosette sampler, which was used to collect particulate organic matter (POM) from water samples (see Jeffreys et al. 2009b).

Natural abundance stable isotope analysis

Vapour phase digestion was used to remove carbonate from POM and sediment samples. Animal tissues were acidified with 1 M HCl prior to analysis. *Linopherus* sp. nov. and filamentous bacteria from the IMP were analyzed using a Finnigan Delta-S isotope ratio mass spectrometer (University of Boston); *Linopherus* sp. nov. and filamentous bacteria from the LMP were analyzed using a Europa Hydra 20/20 isotope ratio mass spectrometer (University of California Davis); δ¹⁵N of sediments were analyzed using a Europa ANCA-NT 20/20 isotope ratio mass spectrometer (Scottish Crop Research Institute, Dundee); δ¹³C of POM and sediments were analyzed using a VG Prism III isotope ratio mass spectrometer (University of Edinburgh). Stable isotope ratios were expressed as (δX) (‰) = (R_{sample}/R_{standard} – 1) × 1000, where X was either ¹³C or ¹⁵N, and R was either (¹³C:¹²C) or (¹⁵N:¹⁴N), respectively. The reference standards for carbon and nitrogen were Pee Dee

Fig. 2. *Linopherus* sp. nov. (A) Image of a whole specimen. (B) Dorsal view depicting branchial pairs. (C) Close-up view of a single branchia depicting branchial rami. (D) *Linopherus* sp. nov. exiting a burrow in a megacore; note the numerous burrow holes

Belemnite and atmospheric N_2 , respectively. Analytical precision was determined using an internal standard (holothurian tissue: *Oneirophanta mutabilis*, Iken et al. 2001), which was sent to each lab: SD values were $\pm 0.16\%$ for $\delta^{13}C$ and $\pm 0.17\%$ for $\delta^{15}N$.

Lipid analysis

To evaluate *Linopherus* sp. nov. diet, lipid extractions were carried out on lyophilized animal tissue ($n = 3$ from each depth) from the LMP, depth range 800 to 950 m, and are described in full in Jeffreys et al. (2009b). Briefly, separate aliquots of tissue were spiked with an internal quantification standard

($5\beta[H]$ -cholic acid) and the total lipid extract was obtained through sonication for 60 min in a solvent mix of dichloromethane and methanol (9:1). Aliquots of lipids were transesterified in methanol containing acetyl chloride at $40^\circ C$ for 12 h to generate fatty acid methyl esters (Christie 1982). The methylated extract was silylated using bis-trimethylsilyltrifluoroacetamide (BSFTA, 1 % trimethylsilyl; 50 μ l; $60^\circ C$; 1 h) to produce sterol trimethylsilyl ethers (Kiriakoulakis et al. 2001) and was spiked with an internal recovery standard ($5\alpha[H]$ -cholestane). Lipid extracts were dissolved in dichloromethane and analyzed using a Trace 2000 Series gas chromatograph (on-column injector; fused high temperature silica column, 60 m $\times$ 0.25 mm inside diameter; 5 % phenyl/95 % methyl

polysiloxane equivalent phase; DB5-HT; J&W) coupled with a Thermoquest Finnigan TSQ7000 mass spectrometer. For full operating conditions see Jeffreys et al. (2009b). Xcalibur software (v. 1.0) was used to acquire and process the data. Relative response factors (RRF) were calculated for 36 alkanolic acids, alkanols, sterols and alkenones, and from these RRFs concentrations of individual compounds were determined in samples by comparison of their peak areas with those of the internal standards (Kiriakoulakis et al. 2004). Lipid concentrations were normalized to total dry tissue weight of *Linopherus* sp. nov.

Pulse-chase experiments

To study the role of *Linopherus* sp. nov. in carbon processing, pulse-chase tracer experiments with ^{13}C -labeled diatoms were carried out on intact sediments from 850 and 950 m with whole benthic communities present. Two approaches were used: shipboard incubations of recovered sediment cores at ambient bottom-water temperature and O_2 (a 2 d experiment at 850 m during the IMP, and a 5 d experiment at 950 m during both IMP and LMP), and an *in situ* incubation (2 d experiment at 950 m during the LMP) using the benthic chamber lander 'Elinor' (Black et al. 2001). For a detailed description of the methods see Woulds et al. (2007). Briefly, shipboard experiments were carried out in the dark using duplicate megacores in a controlled temperature laboratory. Tracer experiments were initiated by introducing a slurry of ~80% ^{13}C -labeled diatoms onto the sediment surface, to a dose of 650 to 1000 mg C m $^{-2}$ (290 mg C m $^{-2}$ for the *in situ* experiment). During incubations, core top water was stirred and DO concentrations were maintained through the use of 'oxystat' gills. At the end of the experiment, megacores were sectioned as above. *Linopherus* sp. nov. were collected and prepared for isotope studies as described for natural abundance isotope studies. The *in situ* benthic lander experiments are described in detail in Woulds et al. (2007). A miniature camera system was fitted inside the lander frame to provide diagnostic information related to the experiments. The box-core recovered at the end of the *in situ* experiments was sub-sampled with 2 megacorer tubes, and *Linopherus* sp. nov. was collected and prepared for analysis as described above. Samples from ^{13}C pulse-chase experiments were analyzed for ^{13}C using a Europa Scientific tracer-mass isotope ratio mass spectrometer (IRMS; Crew) with a Roboprep Dumas combustion sample con-

verter. Carbon contents were determined from IRMS peak areas and calibrated against standards of acetanilide, which were tailored to suit very low C samples. Linearity curves typically had r^2 values greater than 0.99 and $\delta^{13}\text{C}$ had an average SD of 0.7‰ ($n = 27$).

Statistical analyses

Enhanced spatial sampling resolution was carried out during this study at the expense of replication. This resulted in limited statistical analyses for some parameters owing to the absence or low number of replicate samples at each depth and time period. Initially we tested for temporal differences in the density (ind. m $^{-2}$), biomass (wet weight, ind. mg $^{-1}$), number of chaetigers (body length) and respiratory area (no. branchial pairs mg $^{-1}$) of *Linopherus* sp. nov. at 850 and 950 m. We were unable to make temporal comparisons at other sites owing to small sample numbers. If no significant temporal variation was noted at 850 and 950 m, data from both seasons were then pooled in order to make comparisons of density, biomass and body length between sampling depths (i.e. 700, 850, 900, 950 and 1000 m for density, and 800, 850, 900 and 950 m for biomass and body length). Spatial differences in respiratory area were tested for in each season separately. Comparisons of *Linopherus* sp. nov. distribution and biomass within the sediment column were also made where possible. The distribution and variance of the data were ascertained using the Shapiro-Wilks test and Levene's homogeneity of variance test. If data had normal distribution and equal variance, a 1-way ANOVA was performed to ascertain statistical differences. If the data did not meet the assumptions of an ANOVA, a Kruskal-Wallis test or Mann-Whitney *U*-test was carried out. Relationships between environmental parameters (O_2 , %C $_{\text{org}}$, %TN, total phytopigments and grain size) and biological variables (density, biomass and respiratory area) were examined using Spearman's rank correlation.

