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Linking 3D *Perinereis* burrows with physicochemical factors on a tidal flat (Pearl River Delta (PRD), China)

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Abstract

This study employed a comprehensive approach involving field sampling, grain-size analysis, physicochemical measurements, CT scanning and three-dimensional reconstruction in order to investigate the intertidal flats of the Pearl River Delta (PRD). As *Perinereis* is an important group of bioindicators in relation to environmental factors, sedimentological and ichnological analyses were conducted at 6 out of 63 stations. These analyses aimed to clarify the composition and distribution characteristics of *Perinereis* traces, reveal their response relationships with physicochemical factors including sediment grain size, salinity, turbidity and total organic carbon (TOC) content, and further illustrate their environmental significance. The results showed that: (1) *Perinereis* traces are predominantly composed of grazing and dwelling burrows. These burrows display diverse morphologies, including simple cylindrical, Y-shaped, U-shaped and complex branching networks. Some branches exhibit swellings at junctions. (2) Salinity has a significant influence on the burrow diameter of *Perinereis*, exhibiting a positive correlation in the low to moderate salinity range (0.28–3.97 PSU). *Perinereis* growth plateaus when salinity exceeds a threshold of approximately 18 PSU, indicating a physiological tolerance limit. (3) Burrow diameter and sediment bioturbation exhibit no significant correlations with sediment grain size, salinity, turbidity or total organic carbon content. This may be attributed to infaunal interactions (e.g. associations with Macrophthalmus japonicus) and the combined influence of multiple environmental factors. This study improves our understanding of the

biology and ichnology of modern *Perinereis* traces. It provides a quantitative basis for interpreting the interactions between infauna and their environment in intertidal flat ecosystems.

Keywords: Deltaic tidal flat, Neoichnology, *Perinereis*, Physicochemical factors, Paleoenvironment

INTRODUCTION

Neoichnology focuses on the interactions between modern organisms and sediments, integrating research findings from the fields of biology, sedimentology and paleontology (Dashtgard, 2011a, b; Knaust and Bromley, 2012; Adikaram et al., 2017; Kumar, 2017; Brown et al., 2024). It provides a theoretical framework for analyzing infauna and their burrowing behaviors. This facilitates an understanding of the dynamic relationships between infaunal activities and physicochemical factors. Studies of biogenic structures in modern tidal flats may reveal the relationship between such structures and the physicochemical factors of coastal zones. They may also be used to tentatively reconstruct sedimentary and environmental conditions during biological colonization by analyzing burrow characteristics (Virtasalo et al., 2011; Benkhedda et al., 2021). Particularly in tidal flat areas near estuaries, bioturbation patterns typically reflect gradient changes in sediment grain size, salinity, and turbidity associated with freshwater input (Buatois et al., 2012, 2019; Demircan and Uchman, 2016; Sendra et al., 2019; Moyano et al., 2020; Morelle et al., 2024). Although considerable progress has been made in understanding these relationships over recent decades (Buatois and Mángano, 2011; Dashtgard, 2011a, b; Ayranci et al., 2014; Wang et al., 2019, 2024, 2025), there remains a need to extend neoichnological research to understudied taxa in regions worldwide, given the inherent variability of trace makers and the complexity of environmental conditions.

The physicochemical gradients of deltaic tidal flats create a dynamic and highly stressful environment that exerts a strong selective influence on infaunal communities and their biogenic sedimentary structures (Passarelli et al., 2012, 2014). Polychaetes are fundamental bioturbators in such environments. Unlike predators or filter feeders, they can disrupt the chemical balance of sediments through feeding, digestion, excretion, and transporting sediments. They can also migrate through deep sediments, excrete nitrogen, and reconstruct microbial communities by transporting intestinal bacteria. These activities are crucial in driving sediment biogeochemical processes (Cournane et al., 2010; Koo and Seo, 2017; Fang et al., 2018). Previous studies have extensively investigated the relationships between the morphological characteristics of benthic organisms and the distribution of their burrows, as well as the relationships between these characteristics and physicochemical factors. For instance, Wang et al. (2025) found that in the Pearl River Delta (PRD), the burrows of Macrophthalmus japonicus are more complex and their bioturbation is more intense in environments with fine-grained substrates, high TOC content, freshwater to lower mesohaline salinity, and suitable turbidity. Olabarria et al. (1998) observed that the dominant bivalve organisms in the Rías Bajas in northwestern Spain are more prevalent in coarse sand and gravel substrates, as well as in the intertidal zone and in fine sand substrates with an organic matter content of 1–2%. Rowshan et al. (2023) showed that polychaete abundance is higher and the community structure is more dominant in silty loam substrates, environments with high total organic matter (TOM) content, and high salinity in the shallow waters of the southern Caspian Sea, Iran. *Perinereis* are widely distributed across the tidal flat burrow systems of the Pearl

River Delta (PRD) (Wang et al., 2019; Pang et al., 2021). However, research into the burrows of this species in the PRD is currently limited, particularly with regard to systematic quantitative analysis of the relationship between burrow characteristics and environmental physicochemical factors. This makes *Perinereis* an ideal model organism for exploring how deltaic environments regulate biogenic sedimentary structures.

