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E-mail: hyxbe@263.net

Estimating the macrobenthic species richness with an optimized sampling design in the intertidal zone of Changjiang Estuary

Tingting Zhang[1], Feng Zhao[1], Sikai Wang[1], Tao Zhang[1], Jianyi Liu[1], Yu Gao[1], Ping Zhuang[1]*

[1] East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences; Key Laboratory of Fisheries Ecology of the Yangtze Estuary; Scientific Observing and Experimental Station of Fisheries Resources and Environment of East China Sea and Yangtze Estuary, Shanghai 200090, China

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Abstract

It is a challenge in the field sampling to face conflict between the statistical requirements and the logistical constraints when explicitly estimating the macrobenthos species richness in the heterogeneous intertidal wetlands. To solve this problem, this study tried to design an optimal, efficient and practical sampling strategy by comprehensively focusing on the three main parts of the entire process (to optimize the sampling method, to determine the minimum sampling effort and to explore the proper sampling interval) in a typical intertidal wetland of the Changjiang (Yangtze) Estuary, China. Transect sampling was selected and optimized by stratification based on pronounced habitat types (tidal flat, tidal creek, salt marsh vegetation). This type of sampling is also termed within-transect stratification sampling. The optimal sampling intervals and the minimum sample effort were determined by two beneficial numerical methods: Monte Carlo simulations and accumulative species curves. The results show that the within-transect stratification sampling with typical habitat types was effective for encompassing 81% of the species, suggesting that this type of sampling design can largely reduce the sampling effort and labor. The optimal sampling intervals and minimum sampling efforts for three habitats were determined: sampling effort must exceed 1.8 m^2 by 10 m intervals in the salt marsh vegetation, 2 m^2 by 10 m intervals in the tidal flat, and 3 m^2 by 1 m intervals in the tidal creek habitat. It was suggested that the differences were influenced by the mobility range of the dominant species and the habitats' physical differences (e.g., tidal water, substrate, vegetation cover). The optimized sampling strategy could provide good precision in the richness estimation of macrobenthos and balance the sampling effort. Moreover, the conclusions presented here provide a reference for recommendations to consider before macrobenthic surveys take place in estuarine wetlands. The sampling strategy, focusing on the three key parts of the sampling design, had a good operational effect and could be used as a guide for field sampling for habitat management or ecosystem assessment.

Key words: species richness estimation, sample strategy, transect sampling optimization, Monte Carlo simulation, species accumulative curves, Changjiang (Yangtze) Estuary

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1 Introduction

Determination of the richness and abundance of macrobenthic species is important in both benthic ecology and conservation programs, as it provides data for the calculation of biodiversity, biomass, and productivity as well as for the estimation of population trends in habitat management and assessment of ecosystem status (Aarnio et al., 2011; Schooler et al., 2014). Changes in the macrobenthos community integrate their responses to ecosystem function, food web dynamics, and resilience to climatic or anthropogenic environmental change on a variety of temporal and spatial scales (Costa et al., 2001; Schooler et al., 2014). Thus, the determination of the richness and abundance of the macrobenthos has also been used as a good indicator while assessing the ecological health of benthic ecosystems (Dowd et al., 2014; Ferguson and Rakocinski, 2008).

Species abundance is usually measured as the number of individuals found per sample, but species richness is quantified by a set of samples. Thus, the macrofaunal abundance would be informatively derived with data from every sample from projection approaches such as geostatistics methods. However, such approaches do not provide a solution to the problems encountered with estimating species richness (Shen et al., 2012). Quantitative assessment of species richness is usually accomplished by careful sampling designs, which requires an adequate number of samples.