We tested for differences in the trophic ecology of *Linopherus* sp. nov. using the stable isotope and lipid data. Comparisons of isotopic data across water depth zones (LMP only) and between seasons (950 m only) were analyzed using 1-way ANOVA and Mann-Whitney *U*-tests, respectively. Lipid profiles of *Linopherus* sp. nov. (the proportion of each compound relative to total lipid) were compared between depths on log-transformed Euclidean distance similarity matrices using non-metric multidimensional

Table 1. Bottom water oxygen concentrations and sediment organic chemistry (surficial layer, 0 to 0.5 cm) for the Pakistan margin lower oxygen minimum zone. IMP: inter-monsoon period; LMP: late monsoon period; C:N: C:N molar ratio; Total phytopigments: sum of chlorophyll and pheopigments (obtained by bulk fluorescence HPLC); nd: not determined

Depth (m)	CTD O ₂ (μM)		<i>In situ</i> O ₂ (μM)		%C _{org}		% TN		C:N		Total phyto-pigments (mg g ⁻¹)	% Clay
	IMP	LMP	IMP	LMP	IMP	LMP	IMP	LMP	IMP	LMP	IMP	IMP
700	5.22	5.94	nd	nd	2.64	2.46	0.31	0.38	9.93	9.36	1.63	32.05
750	5.31	6.02	nd	nd	2.74	nd	0.35	nd	9.2	nd	nd	34.92
800	5.44	6.11	nd	nd	3.14	2.78	0.39	0.35	9.42	9.37	2.09	35.37
850	5.58	6.25	nd	<0.01	3.11	3.14	0.38	0.36	9.45	9.56	1.05	30.51
900	5.71	6.56	nd	nd	3.51	3.49	0.44	0.44	9.37	9.16	0.72	33.26
950	5.94	6.78	1.7 ± 0.5	2.8 ± 0.7	3.53	3.61	0.42	0.44	9.70	9.70	nd	nd
1000	6.52	7.76	nd	9.3 ± 3.7	2.69	3.14	0.33	0.38	9.47	9.53	1.39	32.58
1050	7.32	8.88	nd	nd	3.10	3.16	0.38	0.40	9.41	9.20	1.52	29.44
1100	nd	nd	nd	nd	3.17	3.14	0.38	0.39	9.80	9.38	2.17	26.64

scaling. Compounds that contributed a mean of less than 0.5 % to the profile were omitted from statistical analyses. Differences in the lipid profiles were ascertained using analysis of similarities (ANOSIM). The compounds responsible for these differences were determined using SIMPER analyses (Clarke & Warwick 2001). All multivariate statistical analyses were performed using PRIMER 6 software (PRIMER-E; Clarke 1993). Within the pulse-chase experiments, the relationship between the biomass (dry weight) of an individual *Linopherus* sp. nov. and its uptake of labeled tracer during the experiments was examined using simple linear correlation analyses ($\alpha = 0.05$). All univariate statistical tests were carried out using SPSS Statistics 20.

RESULTS

Oxygen and organic matter gradients

Detailed descriptions of the environmental conditions at the PM can be found in Brand & Griffiths (2009), Breuer et al. (2009), Cowie et al. (2009), Jeffreys et al. (2009a), Levin et al. (2009b) and Woulds & Cowie (2009). Briefly, DO concentrations based on CTD casts, increased gradually from low values of ~5–6 μM at 700 m to ~7–9 μM at 1050 m (Table 1). *In situ* lander measurements of DO (mm above the sediment–water interface) increased from <0.01 μM at 850 m to 9.3 μM at 1000 m. At 950 m, *in situ* DO increased from 1.7 μM during the IMP to 2.8 μM during the LMP.

Organic carbon (%C_{org}), used here as a proxy for the concentration of OM in surficial sediments (0 to 0.5 cm), increased from ~2.5 % at 700 m to ~3.6 % at 950 m and then decreased to ~3.1 % at 1050 m. Total

nitrogen concentrations mirrored those of organic carbon (Table 1). C:N ratios ranged from 9.2 to 9.9 and exhibited no obvious trend with water depth. Grain size was not correlated with %C_{org} and displayed no consistent trend across the lower OMZ boundary (Table 1).

Sedimentary $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values ranged from –21.1 to –20.1 ‰ and from 6.8 to 8.0 ‰, respectively (Table 2). Stable isotopic signatures of bacteria ranged from –27.8 to –16.0 ‰ and from –0.4 to 7.2 ‰ for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively (Table 2). Isotopic signatures of food sources, including bacteria, POM and sediment were clearly differentiated (Table 2). Mean isotopic values of surficial sediments at 950 m displayed no significant difference between seasons. $\delta^{13}\text{C}$ values of surficial sediments were isotopically lighter at shallower depths, 700 to 900 m, compared with depths of 950 to 1100 m (Table 2). In contrast, $\delta^{15}\text{N}$ values of surficial sediments showed no consistent pattern with water depth across the lower OMZ (Cowie et al. 2009).

Table 2. Stable carbon and nitrogen isotopic values (‰) of food sources at the Pakistan margin lower oxygen minimum zone. POM: particulate organic matter collected from bottom waters; nd: not determined

Depth (m)	Sediments		—POM—		Bacteria	
	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
700	–20.7	7.4	nd	nd	nd	nd
750	–20.6	6.8	nd	nd	nd	nd
800	–21.1	6.8	nd	nd	–25.5 to –27.8	–0.4 to 7.2
850	–20.7	7.3	nd	nd	–17.6	1.3
900	–20.8	7.1	nd	nd	nd	nd
950	–20.5	7.9	–23.2	8.4	–16.0	2.0
1000	–20.4	7.3	nd	nd	–25.1	6.5
1050	–20.5	7.0	nd	nd	nd	nd
1100	–20.1	8.0	nd	nd	nd	nd

Fig. 3. *Linopherus* sp. nov. (A) Density, (B) length, (C) individual biomass size and (D) approximate respiratory area (BP: branchial pairs), as a function of water depth within the lower oxygen minimum zone transition zone. Error bars represent ± 1 SD. (A) Averages for each depth (seasonal data have been pooled); $n = 3$ at 700, 850 and 900 m, 1 at 750 m, 2 at 800 and 1050 m, 14 at 950 m and 4 at 1000 m. (B,C) Averages for each depth (seasonal data have been pooled); $n = 7, 35, 11$ and 157 at 800, 850, 900 and 950 m, respectively. (D) Averages for each depth during each season. ●: inter-monsoon period (IMP); ○: late monsoon period (LMP); $n = 6, 22, 10$ and 157 at 800, 850, 900 and 950 m, respectively, during the IMP, and $n = 6$ and 51 at 850 and 950 m, respectively, during the LMP

Phytopigments, biomarkers for fresh OM, were more abundant at lower oxygen concentrations. Similar concentrations were observed both within the OMZ core and at the lower OMZ boundary (Woulds & Cowie 2009). However, no obvious trend

in pigment concentrations was observed within the lower OMZ transition zone (Table 1).