Traditional burrow casting and excavation methods can destroy the original structure of burrows. However, computed tomography (CT) technology can analyze their complex morphology efficiently, providing a reliable way to link bioturbation structures to physicochemical factors (Mazik et al., 2008; Herringshaw et al., 2010; Pennafirme et al., 2019; Howman et al., 2024). This study employed CT scanning and three-dimensional reconstruction technology to systematically and quantitatively analyze the surface traces and morphological characteristics of *Perinereis* burrows in the tidal flats of the Pearl River Delta (PRD). The study will address the following three scientific questions in depth: (1) explore the morphological and structural characteristics of surface traces and dwelling burrows of *Perinereis* in the tidal flats of the Pearl River Delta; (2) clarify the regulatory effects of physicochemical factors such as sediment grain size, salinity, turbidity, and TOC content on the burrow diameter and bioturbation behavior of *Perinereis*. and (3) reveal the patterns underlying the community distribution of *Perinereis* under the comprehensive influence of multi-factor synergy and biological interactions.

METHODS

Study Area

Along the northern coast of the South China Sea, the research area covers the delta-plain tidal flats of the Pearl River Delta (PRD) in southern China, as well as adjacent nearshore islands (Figs. 1A-C). The complex river network of the PRD discharges an average annual water volume of approximately 2823×108 m3 and a sediment load of 72.4×106 t (Liu et al., 2014), discharging into the South China Sea through eight estuarine outlets, namely Humen, Jiaomen, Hongqimen, Hengmen, Modaomen, Jitimen, Hutiaomen, and Yamen. All the estuaries in the study area are tidal. They are characterized by an irregular semi-diurnal tidal cycle, with two flood and two ebb tides per day. Each lunar month includes six days of spring tides and six days of neap tides, with the remainder being intermediate tides. The tidal range is generally between 0.86 m and 1.66 m, classifying the region as microtidal (Zhang et al., 2010, 2017). The study area is located in a coastal region south of the Tropic of Cancer with a subtropical monsoon climate. It has a warm and humid subtropical monsoon climate, with an annual average temperature of 21.5–22.5°C, which meets the classification standard for a subtropical climate (annual average temperature ≥20°C). The annual average precipitation exceeds 1600 mm, resulting in abundant rainfall (Wei et al., 2024). Sampling took place during the winter, from December 2021 to February 2022. The annual average rainfall in the study area exceeds 1,600 mm (Wei et al., 2024), and there are pronounced seasonal variations in hydrological variables. Peaks in summer precipitation (900 ± 120 mm) and runoff (1.35 ± 0.20 × 10^8 m^3) occur first, followed by peaks in spring (350 ± 50 mm, 0.45 ± 0.08 × 10^8 m^3), autumn (250 ± 40 mm, 0.30 ± 0.07 × 10^8 m^3) and winter (100 ± 20 mm, 0.15 ± 0.05 × 10^8 m^3), in descending order (Li

et al., 2020; Xiao and Cui, 2021). Precipitation is highly concentrated in summer, resulting in significant variability in river discharge and tidal currents. The wet season is characterized by strong hydrodynamics, enriched nutrients and frequent scouring of benthic organisms, whereas the dry season features weaker hydrodynamics, increased salinity and abundant aquatic vegetation (Feng et al., 2021; Pang et al., 2021; Yang et al., 2022). The bay contains numerous remnant hills and islands, which form natural barriers at the estuary mouths and effectively attenuate wave energy. Consequently, wave action is generally weak, with an average wave height of approximately 1 m (Jia et al., 2016). Intense weathering in the source regions supplies fine-grained sediments to the river systems. the total annual input of fine-grained sediment transported, deposited, and dispersed within the estuaries and adjacent coastal and shelf areas of the South China Sea exceeds 8400×104 t (Chen et al., 2023).

Figure 1 - Study area. (A) Map of China, red star marks the study area; (B) map of the Pearl River Delta; (C) satellite image showing the tidal flat that forms the study area (image source: http://glovis.usgs.gov/).

Sampling

Figure 2 - Position of stations and surface-sediment distribution on the studied tidal flat of the Pearl River Delta; red stars mark stations where *Perinereis* burrows were present.

Sampling was conducted with Qiao Island as the central point: Underwater sampling was conducted along radial transects, each measuring 12–20 km in length. A total of 63 sampling stations were established across the study area for sedimentological and ichnological analysis (Fig. 2). Sediment samples were collected using vertical coring with PVC tubes that are 15 cm in length and 7.5 cm in diameter. Bioturbation areas were avoided during sampling, and the pipe mouths were sealed after collection to preserve the morphology of burrows. Surface sediments were collected at a depth of 0-5 cm below the sediment surface, and two parallel samples were prepared. These samples were sealed and stored in sterile polyethylene bags for analysis of indicators such as particle size and total organic carbon (TOC). Two parallel surface water samples were also collected and sealed in 100 ml plastic bottles for later use to determine parameters such as salinity and turbidity. All samples were stored at ambient temperature in light-proof conditions to preserve their original composition before

