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Variations of sediment toxicity in a tidal Estuary: A case study of the South Passage, Changjiang (Yangtze) Estuary

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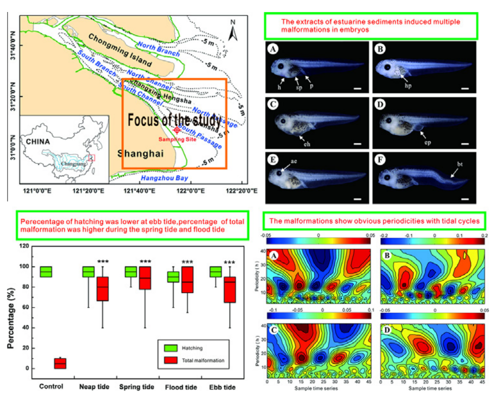
HIGHLIGHTS

- The extracts of estuarine sediments induced multiple malformations in embryos.
- The sediment toxicity was higher during the spring tide and flood tide.
- The phenotypes of malformation show obvious periodicities with tidal cycles.
- The toxicities were impacted by grain fraction, sediment resuspension.

GRAPHICAL ABSTRACT



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ABSTRACT

Sediments in estuaries, especially those containing a large reservoir of contaminants released from urban and industrial activities, have had great impacts on benthic fauna and associated species. A better understanding of the toxicity of contaminants in estuarine sediments is of great significance to ecological assessments. Here, based on the collected sediments from neap to spring tides in the South Passage, Changjiang Estuary, the toxicity of the sediments was first studied using the frog embryo teratogenesis assay—*Xenopus* (FETAX). The results showed that the extracts of estuarine sediments induced multiple malformations in the embryos and that the phenotypes of malformation had two distinct patterns of variations corresponding to the tidal cycles. The phenotypes in the first pattern were dominated by hypopigmentation and edema of the heart, and the pattern was mainly controlled by fine-grained fractions. The phenotypes in the second pattern were dominated by edema of the heart and enlarged proctodeum, and it was mostly controlled by coarse-grain fractions. The sediment toxicity was higher during the spring and flood tides, which may be influenced by the grain size and sediment resuspension. Furthermore, obvious periodicities existed in the changes of the percentages of hatching (14–16 h and 6 h), enlarged proctodeum (15–18 h), and bent tail (5–7 h) due to the influence of tidal cycles. Moreover, our results also suggested that FETAX is an appropriate cost-effective biological monitoring tool to assess estuarine ecological health in contaminated sediments.

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1. Introduction

Estuaries are formed in the narrow zone at the land–ocean margins, and they are environmentally sensitive and vulnerable due to combined influences of human activities, such as damming of the drainage basin and extraction of natural gas, water in the coastal area, natural processes including the effects of compaction of the sediment column and global sea-level rise (Ericson et al., 2006; Syvitski et al., 2009; Dai et al., 2014a). Most estuaries around the world have been facing serious challenges since the anthropogenic era due to the large-scale release of contaminants from upstream urban and industrial activities. As a great reservoir of contaminants, sediments from estuaries create potential exposure to toxic chemicals for organisms living in both the sediments and water-column (Birch, 2000; Dafforn et al., 2012).

However, sediments can also be a source of contaminants when they are disturbed or relocated in estuarine ecosystems (Latimer et al., 1999; Roberts, 2012). Contaminants that are resuspended or released from sediments may also be available for uptake by aquatic organisms with further impacts on estuarine species (Eggleton and Thomas, 2004; Roberts, 2012). Thereafter, contaminants in sediments from the estuaries pose great impacts on benthic fauna and associated species. A better understanding of the toxicity of contaminants in estuarine sediments is of vital importance to ecological assessments.

Recent efforts have aimed at comparing the efficacy of different monitoring tools to assess the toxicity of contaminants in sediments (Luoma et al., 2010). Chemical monitoring tools are usually used in impact assessments with a particular type of contaminant or exposure media, such as sediments and water samples (Birch, 2000). However, biomonitoring tools, which are increasingly applied in the evaluation of contaminant hazards in a number of areas, are more accurate representations of potential exposure than chemical measurements of the exposure media (Romeo et al., 2003; Dafforn et al., 2012). Acute bioassay tools for estuarine sediments have been designed for algae, amphipods, copepods, sea urchins and fishes (Moreno-Garrido et al., 2007; Bellas et al., 2011). However, little work has been conducted on the variation of toxicity in sediments from estuarine environments by the frog embryo teratogenesis assay—*Xenopus* (FETAX).