Lipid distributions and biomarkers in surficial sediments at 950 m displayed evidence of temporal variation in relation to the POM flux (Jeffreys et al. 2009a). No clear trend was observed in lipid distributions of the sediments across the PM (140 to 1850 m) except at ~1200 m (the edge of the lower OMZ boundary), where the lowest concentrations of labile lipids were detected.

Linopherus sp. nov. density, biomass and body size

The dominant species at the lower OMZ is an undescribed polychaete species within the family Amphinomidae and the genus *Linopherus* (Fig. 2). *Linopherus* sp. nov. was present from 800 to 1000 m and was not found in samples collected above or below these depths. Peaks in densities were observed at 850 m, ranging from 2348 to 5664 ind. m⁻² (Fig. 3A, O₂: 5.4 to 6.1 μ M). Densities were significantly higher at 850 m compared with those at 900, 950 and 1000 m (both seasons combined, Kruskal-Wallis test: $p = 0.003$). *Linopherus* sp. nov. accounted for 100% of the macrofauna at 800 m, ~84% at 850 m, ~44% at 900 m, ~18% at 950 m and only 2% at 1000 m. For macrofaunal species occurring alongside *Linopherus* sp. nov. and community diversity metrics, see Tables S1 & S2 in Supplement 1 at www.int-res.com/articles/suppl/m470p079_supp.pdf, which have been adapted from Levin et al. (2009b). Diversity and evenness of the macrofaunal community was lowest at depths where *Linopherus* sp. nov. was the dominant species, e.g. 800 and 850 m (Levin et al. 2009b; Table S2). A seasonal comparison of *Linopherus* sp. nov. density was only possible at 950 m, where no significant difference in density was observed (Mann-Whitney U -test: $p = 0.062$). Spatial distribution patterns were similar during the IMP and LMP across the lower OMZ boundary.

Individuals were found from 0 to 10 cm in the sediment cores; vertical distribution varied with water depth. Individuals were concentrated near the sediment–water interface at shallower depths and worms were found deeper in the sediment with increasing water depth and oxygen concentration of overlying waters. At 800 m, 100% of *Linopherus* sp. nov. were found in the top 1 cm of the sediment. At 850 m, 82 and 18% of individuals were found in the upper 2 cm and at 3 to 5 cm of the sediment, respectively. In contrast, at 950 m, 30% of individuals were

found in the upper 2 cm and 70 % at 3 to 5 cm within the sediment.

Linopherus sp. nov. are relatively large worms for the deep sea. Specimens collected in this study had 7 to 31 chaetigers and wet weights of 0.7 to 45 mg (Fig. 3B,C). No significant temporal variation was found in the number of chaetigers (Mann-Whitney *U*-test: $p = 0.282$ and $p = 0.611$ for 850 and 950 m, respectively). The species appears to exhibit allometric size structuring with depth and oxygen concentration (Fig. 3B,C). Combining data from both seasons, *Linopherus* sp. nov. had significantly fewer chaetigers at shallower depths (800 and 850 m compared with 900 and 950 m, Kruskal-Wallis test: $p < 0.0001$; Fig. 3B). Approximately 60 % of polychaetes at 850 m had <15 chaetigers compared with ~30 % of polychaetes at 900 and 950 m.

The biomass of *Linopherus* sp. nov. did not vary temporally at either 850 m ($F_{1,20} = 1.925$, $p = 0.181$) or 950 m (Mann-Whitney *U*-test: $p = 0.076$). Significant spatial variation in the biomass of *Linopherus* sp. nov. was evident; worms were lighter at shallower depths (Kruskal-Wallis test: $p = 0.019$; mean wet weights were 12.6 mg at 800 m, 16.3 mg at 850 m, 21.6 mg at 900 m and 22.6 mg at 950 m; Fig. 3C).

Larger worms (based on the number of chaetigers) were found deeper within the sediment (below 2 cm), at the shallower sites (seasonal data and 800 and 850 m combined, Kruskal-Wallis test: $p = 0.052$). The mean number of chaetigers was 16.6 for worms at 1.5 cm and 22.0 at 3.5 cm within the sediment column. No significant trend in *Linopherus* sp. nov. biomass was observed downcore ($F_{2,25} = 1.059$, $p = 0.362$ for 800 and 850 m combined and $F_{3,58} = 1.975$, $p = 0.128$ for 900 and 950 m combined).

Branchial morphology

Linopherus sp. nov. possesses numerous dendritic branchial tufts starting at chaetiger 3 or 4 (Fig. 2A,B). Each branchia has multiple rami on its dorsal surface (Fig. 2C). The number of branchial pairs ranged from 3 to 14. Approximate respiratory area (the number of branchial pairs [BP] normalized to wet weight in milligrams, BP mg⁻¹) was estimated for each individual and ranged from 0.24 to 7.14 BP mg⁻¹ (Fig. 3D).

No temporal variation in approximate respiratory area was observed in worms at 850 m (Mann-Whitney *U*-test: $p = 0.121$). At 950 m, approximate respiratory area was significantly higher during the IMP at 0.67 BP mg⁻¹ compared with 0.49 BP mg⁻¹ during the LMP (Mann-Whitney *U*-test: $p = 0.003$). During the

IMP, approximate respiratory area was highest at 850 m followed by 800, 900 and 950 m (Kruskal-Wallis test: $p = 0.017$; Fig. 3D). There was no significant difference in approximate respiratory area between 850 and 950 m during the LMP (Mann-Whitney *U*-test: $p = 0.327$). Approximate respiratory area did not vary with living depth in the sediment (Kruskal-Wallis test: $p > 0.1$ at both 850 and 950 m during both seasons).

Bioturbation and lifestyle

Linopherus sp. nov. constructs distinctive, semi-permanent burrows (Fig. 4). Burrows were rare (<30 burrows 10 cm⁻²) but visible from 700 to 800 m, where uniform, unbroken laminations were the dominant sedimentary feature. Burrow counts increased from ~110 burrows 10 cm⁻² at 800 m to ~162 burrows 10 cm⁻² at 850 m, where densities of *Linopherus* sp. nov. were highest. At these depths sediments were criss-crossed by a network of burrows up to 1 cm long and 0.5 to 2 mm wide, and sediments were bioturbated to a depth of ~8 cm (Fig. 4). However, laminations were still visible, suggesting that bioturbation is limited to movement of materials in and out of burrows. Between depths of 700 and 1050 m, burrow counts and the density of *Linopherus* sp. nov. were positively correlated (Spearman's rank correlation coefficient [r_s] = 0.812, $p = 0.05$; Levin et al. 2009b). While it is not possible to be sure that all burrows visible in X-radiographs can be ascribed to *Linopherus* sp. nov., it is likely that most large visible burrows between 800 and 1000 m water depth were made by this species.