analysis. To collect well-preserved, representative biogenic sedimentary structures, 15 cm-long PVC core tubes with an inner diameter of 7.5 cm were pressed into the sediment during low tide to collect samples in situ. The cores were then sealed within the tubes to create an anoxic environment that would effectively inhibit bioturbation. According to the density of *Perinereis* burrows in the substrate, 35 sediment cores were collected in varying quantities from 18 sampling stations. Six stations with typical sedimentary environments where *Perinereis* were found were selected for sampling according to the distribution characteristics of tidal flat microenvironments in the Pearl River Delta (PRD). The exact locations of these stations were determined using a handheld GPS device and marked on Google Earth Pro. Columnar samples were collected in accordance with the principles of covering the tidal flat microenvironments and ensuring the representativeness of the samples. Meanwhile, points with well-preserved, morphologically typical *Perinereis* burrows were selected to meet the requirements of subsequent experiments such as CT scanning and three-dimensional reconstruction. The number of columnar samples collected and the specific coring points were mainly determined by the integrity of the preservation and representativeness of the *Perinereis* burrows, as well as the characteristics of microenvironmental disruption at each station. Only points with complete burrow morphology that were easy to collect and difficult to damage were sampled using PVC pipes.

Grain Size Analysis

A total of 58 surface sediment samples for grain size analysis were collected from the 63 sampling stations (Table 1). The depth of the core samples is 0–15 cm, with one or two cores collected at each sampling point. For well-preserved, representative *Perinereis* burrows, additional directional core sampling was conducted using PVC pipes with a diameter of 5 or 7.5 cm and a height of 15 cm. The sediment at station 63 consisted of gravel, while stations 17, 53, and 55 were characterized by coarse sand sediments, both of which render them unsuitable for our grain size analytical instrumentation. Furthermore, sampling at station 43 was hindered by excessive current velocities, which prevented successful underwater sampling operations. All sediment samples were oven-dried at 105°C for 24 hours, and approximately 0.2 g of each sample was transferred to pre-cleaned glass beakers. To remove organic matter, 10% hydrogen peroxide (H_2O_2) was added, followed by 10% hydrochloric acid (HCl) to eliminate carbonates. Following chemical pretreatment, samples were left to settle. Before analysis, a 0.5% calgon solution was added to disperse the clay and silt-sized particles, and the suspensions were ultrasonicated for 10 minutes (40 kHz) to ensure complete disaggregation. The grain size test was performed using a Mastersizer 2000 (Malvern Instruments Ltd., Worcestershire, UK), with triplicate measurements performed for each sample to ensure reproducibility (Bertrand et al., 2014; Li et al., 2021; Song and Li, 2023).

Table1 - Grain size parameters (mean grain size, standard deviation (SD), and skewness), turbidity (in nephelometric turbidity units (NTU), salinity (in parts per thousand) and total organic carbon content (TOC)samples taken from the Pearl River Delta.

Station	Mean Grain Size(mm)	SD (mm)	Skewness	Turbidity (NTU)	Salinity (PSU)	TOC (%)
1	0.37	0.64	0.12	8.36	30.10	
2	0.35	0.68	0.02	8.15	31.80	

3	0.39	0.61	0.10	7.45	31.80	
4	0.01	0.32	0.12	12.02	12.90	
5	0.02	0.16	-0.06	6.24	11.80	0.40
6	0.01	0.28	0.06	15.53	20.90	1.01
7	0.01	0.28	0.10	5.79	21.60	0.94
8	0.01	0.26	0.03	2.68	23.50	1.03
9	0.01	0.17	-0.23	10.65	26.90	0.85
10	0.26	0.52	0.26	40.32	28.11	
11	0.01	0.24	0.05	45.88	11.80	1.03
12	0.01	0.29	0.13	6.40	18.30	1.16
13	0.01	0.22	-0.04	5.85	20.40	0.92
14	0.01	0.27	0.04	2.44	21.30	0.77
15	0.01	0.28	0.08	3.74	21.00	0.78
16	0.01	0.34	0.17	10.20	17.40	1.18
17				15.63	18.10	1.41
18	0.01	0.35	0.12	12.02	18.80	1.00
19	0.01	0.36	0.16	5.11	18.20	1.03
20	0.01	0.35	0.16	3.90	19.20	1.56
21	0.01	0.25	-0.01	6.94	18.60	1.04
22	0.01	0.20	-0.03	7.00	18.90	0.82
23	0.01	0.18	-0.08	2.69	20.70	0.25
24	0.05	0.18	0.59	3.33	22.00	0.20
25	0.01	0.22	0.07	1.67	22.30	0.57
26	0.01	0.25	-0.02	31.52	20.20	0.83
27	0.01	0.29	0.09	13.94	22.10	0.94
28	0.01	0.28	0.07	6.37	25.00	1.57
29	0.03	0.13	0.04	8.49	26.40	0.99
30	0.01	0.29	0.12	6.07	26.50	1.19
31	0.01	0.33	0.11	34.44	16.20	1.63
32	0.01	0.32	0.09	33.60	15.90	1.24
33	0.01	0.32	0.15	36.76	10.30	1.54
34	0.01	0.32	0.16	48.96	3.44	1.57
35	0.01	0.29	0.13	16.06	8.13	1.59
36	0.18	0.24	0.66	192.23	15.50	
37	0.01	0.30	0.15	19.63	15.80	1.63
38	0.01	0.27	0.01	6.87	12.60	1.35
39	0.02	0.17	-0.02	5.01	9.31	0.55
40	0.02	0.19	0.06	58.74	14.30	2.18
41	0.02	0.17	-0.00	16.39	0.55	0.74
42	0.02	0.21	0.31	7.54	2.74	1.01
43					6.23	
44	0.46	0.71	0.03	75.98	16.80	