FETAX is a high-throughput toxicological test used in several ecotoxicology applications to determine the toxicities of common environmental contaminants, such as heavy metals (Bosisio et al., 2009). In addition, FETAX can be used to determine the ecotoxicities of environmental samples (de Lapuente et al., 2014). *Xenopus tropicalis* is an emerging animal model in developmental biology and ecotoxicology (Berg et al., 2009). This frog is closely related to *Xenopus laevis* with the advantages of a smaller size and shorter life cycle compared with this congener (Hirsch et al., 2002; Shi et al., 2013).

The Changjiang Estuary is the largest estuary and an important industrial center in China (Fig. 1). The morphodynamic patterns of the Changjiang Estuary can be described as "three-order bifurcations and four-outlet diversions into the sea" (Fig. 1). The estuary is a meso-tidal estuary with average tides ranging from 2.4 to 3.2 m (Yan et al., 2011). It is endowed with abundant fish resources (Ostrach et al., 2008; Du et al., 2013). In recent years, however, the amount of pollutants has significantly increased due to the influence of anthropogenic activities (Dai et al., 2011). Consequently, many problems (e.g., severe eutrophication and an expanding hypoxic zone) frequently occurred in the estuarine area (Kemp et al., 2009; Corbett, 2010; Dai et al., 2011). It is difficult to distinguish the specific components of the contaminants in sediments due to the complicated hydrodynamic conditions in the Changjiang Estuary. Because the sediments of the Changjiang Estuary contain a great reservoir of contaminants with environmental indicators for anthropogenic impacts in estuaries, monitoring the toxicity of the contaminants in Changjiang estuarine sediments should provide important evidence on how they affect benthic communities (Dafforn et al., 2012).

In this study, FETAX was used to evaluate the temporal variations of developmental toxicity of sediments from the South Passage, Changjiang Estuary. The patterns of phenotypes of malformation induced by the sediment extracts were explored. Moreover, the influences of grain size, resuspension and tidal cycles on toxicity of sediments are also discussed.

2. Materials and methods

2.1. Sample collection

The sampling site (31°06′51″N and 121°54′41″E) is located in the South Passage, which is one of the four outlets in the Changjiang Estuary. Hourly surface sediment samples and bottom water were collected during the neap tide (16–17 June) and spring tide (22–23 June) in 2013. Water depth, current velocity, and direction of the sampled site were measured by ADCP (Acoustic Doppler Current Profilers) at the same time. Sediment samples for toxicity analysis were collected by a grab sampler from the top 5–10 cm of the surface sediment, and then the sediment samples were packaged in labeled polyethylene bags. Simultaneously, the bottom water for suspended sediment concentration (SSC) analysis was collected from the 30–50 cm above the sediment surface using a hydrophore and pre-cleaned glass bottles (500 ml). All sediment and water samples were refrigerated and subsequently shipped to the laboratory and stored at 4 °C.

2.2. Analysis of the developmental toxicity of sediments

The analysis of the developmental toxicity of sediments can be divided into three steps. First, the sediment extracts were prepared. Based on the methods proposed by Fort et al. (2001), 400 ml of FETAX solution was mixed with 100 g of sediment in a 500 ml brown glass bottle. The aqueous soil mixtures were then tumbled in a rotary extractor for 48 h at 30 ± 2 rpm and at 22 ± 2 °C under dark conditions. The tumbled samples were allowed to settle overnight at 4 °C. The samples were then centrifuged for approximately 20 min at 8000 rpm. The extract was decanted and stored at 4 °C prior to toxicological testing.