On the other hand, it is difficult to explicitly determine the macrobenthos species richness in benthic areas, especially in the intertidal zone, as macrobenthos survey data are limited to low levels of sampling efforts in such regions. One reason is that the intertidal zone is located at the interface between the ocean and the land, which is affected by the combined interrelations between vegetation, currents, tides and salinity. The intertidal zone exhibits considerable spatial and temporal habitat heterogeneity at various scales (Jordana, 1991). Induced by these environmental variables, such as substrate type, elevation, slope, food

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*Corresponding author, E-mail: pzhuang@ecsf.ac.cn

availability and predation, the macrobenthic species inhabiting the intertidal zone are prone to aggregate on a small scale (Braga et al., 2011; Moodley et al., 1998). As a result, the macrobenthos tend to be patchily distributed and vary considerably in heterogeneous intertidal zones. This patchiness, coupled with the small size of most macrobenthos and their rapid burrowing, introduces considerable difficulties when designing sampling in the field, such as yielding a dataset with many "zero-counts" (Schlacher et al., 2008). Another difficulty with sampling in these intertidal areas is that the physical environment is harsh for field sampling. For example, the soil of the middle and low tidal belts in a typical tidal flat is always characterized by mud, which tends to be trapped, and most low tidal areas are often covered with tidal water in the spring tide or flood time. Moreover, the vegetation communities become very bushy and tall during the growing season, and wide tidal creeks are very hard to cross (Fig. 1).

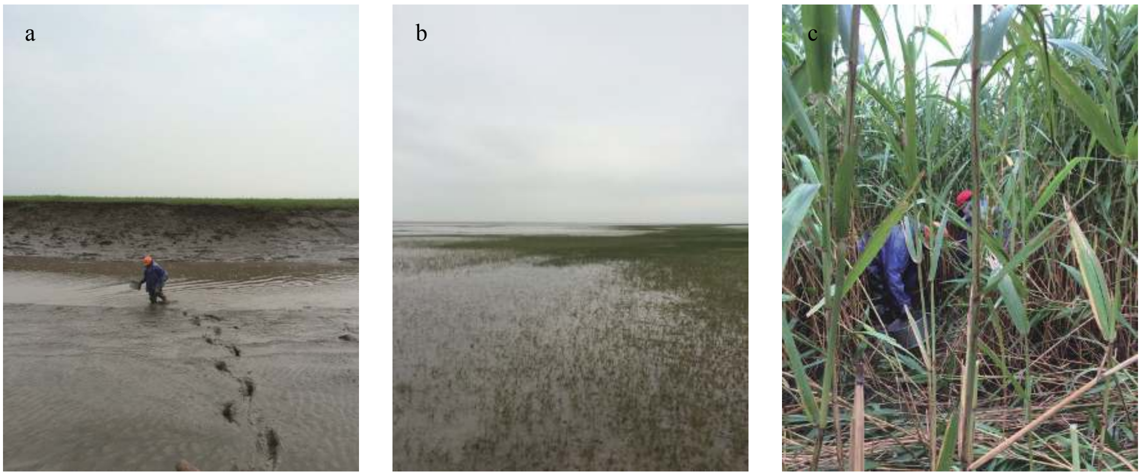

Fig. 1. The unique characteristics of intertidal marsh wetland of estuary. a. Mud easy to be trapped in and wide tidal creek hard to cross, b. flooded low tidal areas, and c. bushy salt marsh vegetation.

In this context, the sampling strategy should be carefully treated and based on theoretical principles or standardized approaches with an adequate number of samples; otherwise, the collected samples will produce a large bias in the results for estimation (Beukema and Dekker, 2012). On the other hand, the consideration of labor and cost effort should also be taken into consideration when determining the sampling design, making sure that these are practical in the field sampling. The assessment of macrobenthic species richness needs a compromise between basic statistical requirements and the logistical constraints of field operations in intertidal areas, which strongly depends on the design of the sampling strategy.