45	0.31	0.64	0.00	142.07	18.00	0.53
46	0.10	0.20	0.52	215.27	12.40	0.90
47	0.46	0.71	0.02	11.41	11.30	
48	0.01	0.32	0.08	163.00	5.48	1.91
49	0.02	0.10	-0.31			1.50
50	0.36	0.68	0.02	148.03	13.90	
51	0.31	0.62	0.02	11.38	10.60	
52	0.25	0.61	0.02	113.90	14.10	
53				192.57	13.60	
54	0.25	0.58	-0.04	129.90	14.20	
55				158.73	11.60	
56	0.02	0.21	0.10	240.27	2.17	0.67
57	0.02	0.25	0.24	44.47	0.28	1.42
58	0.04	0.13	0.06	42.88	0.47	1.38
59	0.01	0.36	0.22	347.17	2.19	2.40
60	0.01	0.35	0.21	117.63	3.97	0.60
61	0.01	0.36	0.16	118.07	9.27	1.58
62	0.01	0.28	0.09	68.42	2.20	2.72
63				116.67	12.10	3.70

Determination of Turbidity, Salinity, and Organic Matter Content

Water samples were collected at 62 sampling stations during low tide for salinity and turbidity measurements. Stations 49 and 50 have hydrological connectivity within the same water body; therefore, only Station 50 was sampled. Salinity was measured using an SX813 conductivity meter and recorded in Practical Salinity Units (PSU). Each point was measured at least three times, and the average value was taken to reduce the impact of instantaneous fluctuations and operational errors. The difference between the three readings had to be less than 0.002 PSU. Turbidity was measured using an NS-type turbidity meter and expressed in nephelometric turbidity units (NTU). Each sample was measured repeatedly at least three times, and the average value was taken as the final result.

Total organic carbon (TOC) content was analyzed from sediment samples taken at 48 stations (Table 1). According to Shetty and Goyal (2022), the loss-on-ignition (LOI) method was used to determine the total organic carbon (TOC) content. The dried samples were ground using a mortar and pestle, further dried for 30 hours at 60 °C, and then heated to 550 °C for 4 hours. The loss in weight from organic matter combustion during LOI was measured using an electronic balance with a precision of ±0.001 g (Wang et al., 2025). Triplicate measurements were performed for each sample, with a relative standard deviation (RSD) < 5% ensuring analytical reproducibility.

Computed tomography (CT) scanning

All 35 collected cores were temporarily stored for one month under ambient temperature, dry, and light-protected conditions. After the completion of all field sampling, they were taken to Nanjing Institute of Soil Research, Chinese Academy of Sciences, for computed tomography (CT) scanning. During transport to the laboratory for scanning, all core tubes were maintained in a strictly upright position to prevent disruption and resuspension of sediments. The Nanotom S CT scanner was

employed with the following parameters: voltage 180 kV, power of 15 W, resolution of 200 nm (0.2 μm), and 3D magnification ranging from 1.5-100×. During scanning, the X-ray source emitted a collimated beam while the core was rotated 360° to acquire multi-angular projections of the target specimen. A total of 4575 projection images were collected per sample, with each 250 ms projection averaged over 8 frames (2 seconds per projection), totaling approximately 153 minutes per scan. Ring artifact correction was enabled to improve image quality.

All three-dimensional reconstructions were completed at the Nanjing Institute of Geology and Paleontology, Chinese Academy of Sciences. To minimize file sizes and reduce computational burdens, the reconstructed 3D volumes were converted to an 8-bit image format using Fiji-ImageJ software. Subsequently, these processed images were imported as a 3D project into VG Studio MAX (Version 2.1, Volume Graphics GmbH, Germany), where a 3D non-linear digital median filter with edge-preserving properties was applied. This filter adopted a 5-voxel window size to mitigate image noise. The final scanned volume exhibited a 3D voxel size of 30 μm. For the sediment core samples, regions of interest (ROIs) that contained burrow structures were segmented via a threshold-based seed point growing algorithm, which enabled the generation of a 3D visualization of the burrow network (Nel et al., 2001; Petrash et al., 2011; Hale et al., 2015; Mimier and Żbikowski, 2017).

Neoichnological assessment

According to field observations and CT image analysis, traces of biological disruption by *Perinereis* was found at only six out of 63 sampling stations *Perinereis* (Table 2). At each station, five 1 m × 1 m quadrats, each with a depth of 20 cm, were randomly excavated. To provide a physical reference scale, a ruler was placed on the surface of the sediment, after which photos of the sediment surface were taken to record the burrow morphology. The average burrow density was determined by counting the number of trace producers and calculating the average. ImageJ software was then used to measure the diameter and cross-sectional area of individual burrows. The occupied surface area (cm^2/m^2) at each station was obtained by multiplying the average burrow density by the total average cross-sectional area. This value was then used to quantify the sediment bioturbation intensity caused by the *Perinereis*. The sediment bioturbation rate (%), according to Dashtgard (2011a), is calculated as (average burrow cross-sectional area × average burrow density) × 0.01. Additionally, the influence of physicochemical factors on average burrow diameter was investigated, as were their correlations. Logistic nonlinear least-squares regression was performed in Origin software to model the relationship between average burrow diameter and salinity. The significance of the model and its parameters was evaluated using ANOVA and t-tests, respectively. The goodness of fit was represented by R^2 and adjusted R^2 ($P < 0.05$).