Second, the embryos were exposed to the extracts of sediments. The use of live organisms in this study followed the protocols approved by the Science and Technology Commission of Shanghai Municipality. The husbandry of *X. tropicalis* adults and breeding of the experimental animals were performed as described previously (Guo et al., 2010). The exposure experiments were conducted following FETAX, with some modifications (ASTM, 1998). A total of 5 ml of sediment extract was put into each well of a plate with 24-well plates. One Ringer's solution medium control was run. Ten embryos were placed in each well. Four replicates were used in the control and each treated group. The plates were incubated at 26 ± 0.5 °C for 24 h under dark conditions, and the medium was renewed at 24 h intervals (ASTM, 1998).

Finally, the embryos were observed and measured. After 48 h of exposure, the surviving embryos were collected from all of the groups and anesthetized with 100 mg L^{-1} MS-222 and then fixed with 4% formalin for 24 h. The embryos were observed under an Olympus SZX16 dissecting microscope (Olympus, Tokyo, Japan), and images were taken with an Olympus DP 25 camera. The main phenotypes represented by the observed malformations were determined.

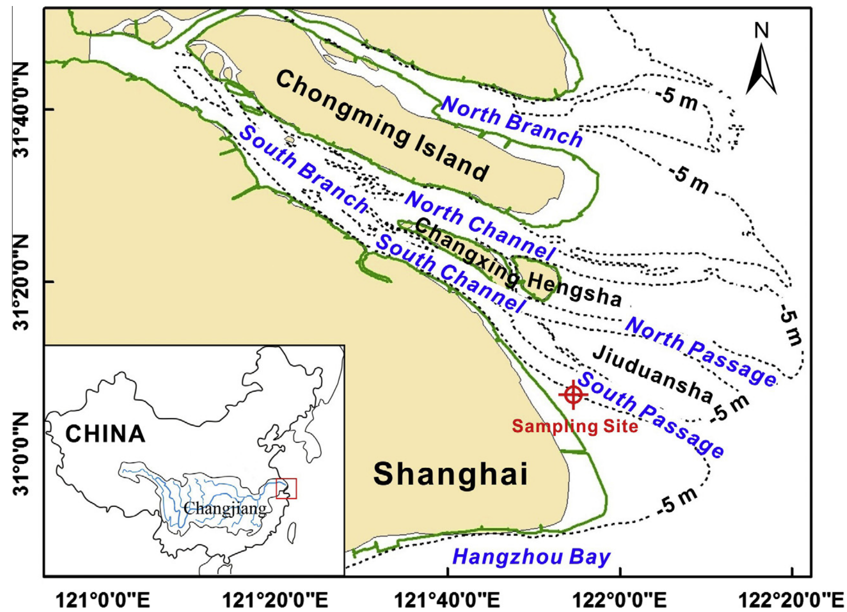

Fig. 1. Location of the Changjiang Estuary and sampling site.

2.3. Data processes and analysis

Empirical Orthogonal Function (EOF) and wavelet analysis were used in this study to analyze the toxicity periodicities and malformation patterns varied with tidal cycles, respectively. The aim of EOF is to find a relatively small number of independent variables that describe as much of the original information as possible (Dai et al., 2013), and it has been widely used in studying the estuarine environment and sediment dynamics (Zarillo and Liu, 1988; Emery and Thomson, 2001; Dai et al., 2010, 2013). Thereby, EOF analysis was used to investigate the dominant patterns of the malformations, as they varied with tidal cycles (in Supplementary material). Subsequently, the wavelet technique (Dai et al., 2014b) was applied to analyze the possible periodicities of the sediments toxicity with tidal cycles because it is a powerful tool in detecting signals and is described as a microscope of signals (Torrence and Compo, 1998). More detailed calculations by wavelet analysis can be found in a previous reference (Torrence and Compo, 1998) (in Supplementary material).

Meanwhile, Boxplot was used to compare the percentages of hatching and total malformation in the different tidal stages. A one-way analysis of variance (ANOVA), followed by Dunnett's test, was also used to determine the significant differences for variables in different tidal stages compared with the control. Moreover, the suspended sediment concentration (SSC, kg m^{-3}) of each bottom water sample was measured by filtering and drying the water samples. The calculation of grain size in the sediments was based on Folk et al. (1970), and subsequently sediment components were obtained by the Udden–Wentworth grain size classification scheme (Wentworth, 1922) (in Supplementary material).