Behavioural observations of *Linopherus* sp. nov. were made from black and white video recordings from benthic chamber lander *in situ* deployments at 850 m (see Video Recording V1 in Supplement 2 at www.int-res.com/articles/suppl/m470p079_supp/). The video recordings reveal an interface-dwelling mode, with amphinomid worms positioned half in and half out of the sediment and moving from side to side. The amphinomid worms seen moving within the water column in the video recordings may have been disturbed by the magnetic stirrer used to mix the chamber water during the experiment.

Trophic ecology and organic matter processing

Collectively the $\delta^{13}\text{C}$ values of *Linopherus* sp. nov. ranged from -8.3 to -19.2‰, falling within the ranges of possible food sources, i.e. sediments and

Fig. 4. X-radiographs of Pakistan margin sediments (as shown in Levin et al. 2009b) taken in May 2003, except at 950 m, which is from October 2003. All of the slabs shown measure $6.9 \times 0.9 \times 14.5$ cm, except that from 900 m, which measures $8 \times 1.2 \times 24$ cm

bacteria (Fig. 5A). Values for $\delta^{15}\text{N}$ ranged from 9.7 to 14.5‰ and were enriched by 2.0 to 7.6‰ compared with surficial sediments and bacteria (Fig. 5A). Depth-integrated stable isotopic signatures of *Linopherus* sp. nov. were not significantly different both across the lower OMZ and within the sediment. Nitrogen isotopic values of *Linopherus* sp. nov. at 950 m were significantly depleted in ^{15}N during the IMP in comparison with the LMP (11.3 and 13.4‰ for the IMP and LMP, respectively; Mann-Whitney *U*-test: $p = 0.007$). Allometric differences in stable isotopic compositions were also detected, with smaller individuals having more depleted $\delta^{15}\text{N}$ signatures (up to 2‰) compared with larger worms (Fig. 5B).

Lipid contents of *Linopherus* sp. nov. ranged from ~ 159 to $2578 \mu\text{g g}^{-1}$ relative to dry tissue weight and generally increased with increasing water depth. Both sterols and fatty acids (FAs) were dominant in terms of lipid class (Fig. 6A,B). Saturated FAs (SFAs) ranged from C_{14} to C_{24} , the dominant homologues being C_{16} and C_{18} . Branched FAs were minor constituents of the total lipids (<2%) and were represented by both *iso*- and *anteiso*-FAs, ranging from C_{15} to C_{19} . Monounsaturated FAs (MUFAs) ranged from $\text{C}_{16:1}$ to $\text{C}_{24:1}$ with $\text{C}_{16:1(\text{n-9})}$, $\text{C}_{18:1(\text{n-9})}$ and $\text{C}_{20:1(\text{n-11})}$ being the dominant homologues. Polyunsaturated FAs (PUFAs) ranged from $\text{C}_{18:2}$ to $\text{C}_{23:2}$, and distributions were dominated by $\text{C}_{20:4(\text{n-6})}$, $\text{C}_{20:5(\text{n-3})}$ and

Fig. 5. (A) Dual isotope plot depicting average stable $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotope values of *Linopherus* sp. nov. and its food sources. POM: particulate organic matter; SOM: sedimentary organic matter; IMP and LMP: inter- and late monsoon periods, respectively. (B) $\delta^{15}\text{N}$ isotope plot of large (>15 chaetigers, $n = 12$) and small (<15 chaetigers, $n = 2$) *Linopherus* sp. nov. from 850 and 950 m during the LMP. Error bars represent ± 1 SD

$\text{C}_{22:6(n-3)}$. SFAs, MUFAs and PUFAs were present in roughly equal proportions (Fig. 6B). Sterol distributions were dominated by cholesterol ($\text{C}_{27}\Delta^5$) and ranged in carbon number from C_{26} to C_{29} ; they were present in both saturated and unsaturated forms. The most abundant sterols following cholesterol were $\text{C}_{27}\Delta^{5,22}$ and the brassicasterol ($\text{C}_{28}\Delta^{5,22}$). Alcohols accounted for <2 % of the total lipid and were present in both saturated and unsaturated forms, ranging from C_{14} to C_{20} . The $\text{C}_{16:0}$ and $\text{C}_{18:0}$ α -hydroxy acids were present in worms at 900 and 950 m in minor amounts (<1 % total lipid).

FAs and sterols of *Linopherus* sp. nov. displayed clustering by depth, except for 900 m (as shown by 3-dimensional scaling, Fig. 6C,D). The effect of depth on both the sterol and FA concentrations of *Linopherus* sp. nov. was weak (ANOSIM: $R = 0.327$, $p = 0.031$ and $R = 0.361$, $p = 0.033$ for sterols and FAs, respectively). Differences in sterol distributions were found between 800 and 850 m, and between 950 m and the 2 shallower depths. These differences were driven by the phytosterols $\text{C}_{28}\Delta^{5,22}$, $\text{C}_{29}\Delta^5$ and $\text{C}_{28}\Delta^5$ and the invertebrate sterols $\text{C}_{27}\Delta^5$ and $\text{C}_{27}\Delta^{5,22}$, these being most abundant in worms at 950 m and more abundant in worms at 800 m compared with 850 m (SIMPER analysis, Table S3 in Supplement 1). Differ-

ences in FA distributions were found between 800 and 850 m and between 950 m and the 2 shallower depths. Differences between 800 and 850 m were driven by the FAs $\text{C}_{22:6(n-3)}$, $\text{C}_{20:5(n-3)}$, $\text{C}_{22:5(n-3)}$ and $\text{C}_{16:1(n-7)}$, which were more abundant in worms at 850 m (SIMPER analysis, Table S4 in Supplement 1). Differences between 950 m and the 2 shallower sites were accounted for by the FAs C_{16} , $\text{C}_{16:1(n-7)}$, $\text{C}_{20:1(n-9)}$, $\text{C}_{20:1(n-11)}$, $\text{C}_{18:1(n-9)}$ and $\text{C}_{22:6(n-3)}$; worms at 850 m had higher abundances of these FAs compared with those at 950 m. Worms at 800 m had higher abundances of the C_{16} FA compared with those at 950 m, which in turn had higher abundances of zooplankton and invertebrate FAs, e.g. $\text{C}_{20:1}$ and $\text{C}_{18:1(n-9)}$ (SIMPER analysis, Table S3).