Table2 - Quantification of *Perinereis* bioturbation: burrow morphometrics (diameter, cross-sectional area, density) and calculated sediment disruption (%) across sampling stations on the Pearl River Delta

Station	Average burrow diameter (cm)	Average burrow cross-sectional area (cm^2)	Average burrow denstity (number of burrows/m^2)	Cross-sectional area* burrow density (cm^2/m^2)	Sediment disruption (area)%

45	0.336	0.089	208	18.5	0.19
56	0.256	0.051	4900	250	2.50
57	0.102	0.008	300	2.4	0.02
58	0.157	0.019	7900	150	1.50
60	0.257	0.052	5100	265.2	2.65
62	0.353	0.098	2800	274.4	2.74

RESULTS

Sedimentology

Figure 3 - Mean grain size and sorting values for all samples collected at the tidal flat; red stars denote samples from stations where *Perinereis* was present.

Overall, *Perinereis* was predominantly found in fine-grained substrates, such as silt, clay, and silty sand (Fig. 2), all of which exhibit poor sorting (sorting index > 1; Fig. 3).

The upper intertidal zone of the study area is characterized by poorly sorted muddy sediments, with arcuate ridge ripples (Fig. 4A) and soft-sediment deformation structures present. The dense reeds and shrubs surrounding the muddy tidal flat (Fig. 4B) provide abundant organic matter for benthic organisms, leading to the formation of numerous burrow openings on the sediment surface.

The middle intertidal zone mainly consists of poorly sorted silty sediments and features typical tidal bedding, including lenticular bedding, flaser bedding and ripple cross-bedding. Meandering tidal

creeks traverse the intertidal zone (Fig. 4C) and transport fine-grained suspended sediments during flood tides. These sediments are subsequently deposited during ebb tides, forming extensive muddy deposits. Scattered shallow pools filled with water are also common in this zone.

The lower intertidal zone is characterized by well-sorted sandy sediments ranging from medium to fine sand (mean grain size: 0.1–0.5 mm). Various sedimentary structures have formed in these sandy deposits, including parallel bedding (Fig. 4D), trough cross-bedding and current ripples (Fig. 4E). Oscillation ripples were also clearly visible at the Dong'ao Island site (Fig. 4F).

Figure 4 - Sedimentological features on the studied intertidal flat. (A) Curved ridge ripple in silty mud (station 53). (B) the mudflat with dense shrubs and reeds, featuring a high density of burrow openings (white arrows with black outline) (station 59). (C) The meandering tidal creeks (indicated by the yellow arrows) traverse the intertidal flat, and there are many small water-filled pools (indicated by the white arrows) on the surface of the middle intertidal flat (station 49). (D) sandy parallel bedding (station 10). (E) current ripples (station 45). (F) oscillation ripples (outlined by white dashed line) (station 2).

Physicochemical factors

The physicochemical factors measured include salinity, turbidity, and total organic carbon (TOC) content. Salinity increases gradually from the west to the east and decreases from the south to the north. Overall, the study area is dominated by saltwater and brackish environments, with only a few stations (Stations 41, 57 and 58) showing significantly lower salinity due to the input of freshwater from the land. According to salinity, the area can be divided into three categories: oligohaline (< 0.05 Practical Salinity Units (PSU)), mesohaline (0.05–16 PSU), and polyhaline (> 16 PSU), with respective means and standard deviations of 0.38 ± 0.13, 9.68 ± 4.76, and 22.04 ± 4.40.

Turbidity can serve as an indicator of terrestrial inputs (e.g., riverine sediment transport) and hydrodynamic sorting processes. The water in the tidal flats of the study area is generally turbid, with turbidity gradually increasing from the sea to the land (from east to west). There is, however, no obvious systematic change along the north-south transect (Fig.5B). According to turbidity, the water can be divided into four categories: <20 NTU, 20–80 NTU, 80–170 NTU and >170 NTU. Their respective means and standard deviations are 8.37 ± 4.57, 46.83 ± 14.11, 134.22 ± 19.23 and 237.50 ± 64.42.

Total organic carbon (TOC) content ranges from 0.20% to 3.70%, with most stations falling within the 0.40% to 2.00% range, indicating an overall low to moderate level of organic matter. The highest TOC content (3.70%) occurs at station 63, and the lowest (0.20%) at station 24 (Table 1), reflecting significant spatial variability in organic matter enrichment. High TOC values are mostly found in areas strongly influenced by terrestrial inputs and relatively low salinities (2.17–14.30 PSU). In contrast, low TOC values are concentrated at station 23 (0.25%) and station 24 (0.20%), as well as some high-salinity stations.