Generally, the design of the sampling strategy contains three main parts: to determine the sampling approach, to define the sample size and to arrange the sampling interval. Other aspects easy to judge according to specific situations, such as sample device, mesh size, sampling time, and sampling depth, are not discussed in this study (Fanini and Lowry, 2016; Ferraro and Cole, 2004; Ferraro et al., 2006; Hammerstrom et al., 2012; Schlacher et al., 2008; Schlacher and Wooldridge, 1996; Souza and Barros, 2015). First, sampling methods include a range of standardized approaches that have already been applied to the estimation of species richness, such as random (Shen et al., 2012), stratified (Schooler et al., 2014), systematic (Dowd et al., 2014) and transect sampling designs (Beukema and Dekker, 2012). Each method has its own strengths and weaknesses related to the sampling area, efficiency, labor and cost. For intertidal areas, transect sampling has become a well-recognized strategy commonly used to survey macrobenthos (Beukema and Dekker, 2012; Schoeman et al., 2008; Varfolomeeva and Naumov, 2013). Transect sampling in intertidal areas is often considered practical and efficient because these areas are spatially variable, and the variation presents itself as obvious environmental gradients (such as salinities, heights, sediment coarseness) along the axes perpendicular to shore (from high tide level to low tide level). Provided that an adequate number of across-shore transects are sampled, each of the likely across-shore "niches" present on the gradient will be represented in a pool of fewer samples by taking a sequence of samples along an across-shore line (Schlacher et al., 2008). However, transect sampling is performed using numerous and continuous quadrats with the same intervals; thus, it has rarely been adopted in the intertidal zones of estuarine wetlands, as such sampling is not practical in the harsh physical field environment. An optimized transect sampling was designed versus a simple transect survey to solve the difficulties in harsh environments. This approach is called the within-transect stratification sampling design, which has stratification within the transect according to habitats types related to species distribution. It is assumed that the variation in density is small when the collection of habitat types within the transect is the same. Skibo (2005) applied this within-transect approach with other routine methods (e.g., random sampling, simple transect sampling) to estimate the richness of red sea urchins in benthic subtidal areas by stratifying according to the substrate types of the strata. The results showed that this approach performed better in the sampling effort, precision and marginal cost, as it captured all aggregation patterns of urchins within a transect that may exist in different substrate types. Second, sampling size has become an important issue, as it provides sufficient precision of the "true" species richness. Until now, some studies still followed the sample size conventions of the sampling team (e.g., three to five samples at each tidal level along the high-low tide transects) (Chen et al., 2009; Lv et al., 2014, 2016; Wang et al., 2010). This tended to result in negatively biased estimates for macrofaunal richness as accuracy decreased at low levels of sampling effort (Schoeman et al., 2003). Fortunately, more and more researchers have explored the relationship between total sampling effort and macrobenthic species richness by using extrapolation procedures, such as species accumulation curves (Beukema and Dekker, 2012; Muxika et al., 2007; Schoeman et al., 2003, 2008; Schooler et al., 2014). Jaramillo et al. (1995) recommended that a sampling effort of 4 m^2 would be appropriate for estimating macrobenthic richness in an intertidal sandy beach by using species accumulative curves. In addition to the ability to determine the minimum sampling effort based on a balance between accuracy, bias, and precision, the advantages of this method include that it provides more accurate estimates of species richness than observed values and allows for the comparison of species richness on different spatial or temporal scales (Colwell et al., 2012). Many studies have not paid much attention to the third part, the determination of the sampling interval. Ecologists have traditionally arranged uniform intervals to take a sequence of samples along transects, and these intervals range between 1 and 25 m, depending on the aims of the studies (Schlacher et al., 2008). This fixed interval method, however, is usually irrespective of the heterogeneous environmental gradient (morphology, sediment, vegetation distribution), inappropriate to capture species patchiness distributed at a small scale, and likely to result in spatial autocorrelation among the individual samples (Schlacher et al., 2008). Instead of arbitrarily determining fixed sampling intervals, Schoeman et al. (2003) applied a repeated resampling technique of datasets, Monte Carlo simulation, to gain an understanding of the range of results that might be possible for any given sampling method under consideration and to determine the proper sampling intervals and quadrat numbers needed for sampling a sandy beach.