3. Results

3.1. Hydrological characteristics

The tidal current in the South Passage is asymmetric because the flood tide duration is shorter than the ebb tide (Fig. 2A). The water level ranged from 6.44 to 8.94 m in the neap tide and from 5.86 to 10.05 m in the spring tide (Fig. 2A). The current velocity ranged from 12.16 to 112.07 cm s^{-1} in the neap tide and from 3.64 to 130.15 cm s^{-1} in the spring tide. Moreover, the velocity

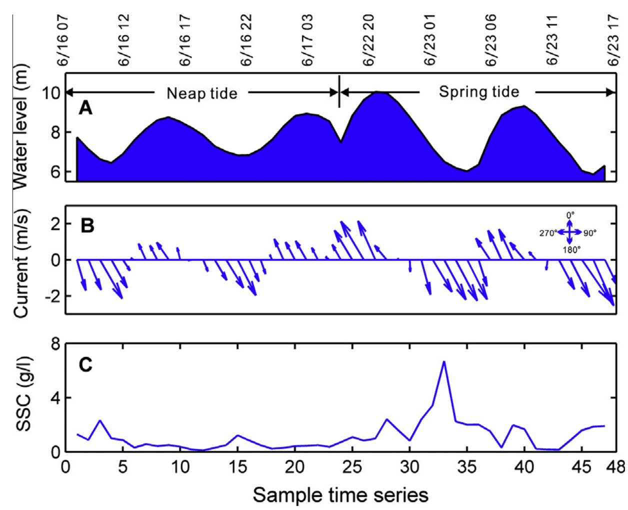

Fig. 2. Time series of water level, current vector and SSC during the study period. The length of the arrow represents the speed of the current, and the angle of the arrow is the direction of the current.

during the ebb tide is much higher than that during the flood tide (Fig. 2B). SSC ranged from 0.12 to 2.34 mg L^{-1} in the neap tide and 0.17 to 6.69 cm s^{-1} in the spring tide, with averages of 0.63 mg L^{-1} and 1.65 mg L^{-1}, respectively (Fig. 2C). In addition, high SSC occurred when the velocity was high (Fig. 2B and C).

The mean grain size of sediments over the study period, including neap and spring tide, was 12.72 μm (Table S1 in Supplementary material). Compared with the sediments during the neap tide, the spring tide presented a higher percentage of fine-grained fractions (clay and fine-silt). Moreover, the grain size ranged from 10.50 to 23.25 μm during the neap tide and from 6.66 to 18.84 μm during the spring tide. The sediments during spring tide were finer than those of neap tide (Table S1 in Supplementary material).

3.2. Phenotypic characteristics

Multiple malformations were observed in the experimental groups treated with sediment extracts. The main phenotypes of