During the ^{13}C pulse-chase experiments of Woulds et al. (2007), *Linopherus* sp. nov. was observed to rapidly ingest large amounts of labeled algae. This was evident in 2 ways: (1) by examination of individuals under the microscope, *Linopherus* sp. nov. was observed with guts full of green labeled phytodetritus, and (2) through their uptake of labeled phytodetritus in the control experiments (T0), which were ended within 2 h of the addition of the labeled algae. In Expt T0 at 950 m during the LMP, the population of *Linopherus* sp. nov. within a single core (78 cm^2)

Fig. 6. *Linopherus* sp. nov. lipid distributions — (A) $\mu\text{g g}^{-1}$ tissue and (B) as a percentage relative to total lipid — as a function of depth. Compound classes are: saturated fatty acids (SFAs), branched fatty acids (BrFAs), monounsaturated fatty acids (MUFAs), polyunsaturated fatty acids (PUFAs), sterols and alcohols. Error bars represent mean $\pm$ 1 SD, $n = 3$. (C) 3D scaling ordination of the sterol distribution of *Linopherus* sp. nov. (D) 3D scaling ordination of the fatty acid distribution of *Linopherus* sp. nov.

ingested $\sim 5\%$ (240 μg of algal C) of the added label within 2 h. In the longer experiments ($t = 2$ to 5 d), uptake of algal C by *Linopherus* sp. nov. ranged from 400 to 626 μg and accounted for between 36 and 85 % of total macrofaunal uptake. In the 5 d *in vitro* experiments at 950 m, uptake of labeled carbon by *Linopherus* sp. nov. was higher during the IMP compared with the LMP (64 and 36 % of total macrofauna uptake during the IMP and LMP, respectively). The proportion of label taken up by *Linopherus* sp. nov. during the 2 d *in vitro* experiment at 850 m and the 5 d *in situ* experiment at 950 m was similar at $\sim 85\%$ of the total macrofaunal uptake (Fig. 7A). In the ship-board experiments (at 850 and 950 m and during both seasons), *Linopherus* sp. nov. found deeper in the sediment (at 2 to 3 cm) ingested more labeled

algae than those found at the surface. Conversely, during the *in situ* experiment at 950 m, *Linopherus* sp. nov. found at the surface ingested greater amounts of label than those found deeper in the sediment (Fig. 7B). Positive correlations were observed at 950 m between *Linopherus* sp. nov. biomass (dry weight) and *Linopherus* sp. nov. uptake of label during both the IMP and LMP (Table 3). No correlation was apparent in the experiments at 850 m.

Total resource use of ^{13}C -labeled algae by macrofauna across the PM was low at 140, 1200 and 1850 m (Table 4). This coincides with the high species diversity at 140 and 1850 m (Table 4). At sites where *Linopherus* sp. nov. was dominant and species diversity was low (i.e. 850 and 950 m), total resource use was high (Table 4).

Fig. 7. *Linopherus* sp. nov. (A) Percentage of added C recovered from *Linopherus* sp. nov. in the ^{13}C pulse-chase experiments (shipboard and *in situ*) compared with that recovered from its nearest competitor in each case, and from the total metazoan macrofaunal population. 2 d and 5 d indicate the duration of shipboard experiments. (B) Uptake of ^{13}C by *Linopherus* sp. nov. across different sediment horizons from pulse-chase experiments at 950 m. *In vitro* experiments are shipboard incubations. Adapted from Woulds et al. (2007).

IMP: inter-monsoon period; LMP: late monsoon period

Interaction with oxygen and organic matter

The density of *Linopherus* sp. nov. across the lower OMZ boundary was not correlated with any of the measured environmental parameters (Table 5). Individual biomass of *Linopherus* sp. nov. was correlated with both oxygen and organic carbon across the lower OMZ boundary ($r_s = 1.0$, $p < 0.01$ for both oxygen and organic carbon; Table 5). During the IMP, respiratory area of *Linopherus* sp. nov. was significantly negatively correlated with oxygen across the lower OMZ boundary ($r_s = -0.250$, $p = 0.05$; Table 5). At 950 m the decrease in respiratory area of *Linopherus* sp. nov. from the IMP to the LMP was negatively correlated with oxygen concentration ($r_s = -0.249$, $p = 0.03$).

DISCUSSION

Oxygen thresholds and controls on the population structure of *Linopherus* sp. nov.

DO appears to exert threshold effects on the distribution of *Linopherus* sp. nov. across the PM, confining this species to the lower OMZ boundary. Based on the *in situ* Profiler lander deployments, we suggest that *Linopherus* sp. nov. has a lower oxygen threshold of $\leq 1 \mu\text{M}$ (Tables 1 & 2). We suggest that *Linopherus* sp. nov. was not present at depths of 700 and 750 m as oxygen concentrations fell below the lower oxygen threshold of this polychaete. Although there were no *in situ* DO measurements at 700 and 750 m to support this, we base our suggestion on the observations of Breuer et al. (2009), who reported no oxygen at 300 m, and on the lander observations at 850 m (present study), in which oxygen fell below the limits of detection ($0.01 \mu\text{M}$).

Linopherus sp. nov. was not present at depths below 1000 m where *in situ* oxygen concentrations were

Table 3. Correlations (r^2) with p-values between ^{13}C uptake and individual biomass of *Linopherus* sp. nov. during pulse-chase experiments. Italics denote a Spearman's rank correlation. Significant ($p < 0.05$) results are marked with an asterisk. IMP: inter-monsoon period; LMP: late monsoon period

Parameter	IMP				LMP			
	850 m, shipboard		950 m, shipboard		950 m, shipboard		950 m, <i>in situ</i>	
	r^2	p	r^2	p	r^2	p	r^2	p
Weight (mg)	3.87	2.69	2.08	1.33	4.20	1.39	4.74	1.99
^{13}C uptake (μg)	1.34	0.19	15.32	10.25	20.24	9.25	16.85	6.90
Correlation	0.52	0.15	0.73	0.04*	0.88	0.0022*	0.72	0.0055*
n	9		8		6		13	

Table 4. Mean ($\pm$ SD) percentage ^{13}C uptake by the macrofaunal community from 2 d pulse-chase experiments. Values in bold are the proportion (%) of macrofaunal uptake accounted for by *Linopherus* sp. nov. Data are taken from Woulds et al. (2007). Total number of macrofaunal species (S) and total macrofauna biomass (ww: wet weight) are taken from Hughes et al. (2009) and Levin et al. (2009b). Values in bold represent the total biomass of *Linopherus* sp. nov. IMP: inter-monsoon period; LMP: late monsoon period; nd: not determined

Depth (m)	^{13}C uptake by macrofauna (%)		Total number of macrofaunal species (S)		Total macrofaunal biomass (g ww m $^{-2}$)	
	IMP	LMP	LMP	LMP	IMP	LMP
140	17 $\pm$ 2	7 $\pm$ 5	51 $\pm$ 5	45 $\pm$ 3	6.8 $\pm$ 3.5	4.3 $\pm$ 2.0
850	42 (85)	nd	3	2	54.7 (45)	43.3 (30.2)
950	46 $\pm$ 2 (64)	47 $\pm$ 8 (36)	12 $\pm$ 1	13 $\pm$ 1	66.5 $\pm$ 4.5 (22.7)	25.0 $\pm$ 19.8 (16.8)
1000	57 $\pm$ 2	nd	4	12	52.4 (0)	91.8 (0.06)
1200	1 $\pm$ 0.5 ^a	nd	13 $\pm$ 2.6	nd	0.4 $\pm$ 0.8	5.3 $\pm$ 8.3
1850	4 $\pm$ 1	2 $\pm$ 1	53 $\pm$ 6	44 $\pm$ 4	9.3 $\pm$ 14.4	1.6 $\pm$ 0.8