Although many studies have contributed a lot to each part of the sampling design, it is noted that most researchers were prone to focus on one or two aspects, versus the entire process, of the sampling design (Ferraro and Cole, 2004; Schoeman et al., 2003, 2008; Schooler et al., 2014; Skibo, 2005). Without a comprehensive conception of the entire process, the applied methods are not complete, and the conclusions cannot be directly applied to other sampling areas in the field. In this case, the adopted sampling strategy may be not the optimum one. A comprehensive understanding of key parts of the entire sampling design process is therefore required, and an operational and standardized technological strategy needs to be formed in intertidal areas. Meanwhile, the sampling design should be an optimal one that facilitates the provision of good estimates and takes into account the difficulties of sampling in the field.

Based on the practical difficulties of field sampling in the intertidal salt marsh and the previous neglect for the entire process of the sampling strategy, we raised a scientific question. The aim of this study is to explicitly estimate the macrobenthos species richness in the estuarine wetland ecosystem with an optimum and comprehensive sampling strategy. This study focused on the three main parts of the sampling design and tried to provide an optimized, efficient and practical sampling strategy for a typical intertidal wetland in the Changjiang Estuary, China, known as the most famous estuary in the world. In selecting a sampling method and statistical methods, the advanced within-transect stratification, the species accumulation curves and the Monte Carlo simulation were applied to optimize the sampling method, determine the minimum sampling effort and explore the appropriate sampling interval for this study. The intertidal areas in the Changjiang Estuary wetland are characterized by three pronounced habitat types: salt marsh vegetation, mud flats and traversed tidal creeks. Each habitat type is combined with interactions of the gradients of vegetation types, substrate types, salinity or elevation (Ma et al., 2011; Xue et al., 2016). The distributions of the macrobenthos are closely related to the three habitats (Quan et al., 2016). This allows using within-transect stratification sampling to investigate species richness by classifying three strata according to the three typical habitat types. Then, by using Monte Carlo simulations, data collected were used to simulate several sampling intervals from which the optimal sampling interval was chosen for each habitat stratum. The minimum sampling effort was investigated by using accumulative species curves while also considering what the optimal sampling intervals were in each habitat type. Through our study, a comprehensive and directly usable strategy of field sampling can be provided when estimating macrobenthic richness in intertidal areas, a strategy which balances the difficulties found in real-world situations (labor and cost) and the statistical requirements (accuracy and precision). The methods and the recommendations provided in the conclusions can be used as a guide and are operational with significance in field sampling and monitoring for habitat management or ecosystem assessment.

2 Methods

2.1 *Study area*

In this study, the intertidal macrobenthos were surveyed on the eastern shoal of Chongming Island, which is located at the mouth of the Changjiang River (Yangtze River) in China and is the largest estuarine alluvial island in the world (Wu et al., 2005). The eastern shoal of Chongming Island, located between 31°25′–31°38′N and 121°50′–122°05′E, is the most mature intertidal wetland in the Changjiang Estuary, an estuary where numerous studies have been carried out in its intertidal wetland for assessing the ecological health by using macrobenthos as indicators (Chao et al., 2012; Lv et al., 2014; Shou et al., 2013; Wang et al., 2010). It is characterized by the Asian monsoon climate and regularly flooded by semi-diurnal meso-tides with amplitudes of 2.45–4.96 m. The south of the eastern shoal of Chongming Island is the closest to original wetland, which was not affected by the reclamation project undertaken between 2013 and 2014. The width of the intertidal zone can reach five kilometers at its maximum, with three pronounced habitats: salt marsh vegetation, tidal flat and tidal creek (Fig. 2). The salt marsh vegetation distributes stripped vegetation, including *Phragmites australis*, *Spartina alterniflora*, *Scirpus mariqueter*, and *Scirpus triqueter* (Lv et al., 2016). There was a visible difference in habitat type between the high and low tidal levels, with salt marsh vegetation during high tide and tidal flat during low tide (Zheng et al., 2016). There was virtually no salt marsh vegetation covering the tidal flat, except for a scattered *Scirpus mariqueter* community. Thus, the macrobenthic sampling in this study mainly took place in the south of the eastern shoal of Chongming Island (Fig. 2).