Analysis of the relationship between the burrow characteristics of *Perinereis* and environmental factors reveals a significant correlation between burrow diameter and salinity. Burrow diameter increases with rising salinity between 0.28 and 3.97 Practical Salinity Units (PSU), stabilizing above approximately 18 PSU (Fig. 9). At salinities of 0.5–16 PSU, the degree of sediment bioturbation decreases as salinity increases (Fig. 10D). However, burrow diameter and sediment bioturbation show no significant correlations with TOC content, mean grain size or turbidity (Figs. 10A–C).

Figure 5 - Salinity (A) and turbidity (B) values measured at all stations on the Pearl River Delta tidal flat; red stars denote stations where *Perinereis* was present.

Neoichnology of *Perinereis*

Surface traces and dwelling burrows constructed by *Perinereis* mainly occur in the modern intertidal zones of the Pearl River Delta (PRD). Their detailed characteristics are described below.

Upper intertidal zone: On the sediment surface, the fecal pellets of *Perinereis* appear as slender, cylindrical, curved accumulations, measuring around 1 mm in width (Fig. 6D). Their burrow openings are mostly circular (Fig. 6C), measuring 0.5–2 mm in diameter, and the burrows can extend to a depth of 11 cm below the surface (Figs. 7A and 7B). The red and white arrows in Fig. 7C indicate suspected dwelling/activity chambers. These burrows are relatively shallow, generally reaching only about 5 cm below the surface. Three-dimensional images show that the burrows of *Perinereis* consist mainly of fine, densely packed tunnels forming a highly interconnected network with extensive branching, bending, and bifurcation (typically at angles of 45–90°). The green arrows in Figs 7G and 7H show where the tunnels intersect and intertwine. In contrast, the burrows of *Macrophthalmus japonicus* are mostly Y- or J-shaped, featuring thick main tunnels (5–10 mm in diameter) and multi-directional branches that form complex connected spaces (Fig. 7C).

Middle intertidal zone: A live red *Perinereis*, measuring approximately 10–12 cm in length, was observed on the sediment surface (Fig. 6A), alongside the circular, 7–10 mm openings of adjacent *Macrophthalmus japonicus* burrows (Fig. 6B). The two species often coexist at stations where *Perinereis*

occur, with the burrows of *Perinereis* frequently distributed around the Y-shaped burrows of *Macrophthalmus japonicus* (Figs. 7D and 7E). Some *Perinereis* burrows are also Y-shaped with upward openings, but they are smaller than the crab burrows. Three-dimensional images show that *Perinereis* burrows within the sediment are extremely dense (0.5–1 mm in diameter) with occasional swellings at branch points (Fig. 7F, green arrow).

Lower intertidal zone: The substrate consists of sandy sediment mixed with fine gravel. Linear traces of *Perinereis* can be seen on the surface as slender features measuring around 2 mm in width (Fig. 6E). Due to the strong tidal and wave dynamics, bioturbation structures are poorly preserved and *Perinereis* burrows and traces are relatively scarce.

Figure 6 - Traces of *Perinereis* on the sediment surface: surface crawling traces (marked with red arrows) and openings of subsurface burrows (marked with black arrows). (A) red-colored trace-maker *Perinereis*. (B) burrow entrance of *M. japonicus*. (C) burrow entrance of *Perinereis*. (D) *Perinereis* faeces. (E) crawling traces.

Figure 7 - CT images of *Perinereis* from the Pearl River Delta intertidal flat. (A), (B) simple *Perinereis* burrows. (C) simple, rarely branched, nearly vertical burrows of *Perinereis* (marked with red arrow) and simple J-shaped *M. japonicus* burrow (marked by white arrow). (D) simple *Perinereis* burrows (marked with red arrow) and simple J-shaped *M. japonicus* burrows (marked by white arrow). (E) complex *Perinereis* burrows (marked with red arrow) and simple Y-shaped *M. japonicus* burrows (marked by white arrow). (F) (G) and (H) complex burrows of *Perinereis* with swelling part (marked by green arrow).

Figs. 8A and 8B show field observations of *Perinereis* burrows in sediments. Figs. 8C and 8D are schematic diagrams of the corresponding morphologies. Simple-type burrows have few scattered branches and a relatively uniform burrow diameter (Fig. 8D). Their morphologies include U-shaped and Y-shaped structures. Complex-type burrows (Fig. 8C) have numerous dense branches with expanded regions at the bifurcations and significant variations in burrow diameter. The combination of multiple U-shaped and Y-shaped branches, together with dense swollen areas, forms a complex three-dimensional network that supports the rapid movement and expanded foraging range of *Perinereis* within the sediment.

Figure 8 - Comparison of dense burrow openings (A) and sparse burrow openings (B) of *Perinereis* on the Pearl River Delta tidal flat; comparison of complex burrows (C) and simple burrows (D).

Table 3 - Characteristics of simple and complex burrows across all *Perinereis* stations based on CT images.