^aResults from a 5 d experiment

Table 5. Spearman's rank correlations (r_s) with p-values in parentheses between environmental parameters and the density, individual biomass and respiratory area (BP: branchial pairs) of *Linopherus* sp. nov. Significant ($p < 0.05$) results are marked with an asterisk

Parameter	Density (ind. m $^{-2}$)	Individual biomass (mg ww)	Respiratory area (BP mg $^{-1}$)
DO (μM)	0.146 (0.729)	1.000 (<0.01)*	-0.250 (0.05)*
%C _{org}	0.610 (0.108)	1.000 (<0.01)*	-0.800 (0.200)
% TN	0.610 (0.115)	0.738 (0.262)	-0.800 (0.200)
Phytopigments (mg g $^{-1}$)	-0.580 (0.228)	0.632 (0.368)	0.316 (0.684)
Grain size (% Clay)	0.111 (0.812)	0.400 (0.600)	-0.800 (0.200)

$\geq 9 \mu\text{M}$. We suggest that *Linopherus* sp. nov. was out-competed at depths of >1000 m as the increase in DO at these depths coincided with oxygen thresholds of other species (Levin et al. 2009b) including megafaunal predators (Murty et al. 2009). *Linopherus* sp. nov. was most abundant at depths of 800 to 850 m (CTD DO = 5.4 to 6.25 μM , Profilur DO $\leq 0.01 \mu\text{M}$; Fig. 3a), where its densities peaked and it dominated the macrofaunal community (Levin et al. 2009b). Surprisingly DO and population density were not significantly correlated. At the Oman and western Indian margins, at similar DO concentrations, *Linopherus* sp. nov. was absent and spionid or cirratulid polychaetes dominated the macrofaunal communities (Levin et al. 2000, Ingole et al. 2010, Hunter et al. 2012).

Linopherus sp. nov. is an unusually large species for the deep sea. However, similarly large polychaetes are found within the food-rich, albeit shallower, Chilean OMZ (González & Quiñones 2000). González & Quiñones (2000) showed that polychaetes inhabiting hypoxic/anoxic environments have more than 1 pyruvate oxidoreductase enzyme, which is utilized in anaerobic metabolism. Furthermore those authors

demonstrated a positive allometric relationship with anaerobic metabolism (utilizing pyruvate oxidoreductase enzymes) in polychaetes inhabiting hypoxic sediments at the Chilean margin. We suggest that the large body size of *Linopherus* sp. nov. in the present study could be related to its capacity for anaerobic metabolism using pyruvate oxidoreductases.

Linopherus sp. nov. displayed evidence of population size structuring within the lower OMZ (Fig. 3B,C). DO and the biomass of *Linopherus* sp.

nov. were positively correlated across the lower OMZ, where smaller individuals tolerated lower DO concentrations. Approximately 60% of *Linopherus* sp. nov. at 850 m had fewer than 15 chaetigers. These may be juveniles or reflect stunted growth. *Lucicutia* sp. copepods in the Arabian Sea display ontogenetic zonation across the OMZ, where young occupy low DO waters (Wishner et al. 2000).

Adaptation to low DO conditions

Smaller-bodied organisms tend to be prevalent in OMZs as they have a greater surface area for oxygen diffusion (Levin 2003, Gooday et al. 2009). Whilst *Linopherus* sp. nov. are relatively large worms, morphological observations of the branchial tufts of *Linopherus* sp. nov. indicate that this species has greatly enlarged respiratory structures. The branchial tufts consisted of numerous branchial pairs (up to 14) with circular branching rami (Fig. 2). Similar respiratory adaptations have been observed in spionid polychaetes from the Oman margin (Lamont & Gage

2000), which had either numerous pairs of branchiae (up to 12) or 3 pairs of branchiae, each bearing numerous plate-like pinnules (Lamont & Gage 2000).

Where DO was lowest on the Oman margin, larger branchial surface areas in proportion to body size were noted in spionid polychaetes (Lamont & Gage 2000). In the present study, *Linopherus* sp. nov. shows a similar adaptation, having larger approximate respiratory areas concurrent with lower DO (Fig. 3D). *Linopherus* sp. nov. apparently enhances oxygen diffusion by increasing its body area/mass ratio, specifically at depths where DO concentrations are lowest. Furthermore, the decrease in *Linopherus* sp. nov. respiratory area during the LMP demonstrates that *Linopherus* sp. nov. may respond quickly to temporal shifts in DO.

Behavioural adaptations to low DO were also noted. *In situ* benthic lander observations revealed that the interface-dwelling mode of *Linopherus* sp. nov. involved this species moving its body from side to side. These movements may increase oxygen exposure across its branchiae.

Organic matter and potential food sources at the OMZ boundary

Relatively high %C_{org} concentrations of sediments across the lower OMZ boundary (Table 1) suggest an abundance of OM/food for *Linopherus* sp. nov. However, C:N ratios and lipid biomarkers reflected a degraded OM source, suggesting that this material is low quality OM. Both phytopigment and lipid distributions across the PM as a whole appear to be heavily influenced by faunal feeding processes (Jeffreys et al. 2009a, Woulds & Cowie 2009). Temporal variation in food availability is evident from lipid distributions and appears to be related to the POM flux (Jeffreys et al. 2009a).

Stable isotopic values for sediments and POM were consistent with material derived from phytoplankton and displayed evidence of water-column denitrification (Fig. 5A; Fry & Sherr 1984, Gaye-Haake et al. 2005). Bacterial isotope values suggest fixation of carbon and nitrogen by oxidation of sulphide and by denitrification (Fig. 5a; Brooks et al. 1987, Robinson & Cavanaugh 1995, Campbell et al. 2003).

Interaction of *Linopherus* sp. nov. with organic matter

The relatively large size of *Linopherus* sp. nov., especially for an OMZ species, suggests it has an

abundant food supply. Food supply (%C_{org}) was strongly and positively correlated with biomass across the lower OMZ. Concomitantly, at 950 m *Linopherus* sp. nov. biomass and density showed small increases temporally alongside increases in C_{org}. These results suggest that *Linopherus* sp. nov. can take advantage of the monsoon-associated food supply to meet its energetic requirements for somatic growth.

OM quality and quantity is influenced by faunal activity (Smallwood et al. 1999, Ginger et al. 2001, Woulds et al. 2007). The relatively degraded food source observed at the PM compared with other OMZ margins results from greater mineral input via the Indus river, heterotrophic reworking of OM in the upper water column and selective feeding and uptake of OM by the benthic fauna (Jeffreys et al. 2009a, Levin et al. 2009b).