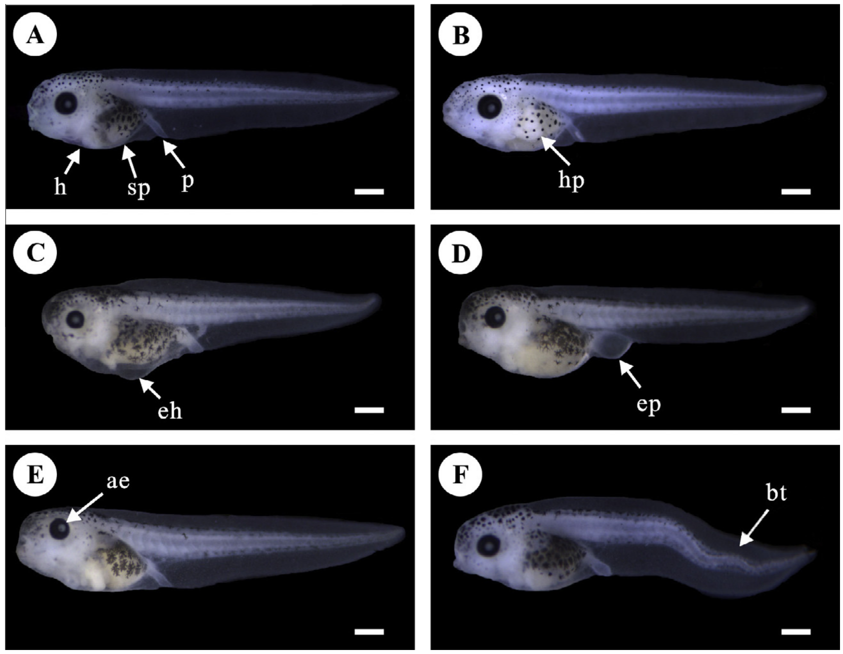

Fig. 3. Morphological photographs of *X. tropicalis* embryos in the control (A) and treatment (B–F) groups after 48 h exposure (ae: abnormal eye, bt: bent tail, eh: edema of the heart, ep: enlarged proctodeum, h: heart, hp: hypopigmentation, sp: skin pigmentation, p: proctodeum, and scale bar: 1.0 mm).

malformations included abnormal eye, bent tail, edema of the heart, enlarged proctodeum and hypopigmentation (Fig. 3). The dominant phenotypes of malformation were hypopigmentation (65%) and edema of the heart (42%), followed by enlarged proctodeum (14%) (Fig. S1 in Supplementary material). During the neap and spring tides, the percentage of hatching showed slight fluctuations, while the percentage of malformations had significant variations (Fig. S1 in Supplementary material).

3.3. Patterns of malformations

EOF analysis showed that the first two eigenvalues explained approximately 83% and 13% of the total variation of malformations during the observation period. Sediments from the South Passage induced two patterns of malformations that varied with time, which can also be described as the principal components of the malformations (Fig. 4). The first pattern was dominated by hypopigmentation and edema of the heart with positive eigenvalues (Fig. 4A). The temporal eigenfunction presented higher values during the spring tide than the neap tide (Fig. 4B). The second pattern was dominated by edema of the heart and enlarged proctodeum (Fig. 4C). The temporal eigenweightings during the spring tide were much lower than those during the neap tide (Fig. 4D).

3.4. Variations in percentages of hatching and malformations

Compared with the control test baseline, no significant difference of the percentage of hatching was observed, while clear changes in percentage (with significant test, $P < 0.001$) of total malformation were detected in the different tidal stages based on ANOVA (Fig. 5). The percentage of hatching was higher during the ebb tide than flood tide. The percentage of total malformation

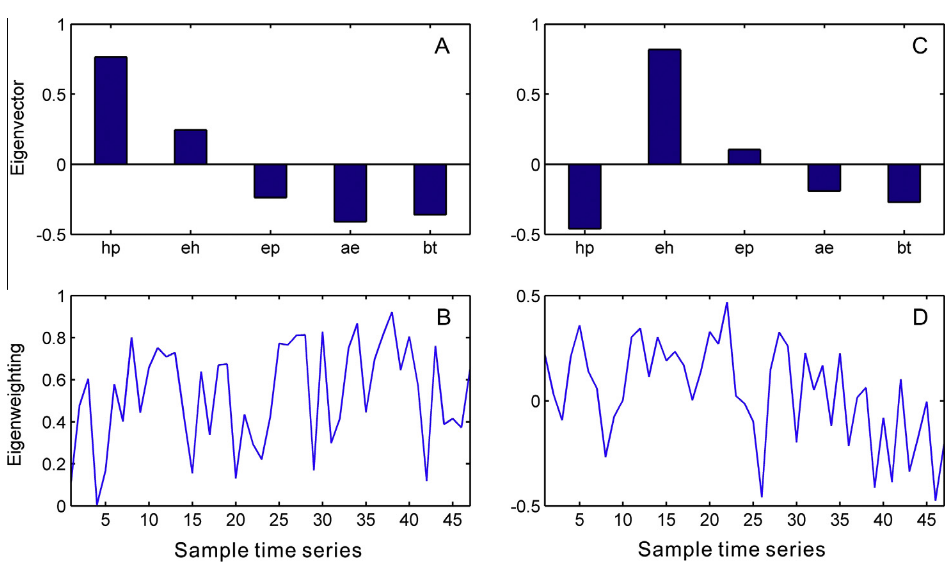

Fig. 4. Results of total percentage of malformations using EOF.