2.2 *Field and laboratory methods*

Three transects were sampled along high to low tidal levels. Several segment lines were set in each transect according to the habitat stratum to conduct the “within-transect stratification sampling”. One “segment” refers to a specific part of one transect, which is located in one specific habitat. Thus, two to three replicate segments were sampled at each habitat type stratum (salt marsh vegetation, tidal flat, tidal creek) along each transect (Fig. 2). As the distributions of the macrobenthos are closely related to the three habitats (Quan et al., 2016), this allows using within-transect stratification sampling to investigate species richness by classifying three strata according to the three typical hab-

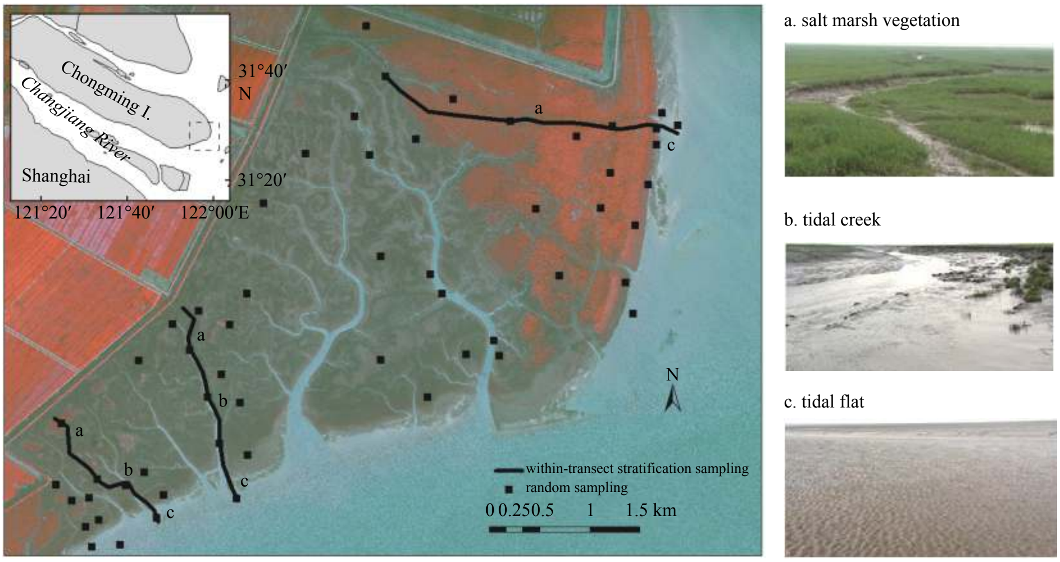

Fig. 2. Study area and the location of the three sampling transects and random sampling sites at large scale. The strata were classified according to the three typical habitats shown as a, b and c. Three salt marsh vegetation, three tidal flat and two tidal creek segments were selected within these transects.

itat types. For the salt marsh vegetation stratum, three segments were sampled, two at high tide and one at middle tidal level. For the tidal creek stratum, two segments were sampled at middle tidal level. For the tidal flat stratum, three segments were sampled, all at low tide. Each line segment comprised contiguous 0.1 m^2 quadrats by using square steel frames (0.32 m by 0.32 m by 0.15 m depth). The continuous sampling in each segment was performed following the routine: three replicates at 1, 2, 3, 5, 10, 15, 20, 50, 100, and 150 m intervals, totaling 3 m^2 per transect (Fig. 3). This is an area close to the referenced sample effort (4 m^2) of macrobenthos in a sandy beach (Jaramillo et al., 1995), and the greatest area that a team of three or four researchers can sample during a single spring low tide. The arrangement of sampling intervals provided enough ranges in which macrobenthic species prefer to aggregate. It was assumed that no migration either into or out of the quadrats occurred during sampling. As the design of sampling strategy was concentrated on sampling interval and sampling effort, the other sampling aspects, such as the season's effect, were be simplified to avoid the variations in this study. All samples were collected around low spring tide and were sampled on five consecutive days. All surveys were conducted from April 18 to April 27, 2016, when macrobenthos are abundant in the region.