Visual evidence from X-radiographs suggests that *Linopherus* sp. nov. does not greatly impact sediment OM processing via bioturbation, as intact laminations are visible at sites where *Linopherus* sp. nov. is present (Fig. 4). However, pulse-chase experiments reveal that *Linopherus* sp. nov. is able to rapidly (<2 h) ingest/remove phytodetritus from the sediment surface in a similar way to foraminifera within the OMZ core (Larkin 2006, Woulds et al. 2007) and pennatulids at the lower OMZ boundary (Jeffreys et al. 2009a). *Linopherus* sp. nov. dominated the uptake of labeled carbon in experiments at the lower OMZ transition zone (Fig. 7, Table 4), suggesting that this species has a significant impact on the processing and the quality and quantity of OM at the sediment surface at the PM.

Nutritional strategies of *Linopherus* sp. nov.

Video observations and X-radiographs demonstrate that *Linopherus* sp. nov. is not a classic infaunal deposit feeder, as sediment laminations are still present and it is seen freely moving in and out of its burrows (Fig. 4, see Video Recording V1 in Supplement 2). Instead *Linopherus* sp. nov. is an opportunistic feeder capable of feeding on fresh phytodetritus, as evident from the pulse-chase experiments and visual observations of gut contents. Lipid biomarkers support this, with fatty acids (C_{20:5(n-3)}, C_{22:6(n-3)}) and sterols (C₂₈Δ^{5,22}, C₂₉Δ^{5,22}) being derived from phytodetritus (>15% total lipids) present in its tissues. The Amphinomidae have been classified previously as predators on hard substratum and carrion feeders in sandy/muddy environments (Fauchald & Jumars

1979). Our observations of *Linopherus* sp. nov. suggest this family has a much broader diet.

The wide range in stable isotope values indicates that *Linopherus* sp. nov. has an omnivorous trophic strategy (Fig. 5a). Assuming a trophic fractionation of 1.0 and 3.4 for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively (Post 2002, McCutchan et al. 2003), stable isotope values for *Linopherus* sp. nov. were 1 to 2 trophic steps removed from the sediments, suggesting both detritivorous and predatory aspects to their diet. The $\text{C}_{18:1(n-9)}:\text{C}_{18:1(n-7)}$ ratio, indicative of the degree of carnivory (Graeve et al. 1997), supports this with a mean value of ~ 3.4 . Cholesterol was the dominant sterol ($>50\%$ of the total sterols) present in *Linopherus* sp. nov. and is also an indicator of carnivory (Nelson et al. 2001), although polychaetes are able to synthesize sterols de novo (Wootton & Wright 1962, Walton & Pennock 1972). The second most abundant sterols included both isomers of the invertebrate biomarkers $\text{C}_{27}\Delta^{5,22}$, which may be biosynthesized by *Linopherus* sp. nov.

The proportion of PUFAs in the tissues of *Linopherus* sp. nov. was similar to those reported for abyssal polychaetes in the Pacific Ocean (Drazen et al. 2008), suggesting a link to the euphotic zone production and ingestion of phytodetritus, as most marine animals cannot synthesize long-chain PUFAs de novo (Pond et al. 2002).

Long-chain MUFAs ($\text{C}_{20:1}$ and $\text{C}_{22:1}$), known to be indicative of calanoid copepods (Dalsgaard et al. 2003), were present in *Linopherus* sp. nov. at the PM and may reflect consumption of copepod remains or faecal pellets associated with the vertical flux, rather than direct ingestion of copepods. Crustacea (i.e. amphipods) were only present from 900 m, where DO was $>5.8\ \mu\text{M}$ (Levin et al. 2009b), so assimilation of these MUFAs from direct ingestion of benthic crustacea seems unlikely.

Bacteria may be another food source for *Linopherus* sp. nov., and large filamentous sulphur bacteria were observed at the lower OMZ transition zone and within the burrows of *Linopherus* sp. nov. A single specimen from 1000 m had an isotopically heavy $\delta^{13}\text{C}$ signature (-8.3‰ , Fig. 5A), which may be indicative of ingestion of bacteria utilizing the rTCA cycle. Bacterial FAs, including $\text{C}_{18:1(n-7)}$, odd-numbered saturated and branched FAs, represented $\sim 10\%$ of the FAs in *Linopherus* sp. nov. tissues, suggesting a bacterial component to the diet. Visual observations and biochemical and experimental data all suggest that *Linopherus* sp. nov. is an indiscriminant opportunistic omnivore that takes advantage of any available food source, although phytodetritus is likely to be its most common food source.

Seasonal and allometric differences were observed in the stable isotopic nitrogen composition of *Linopherus* sp. nov., smaller worms being isotopically lighter in $\delta^{15}\text{N}$ than their larger counterparts at the same sites (Fig. 5B). Similar trends have been observed in shallow-water ampharetid and spionid polychaetes and ascribed to juveniles feeding on benthic diatoms, whilst adults fed on macroalgal detritus (Hentschel 1998). At 950 m at the PM, nitrogen isotope values of *Linopherus* sp. nov. became isotopically lighter during the LMP and densities increased at this site after the SW monsoon, perhaps reflecting a recruitment event.

Lipid distributions varied with depth and worm size. Worms were smaller at the shallower sites (800 and 850 m) and had relatively more abundant phytodetrital biomarkers, e.g. $\text{C}_{22:6(n-3)}$ and $\text{C}_{16:1(n-7)}$, than did larger worms found deeper (900 and 950 m), which had greater proportions of invertebrate biomarkers, e.g. $\text{C}_{20:1(n-11)}$ and $\text{C}_{27}\Delta^5$. Ontogenetic differences in lipid composition have been noted previously for calanoid copepods (Svetlichny et al. 2006, Laakmann et al. 2009), pteropods (Boer et al. 2005), euphasiids (Kattner & Hagen 1998) and an alvinocaridid shrimp (Stevens et al. 2008). In the Arctic, calanoid copepods exhibit differences in trophic strategy, as inferred from lipid distributions, in order to minimize competition (Laakmann et al. 2009). In the Black Sea, vertical migrations of copepods into the OMZ have been linked to the formation of lipid reserves for reproduction (Svetlichny et al. 2000, 2006).

The strategy of *Linopherus* sp. nov. inferred from our data set is that smaller individuals, possibly juveniles occupying sites with the lowest DO concentrations, may (1) obtain refuge from predators, (2) minimize competition for food resources, (3) take advantage of the more abundant phytodetritus and (4) build up lipid reserves. We deduce that these smaller worms/juveniles are primarily reliant on phytodetritus whereas adults are more opportunistic and utilize scavenging as a nutritional strategy alongside feeding on phytodetritus when available.