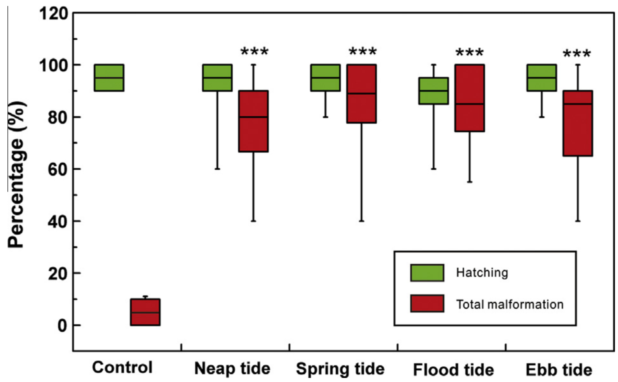

Fig. 5. Variation in percentages of hatching (%) and total malformation (%) during the different tide stages. The box extent and line inside the box denote the 25th and 75th and 50th percentiles. Whiskers denote the maximum and minimum values. One-way analysis of variance (ANOVA), followed by Dunnett's test, was used with $^{***}P < 0.001$ compared to the Ringer's solution medium control group.

was higher during the spring and flood tides than the neap and ebb tides (Fig. 5).

3.5. Periodic changes of sediment toxicity

Toxicities periodicities are the oscillations of the toxicities presented by the contaminants in sediments with the tidal cycles. The wavelet analysis showed that obvious periodicities existed in the changes of the percentages of hatching (14–16 h and 6 h), enlarged proctodeum (15–18 h), and bent tail (5–7 h). However, the periodicity of hypopigmentation was inconsistent (Fig. 6).

4. Discussion

The sediments from the South Passage of the Changjiang Estuary showed distinct developmental toxicity with two patterns of malformation during the observation period. The developmental toxicity of the sediments was higher during the spring and flood tides. Here, the effects of grain size and resuspension on the toxicity of the sediments are further explored, and the tidal cycle influence is also determined.

4.1. Impacts of grain size of sediments

The sedimentary characteristics are important to evaluate the contaminant conditions in estuaries. Studies have proved that many contaminants are closely associated with fine particles in estuarine and coastal environments (Olsen et al., 1982; Gao et al., 2013).

In this study, our analysis suggested that the sediment toxicity from the South Passage was related to the grain size (Table S2 in Supplementary material). Hypopigmentation, the dominant malformation of the first pattern, was positively correlated with fine-grained fractions (clay and fine silt) ($P < 0.05$) (Table S2 in Supplementary material). Sediments were finer during the spring tide than those of the neap tide (Table S1 in Supplementary material). Consequently, hypopigmentation increased during the spring tide, indicating that the first malformation pattern was controlled by fine-grained fractions (Fig. 4B). However, edema of the heart and enlarged proctodeum, the main malformations of the second pattern, showed inverse correlativity with grain size (Table S2 in Supplementary material). Hence, edema of the heart and enlarged proctodeum significantly decreased during the spring tide, suggesting that the second pattern of malformations was mainly controlled by coarse-grained fractions (Fig. 4D).

4.2. Impacts of resuspension actions

Resuspension of contaminated sediments is an important source of contaminants in historically contaminated estuaries (Riggsbee et al., 2008; Kalnejais et al., 2010). Here, the mean bottom velocity of ebb tides was higher than that of flood tides, suggesting that sediments are more likely to suspend during ebb tides (Fig. S2A and C in Supplementary material). This was consistent with the results that suspended sediment concentrations (SSC) were higher during ebb tides than flood tides (Figs. S2B and D in Supplementary material), which might induce higher concentrations of contaminants in suspended sediments during ebb