Station	Morphological structure	swelling phenomenon	symbiotic relationship	Burrow type
45	vertical tubular	no swelling	None	simple
56	vertical tubular and Y-shaped	no swelling	None	simple

57	vertical tubular and Y-shaped	no swelling	None	simple
58	vertical tubular, U-shaped and Y-shaped, multiple branches	with swelling	associated with *M. japonicus*	complex
60	vertical tubular, U-shaped and Y-shaped	no swelling	associated with *M. japonicus*	simple
62	vertical tubular, U-shaped and Y-shaped, multiple branches	with swelling	associated with *M. japonicus*	complex

DISCUSSION

Analysis of the sediment bioturbation caused by *Perinereis* (Table 2 and Fig. 3) shows that this species inhabits fine-grained substrates and exhibits lower burrow density and weaker sediment bioturbation in coarser-grained sediments, such as those at Station 45. *Perinereis* can reduce the shear resistance of fine-grained sediments through setae movement and mucus lubrication, enabling them to burrow efficiently (Nel et al., 2001; Petrash et al., 2011; Mimier and Żbikowski, 2017). However, their limited muscle strength and mucus secretion make it difficult for them to penetrate medium and coarse sands (Francoeur and Dorgan, 2014; Sun et al., 2019), resulting in a significant reduction in burrowing ability. The surface tension of fine-grained substrates is low and burrow stability is poor, meaning that *Perinereis* may rely on the stable burrow structures of *Macrophthalmus japonicus* for survival (Ho et al., 1997; Palomo et al., 2004). However, the bioturbation of *Perinereis* can increase sediment porosity, improve the distribution of organic matter, promote microbial activity and indirectly provide *Macrophthalmus japonicus* with more benthic microalgae or organic detritus (Palomo et al., 2004; Patel and Desai, 2009; Alvarez et al., 2018). This is consistent with the phenomenon observed at Stations 58, 60 and 62, where the two species often occur alongside each other. Together with the statistics on burrow characteristics in Table 3, it can be inferred that suitable environmental conditions and infaunal interactions can improve the settlement success rate of *Perinereis* and promote the formation of complex burrows. To a certain extent, this can also weaken the direct control of sediment grain size on burrow density (Kruger and Woodin, 1993; Palomo and Iribarne, 2000)

Salinity is an important factor affecting the growth and development of infauna. It can also induce spatial heterogeneity by influencing infaunal behavior and distribution (Gingras et al., 2011; Ayranci et al., 2013; Freitas et al., 2015; La Croix et al., 2015; Ayranci et al., 2016). In environments with fluctuating salinity, *Perinereis* must maintain homeostasis through ion and osmotic regulation. Fig. 9A shows a positive overall correlation between salinity and the diameter of individual burrows, with each burrow acting as a statistical unit. Data dispersion at the same salinity reflects individual differences: each salinity value in the figure corresponds to a single sampling station and the shape and color of the data points distinguish the statistical results for burrow diameters from different stations. The distribution of burrow diameters at a salinity of 2.20 PSU is the widest, with the largest number of data points. This is because low-salinity tidal flat areas are characterized by high levels of

organic matter, abundant food resources and relatively low levels of predation. Together, these factors promote faster growth and larger body size in *Perinereis* individuals, as well as increased variability in burrow diameter. When salinity increases, the increased osmotic stress may inhibit growth. However, *Perinereis* tends to develop more advanced tissue structures to meet its physiological needs. This results in an increase in individual diameter, showing characteristics of compensatory growth (Pechenik et al. 2016; Villena-Rodríguez et al. 2024). Fig. 9B uses the average diameter of the burrows at each station as the statistical unit. Fitted using a logistic model, it reveals a nonlinear saturation relationship between salinity and average burrow diameter characterized by 'rapid growth at low salinity and stabilization at high salinity'. Under low to medium salinity (0.28–3.97 PSU), the average burrow diameter increases with rising salinity, which is consistent with consistent with previous studies (Gingras et al., 2008, 2024). When salinity exceeds 18 PSU (e.g. at station 45), the diameter tends to stabilize, indicating that the *Perinereis* have reached their physiological tolerance limit for salinity (Feng et al., 2014). Regression analysis shows that the model has an R^2 of 0.84, an adjusted R^2 of 0.59, an *F*-value for analysis of variance of 24.69 and a *P*-value of 0.0391 (less than 0.05), indicating that the model is significantly effective overall.

Figure 9 – Relationship between burrow diameter (cm) and salinity (PSU) at sampling stations where *Perinereis* was present. (A) Scatter plot of the diameters of multiple burrows at *Perinereis* sampling stations under different salinity values. (B) Linear regression showing the relationship between salinity and average burrow diameter.

The characteristics of tidal flat bioturbation are collectively regulated by sediment grain size, turbidity and total organic carbon (TOC) (Li et al., 2019; Grandjean et al., 2024; Nauta et al., 2024). High turbidity is often accompanied by high sedimentation rates, and easily deposited flocculants can clog the filter-feeding organs of *Perinereis*, significantly affecting them. At the same time, reduced transparency inhibits phytoplankton photosynthesis, resulting in a decline in primary productivity (Cloern, 1987; Davies-Colley and Smith, 2001). The total organic carbon (TOC) content of a sedimentary environment can be used to characterize its nutritional status, food availability, environmental stability, and habitat suitability (Hyland et al., 2005; Li et al., 2016). Although there is a certain correlation between TOC and food sources, it is neither absolute nor precise, and can only be used as a basis for identifying potential food sources. Total organic carbon (TOC) includes both easily decomposable and refractory components, and the total amount of TOC does not directly reflect the