Effect of *Linopherus* sp. nov. on ecosystem responses to gradients in oxygen and organic matter

Understanding ecosystem processes within OMZs is critical as hypoxia alters both the structure and function of benthic ecosystems, leading to loss of biodiversity and elevated dominance (Levin et al. 2009a). Such shifts in community structure can lead

to changes in trophic structure, energy flow pathways, nutrient cycling, bioirrigation and OM cycling and burial (Cardinale et al. 2006, Canuel et al. 2007, Solan et al. 2008, Middelburg & Levin 2009). The PM OMZ is particularly suited for studying the effects of biodiversity on ecosystem functioning (here defined as OM processing) as it has high faunal diversity above and below the OMZ (Hughes et al. 2009), and communities are dominated by a single species at the lower boundary (Levin et al. 2009b).

Previous studies on biodiversity and ecosystem functioning have artificially manipulated biodiversity through removal of species and have concentrated on mixtures of a few species, making extrapolations to the field difficult (Loreau et al. 2001). More recently, Karlson et al. (2010) were able to mimic field conditions in the species-poor Baltic Sea in order to understand the effect of biodiversity on OM processing, using pulse-chase experiments. Those authors concluded that ecosystems with higher species diversity (maximum number of species = 3) incorporated more carbon and nitrogen, displayed more efficient trophic transfer of phytodetritus and were more productive than monocultures. The importance of single species and species-specific traits on ecosystem functioning has also been demonstrated (Solan et al. 2004, Norling et al. 2007, Godbold et al. 2009b). Hence, we now focus on *Linopherus* sp. nov. as a possible model for understanding small-scale ecosystem responses to gradients in OM and DO.

Linopherus sp. nov. is a low oxygen specialist (Levin et al. 2009b). As a result it is the only metazoan found at 800 m and dominates the macrofaunal community at 850 m, where it occurs alongside the spionid polychaete *Prionospio*. Associated with this dominance are low levels of diversity (Levin et al. 2009b). As DO increases, *Linopherus* sp. nov. becomes less dominant and macrofaunal diversity increases; *Linopherus* sp. nov. is absent at DO > 7.8 μM (CTD values). This DO-driven dominance has implications for the quality, quantity and processing of OM. *Linopherus* sp. nov. is an important consumer of OM. Pulse-chase experiments reveal that the dominance of *Linopherus* sp. nov. appears to increase the total resource use (i.e. ^{13}C -labeled algae) by the macrofauna at the lower OMZ boundary compared with other sites at the PM (Woulds et al. 2007). For example, at 140 and 1850 m where macrofaunal diversity was higher, incorporation of carbon by the macrofauna was low, compared with 850 and 950 m where diversity was low, but incorporation of carbon was much higher (Table 4). Uptake of isotopically labeled phytodetritus was elevated at 800 m (com-

pared with 500 and 1100 m) on the Indian margin, largely due to the dominant cirratulid polychaetes (Hunter et al. 2012). Increased total resource use by a dominant species has been demonstrated previously for a wide range of environments (Cardinale et al. 2006) and specifically for coastal echinoderms (Godbold et al. 2009a). The results of Woulds et al. (2007) contradict those of Karlson et al. (2010), who found that higher metazoan macrofaunal species diversity (maximum number of species = 3, minimum number of species = 1) led to enhanced processing of phytodetritus. However, the results shown here from the PM are representative of larger multi-species communities (maximum number of species = 53, minimum number of species = 2) and demonstrate that dominant species in OMZs can have a significant impact on carbon processing.

Macrofauna can also impact OM processing by other members of the benthic community, e.g. meiofauna (Nascimento et al. 2011). At sites where *Linopherus* sp. nov. was dominant, foraminifera and macrofauna were less abundant and their diversity was reduced compared with other areas of the PM, notably the core of the OMZ for foraminifera (Goody et al. 2009, Hughes et al. 2009, Levin et al. 2009b). Although the effects of depth and DO cannot be discounted, the presence of *Linopherus* sp. nov. has implications for the food resources available to other consumers and may affect community structure through competition for resources. Polychaetes can prey on foraminifera (Nomaki et al. 2008) and so foraminifera may also constitute an important food source for *Linopherus* sp. nov., thereby keeping densities low.

Levin et al. (1997) demonstrated that deep-living maldanid polychaetes can transport fresh OM to depths of >10 cm, thereby enabling other deep-living species to utilize this resource. Similarly, subduction of phytodetritus and/or creation of burrows into which phytodetritus may be passively deposited by *Linopherus* sp. nov. could enhance processing of OM through facilitating feeding by deeper-living species.

Lipid distributions of *Linopherus* sp. nov. suggest efficient transfer and processing of OM, reflecting its importance in OM turnover and quality. The presence of phytoplankton biomarkers, i.e. $\text{C}_{22:6(n-2)}$, $\text{C}_{20:5(n-3)}$ and $\text{C}_{16:1(n-7)}$, in *Linopherus* sp. nov. (at 800 and 850 m), together with its apparent feeding behaviour (moving out of its burrows), suggests that selective feeding and uptake of lipid-rich particles occur, leaving lipid-depleted OM to be deposited at the sediment surface. Furthermore, sedimentary pigment distributions provide evidence for digestive

alteration of OM by *Linopherus* sp. nov. At 950 m the pheophytin/pheophorbide ratio decreased with depth, suggesting that pheophorbide was produced by *Linopherus* sp. nov. through the digestion and breakdown of chlorophyll (Woulds & Cowie 2009).

CONCLUSIONS

(1) DO exerts threshold effects on the distribution of the polychaete *Linopherus* sp. nov. This species is a low-oxygen specialist capable of tolerating *in situ* DO concentrations < 1 µM and is not found at oxygen concentrations ≥ 12 µM, where it appears to become outcompeted by other macrofauna.

(2) *Linopherus* sp. nov. displays both ecological and morphological adaptations to low DO. Larger proportions (~60%) of smaller individuals (<15 chaetigers), possibly juveniles, are present at lower DO concentrations; these individuals have a higher surface area:volume ratio for oxygen diffusion than do large individuals. The respiratory structures of *Linopherus* sp. nov. are greatly enlarged, and the approximate respiratory area (normalized to body weight) is greater at lower DO. Lower DO may therefore provide a refuge from predation or cannibalism for juveniles of this species. *Linopherus* sp. nov. is highly mobile and either migrates to water depths with higher DO once it reaches maturity or grows to larger size at higher O₂ levels.

(3) *Linopherus* sp. nov. are opportunistic omnivores relying on phytodetritus, bacteria and invertebrate prey for food. Pulse-chase experiments demonstrated that *Linopherus* sp. nov. can rapidly consume phytodetritus. The large body size of *Linopherus* sp. nov. may be attributed to an abundant food supply and the successful nutritional strategy of these polychaetes.

(4) *Linopherus* sp. nov. is proposed to influence biodiversity through: (i) its tolerance to hypoxia, thereby promoting its dominance, and (ii) competitive exclusion for resources. The presence, dominance and trophic plasticity of *Linopherus* sp. nov. at the lower OMZ transition zone leads to more efficient trophic transfer and processing of OM, which has ramifications for carbon cycling on the PM.

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