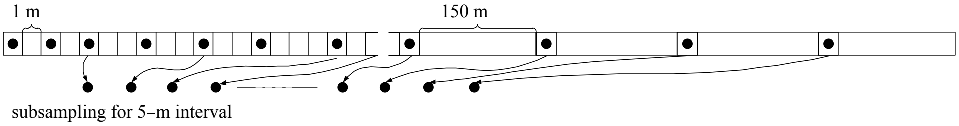

Fig. 3. Subsampling at each segment within each habitat strata, and taking 5-m interval subsampling as an example.

Samples collected in this manner were assigned a unique reference number and sieved through a 0.5-mm mesh to extract resident macrobenthic specimens, which were preserved in buffered formalin for subsequent analysis. In the laboratory, all specimens were examined and identified to the lowest taxonomic level possible and counted.

2.3 *Precision analysis of the within-transect stratification sampling*

To assess the efficiency of the within-transect stratification design in estimating species richness in a precise way, a measure of the true number of species present in the intertidal zone is required. In addition to the samples mentioned above that were collected using the within-transect stratification design, more than 300 samples were collected at 57 sites by random large-scale sampling from June 23 to June 29, 2016 for another general survey (Fig. 2). On this basis, it is possible to treat the samples from the transects as a subset and the samples from the random sampling as the large sample that is closer to the "true number" of species. Thus, the efficiency of within-transect stratification was assessed by comparing that design's results with the species composition of random sampling to determine how well their total number of species match.

2.4 *Sample interval determination*

2.4.1 *Subsample resampling*

To determine the optimal sampling interval in each habitat type, several sampling intervals should be set initially. Herein, the fixed intervals (1, 5, 10 and 20 m) were chosen as the referenced groups, as the length of a segment line was less than 1 000 m. The data acquired from the continuous sampling with incremental intervals (1, 2, 3, 5, 10, 15, 20, 50, 100, and 150 m) in each segment can largely assure enough samples of subsampling. Using intervals that were larger than 20 m were not practical, as these sizes would not allow enough subsamples to be drawn in the following step. After the data were collected from the field and the sampling intervals were determined, the following step was to acquire the biological data for each fixed interval based on a resampling technique called subsampling. The subsampling process involved repeatedly drawing subsamples of data from the original sample collected in the field. It is assumed that all the data could be represented as the ones sampled from the fixed 1-m interval data pool and the data except for the ones whose interval was less than 5 m could be represented as the sample of 5-m interval group (Fig. 3). Thus, all data for the chosen intervals could be collected by such subsampling with enough replicate quadrats, avoiding sampling four times at one segment line. The chosen fixed intervals covered most of the ranges that macrobenthic species preferred for aggregation as reviewed in Schoeman et al. (2003).

2.4.2 *Monte Carlo simulation*

The biological data from the subsampling was still limited, as conducting subsampling just acquired one group of data within each segment for each fixed interval. To gain a full understanding of the range of results that might be possible for each fixed interval group, the sub-datasets of the fixed intervals were repeatedly resampled using a Monte Carlo simulation. The Monte Carlo approach is based on computational algorithms that rely on repeated sampling to obtain numerical results and determine the properties of some phenomenon (or behavior). The Monte Carlo method simulates samples from a probability distribution for each variable and produces hundreds or thousands of possible outcomes based on this probability distribution. The results are analyzed to obtain the probabilities of the occurrence of different outcomes. In principle, the Monte Carlo method can be used to solve any problem having a probabilistic interpretation. In this case, Monte Carlo simulation was used to estimate the probability of species occurrence with the probability distribution of limited subsampling data at different intervals. By 100 virtual iterations on the basis of probability distribution, the probabilities of different outcomes occurring were acquired at each fixed interval. With the simulated results, the number of species was easy to be estimated at each fixed interval, which helped to optimize the sampling strategy. For each fixed interval at each segment, 100 virtual iteration samplings were constructed by Monte Carlo simulations, using the Crystal Ball software (Au et al., 2010; Gonzalez et al., 2005), a Visual Basic program, developed by Oracle Corporation.