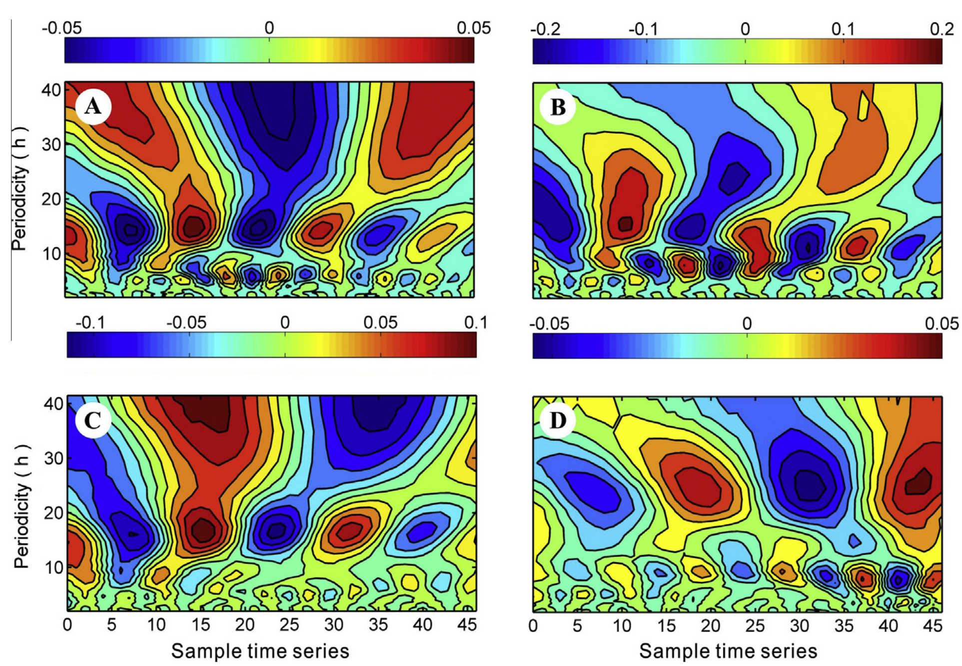

Fig. 6. Periodic changes in the percentages of hatching (A), hypopigmentation (B), enlarged proctodeum (C) and bent tail (D) with wavelet analysis.

tides (Fig. S2 and Table S3, in Supplementary material). Resuspension of sediments may lead to the coarsening of the bed-located sediments, and coarse-grained sediments tend to have relatively low toxicity. This indicates that the sediments of ebb tides showed lower toxicity than those of flood tides. Meanwhile, the sediments during spring tide were finer than those during neap tide, indicating the spring tide sediments tend to show relatively higher toxicity due to the impacts of relatively fine grain size components in the sediments.

4.3. Impacts of tidal cycle

Continuous tidal cycle may lead to significant fluctuations of water level and hydrodynamic conditions, thereby controlling the sediment process in estuaries. Consequently, tidal currents have potential influence on sediment-bound contaminants, which could potentially affect the periodic changes of contaminants in sediments (Cailleaud et al., 2009; Guédron et al., 2012). The South Passage is dominated by an irregular semi-diurnal tide. Different tidal durations indicate variations of tidal cycles. The specific periodicities of sediment toxicity found in this study might be due to impacts by the tidal cycles (Fig. S2 in Supplementary material).

As mentioned above, grain size, sediment resuspension and tidal cycles may influence the contaminants and toxicity of sediments. Therefore, it is necessary to consider the system hydrodynamic characteristics in aquatic environment research, which have been ignored by many studies.

5. Conclusions

In brief, our results suggest that the extracts of sediments from the Changjiang Estuary induced special multiple malformations in embryos and that the phenotypes of malformation had two distinct patterns of variations based on the tidal cycles. A correlation analysis indicated that the two patterns were mainly controlled by fine-grained and coarse-grained fractions. Furthermore, the toxicity of the sediments was higher during the spring and flood tides than during the neap and ebb tides, which could be induced by the estuarine sediment resuspension actions. Moreover, our results also suggest that the toxicity in estuarine sediments can be monitored by high-trough tests (e.g., FETAX). Since resuspension and tidal cycles are common in large estuaries around the world, our study should be significant for the monitoring and assessment of areas with similar estuarine environments.

Acknowledgements

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2015.01.007.

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