amount of active organic carbon available to infauna. This study demonstrates that it is only the easily decomposable components of TOC that serve as effective carbon and food sources for *Perinereis* (Li et al., 2022; Wang et al., 2024). Analysis of six *Perinereis* distribution stations in the intertidal zone of the Pearl River Delta (PRD) showed no significant correlation between physicochemical factors and bioturbation intensity. This differs from the traditional view of 'multi-factor synergistic regulation'. However, this conclusion is limited by the regional sedimentary environment and sample size, and is therefore mainly applicable to the study area. Spearman rank correlation analysis using Origin software (N = 6) revealed that only mean grain size was strongly negatively correlated with bioturbation rate (r_s = -0.74, P = 0.09). While this did not reach the significant level of P<0.05, P<0.1 indicates that the bioturbation rate decreases with increasing mean grain size. There were extremely weak correlations between the bioturbation rate and TOC (r_s=0.20, P=0.70), turbidity (r_s=0.37, P=0.47) and salinity (r_s=0.37, P=0.47), and these were not statistically significant. Overall, mean grain size is the environmental factor with the strongest correlation, while the regulatory effect of other factors is insignificant (Figs. 10A–D). This may be related to infaunal dependence on sediment physical characteristics. Fine-grained sediments facilitate drilling and the stability of dwelling tubes, which can increase the intensity of sediment bioturbation. Meanwhile, the loose structure of coarse-grained sediments is not conducive to burrow maintenance, leading to a decrease in bioturbation rates (Ho et al., 1997). Therefore, mean grain size is the main factor affecting disruption behavior, with the roles of TOC, turbidity and salinity being relatively indirect or offset by flexible feeding strategies (Dorgan et al., 2008).

Figure 10 – Relationship between TOC content (A), mean grain size (B), turbidity (C), salinity (D), and sediment disruption at stations where *Perinereis* was present in the Pearl River Delta tidal flats. Red stars denote sediment disruption at stations where *Perinereis* was present.

As typical biogenic traces, the burrows of the polychaete *Perinereis* preserve behavioral responses to environmental conditions (Kulkarni and Panchang, 2015) Burrow density is sensitive to population dynamics and short-term catastrophic events; a sudden decrease may indicate population collapse caused by anoxia, toxic pulses, or rapid sediment burial (Bromley and Ekdale, 1984; Dworschak and Rodrigues, 1997; Martin, 2004). Burrow diameter reflects individual physiological and behavioral adjustments and may indicate chronic, sublethal environmental stressors (Rodríguez-Tovar et al., 2014; Blankson et al., 2017; Koo and Seo, 2017; Tian et al., 2019).

CONCLUSION

This study examined the burrow systems of *Perinereis* in the intertidal zones of the Pearl River Delta (PRD), China. The results suggest that the benthic burrows of *Perinereis* exhibit a wide range of morphologies, including simple cylindrical, Y-shaped, U-shaped, and complex reticulated structures, which may exhibit local swellings at branch points. This morphological variation reflects the organism's ability to flexibly adjust its behavioral patterns in response to local microenvironmental conditions. Salinity was identified as the environmental factor significantly correlated with burrow diameter, with a nonlinear, saturating relationship: low to moderate salinity promotes burrow

enlargement, whereas burrow diameter tends to stabilize at high salinity levels approaching the species' physiological tolerance limit. However, salinity had no significant effect on burrow density.

Neither burrow diameter nor sediment bioturbation showed a significant correlation with single factors such as sediment grain size, salinity, turbidity, or total organic carbon (TOC) content. This suggests that these parameters are jointly regulated by biotic interactions and multiple environmental factors. The bioturbation rate was strongly negatively correlated with mean grain size, indicating that mean grain size is the primary factor controlling *Perinereis* bioturbation behavior. The influences of TOC, turbidity and salinity are relatively indirect, or may be offset by the infaunal flexible feeding strategies.

In terms of potential paleoenvironmental implications, the morphology of *Perinereis* burrows may indicate low-energy, nutrient-rich microenvironments in ancient intertidal zones. Differential responses in burrow density and diameter may reflect population dynamics and long-term environmental stress in paleo-ecosystems, which may offer preliminary insights for future paleoenvironmental reconstruction studies.

AI USE DISCLOSURE

During manuscript compilation, AI resources were only utilized to refine English diction and enhance overall manuscript legibility. All research conceptualization, original text composition, experimental design and statistical data analysis were accomplished independently by the author team; every figure and table was self-generated, with no artificial intelligence adopted for graph drafting or computational processing.

DATA AVAILABILITY STATEMENT

The authors confirm that all relevant data utilized in the research described herein are included within the article.

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AUTHOR CONTRIBUTION

Y.Y.W.: Conceptualization; Investigation; Data curation; Formal analysis; Visualization; Writing – Original draft preparation.
Z.Z.: Methodology; Formal analysis; Data curation; Validation; Writing – Reviewing and Editing.
M.M.: Investigation; Data curation; Methodology; Validation.
Y.M.Z.: Supervision; Methodology; Data curation; Validation; Writing – Reviewing and Editing.
G.C.Z.: Supervision; Project administration; Writing – Reviewing and Editing.

All authors: Approved the final version of the manuscript.

CONFLICTS OF INTEREST

We declare that we have no financial or personal relationships with other people or organizations that could inappropriately influence our work. There are no professional or other personal interests of any nature.

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