For each of these virtual iterations simulated for each fixed interval in one segment, species richness was estimated following Colwell et al. (2012) as the expected number of species (S_{sample}) with t subsampling quadrats:

$$S_{\text{sample}} = S_{\text{obs}} - \sum_{i=1}^{t} (1 - Y_i)^t \,, \tag{1}$$

where S_{obs} represents the number of observed species for each segment and Y_i indicates the observed species incidence frequencies.

For each fixed interval with 100 iterations, a normalized deviation ($\varDelta$) was calculated for the estimated species richness using the following formula:

$$\varDelta = \frac{\text{estimate} - \text{true value}}{\text{true value}} \times 100\%, \tag{2}$$

where the "estimate" was the expected number of species (S_{sample}) of each irritation and "true value" was substituted by the true number of species of the segment. According to this formulation, a $\varDelta$-value of zero indicates no difference between the estimated and actual number of species, a negative value indicates that the true value of the variable was underestimated on that virtual transect, and a positive value indicates an overestimate. The normalization of estimates has the additional advantage that estimates of variables can be compared on identical scales (%), irrespective of their true value. If the estimates from most iterations are close to the true value ($\varDelta$-values are close to zero), then data from a single field sample using that method could provide the basis for a good estimate of the variable under consideration. To provide quantitative representations of $\varDelta$-value distributions that are also easy to interpret and compare visually, box plots were compiled for the frequency-distributions of $\varDelta$ for each simulated sampling method at each fixed interval.

2.5 *Sampling effort optimization*

The convenient tool for estimating the minimum sampling effort is to construct the species accumulative curve: plotting the increasing number of species with the increasing surface area sampled reveals an asymptotic value of the species number by extrapolation. If these total areas sampled are enough to include a substantial portion of the number of species actually present, the asymptotic value could be a good representation of the true species richness (Beukema and Dekker, 2012; Colwell et al., 2004; Gotelli and Colwell, 2001). The total area corresponding to the asymptotic value is the minimum sampling area required. Thus, the species accumulative curves were used to determine the minimum sampling effort in each habitat type with different fixed intervals. The software Estimate S 9.0 (http://purl.oclc.org/estimates) was used to generate species accumulative curves.

3 Results

3.1 *Comparison of within-transect sampling and random sampling*

A total of 28 species were recorded in the three transects (22 species) and random sampling (24 species) (Table 1). In both of the sampling techniques, the gastropods (11 species) represented the most abundant group, followed by Crustacea (6 species), Polychaeta (5 species) and Bivalvia (4 species). Other casual groups observed included Insecta (1 species) and Nemertinea (1 species) (Table 1). Among these, 18 species observed in the random sampling were also observed in the within-transect stratification sampling, which accounted for 75% of the total species in the latter sampling. Six species from the random sampling were excluded from the three transects, among which most were rare species (*Cerithidea largillierli*, *Nerita polita L.*, *Lunatia gilva*, and *Moerella iridescens*) (Liu, 2007). Additionally, four species from the three transects were excluded from the random sampling, and except for *Assiminea* sp., these excluded species were all rare species (*Potamilla torelli*, *Heteromastus filiformis*, and *Neritina violacea*) (Liu, 2007) (Table 1). If the rare species are ignored, the reduplicative species of the within-transect sampling can account for up to 81% of the total species in the random sampling. In other words, the within-transect sampling method was effective in collecting most of the representative species of the marsh wetland with less labor and sampling effort. The estimation of species richness with this method can effectively represent the species richness in the entire area.

3.2 *Estimates for species richness at different intervals*

All estimates of macrofaunal species richness were negatively

Table 1. Counts of various taxa encountered on within-transect stratification sampling and random sampling

			Random sampling	Within-transect stratification sampling
Annelida	Polychaeta	*Capitella capitata*2)	17	–
		*Tylorrhynchus heterochaetus*2)	71	–
		*Dentinephtys glabra*1)	8	6
		*Potamilla torelli*2)	–	1
		*Heteromastus filiformis*2)	–	5
Mollusca	Gastropoda	*Assimima violacea*1)	714	836
		*Assimima latericea*1)	30	46
		*Stenothyra glabra*1)	718	243
		*Pseudoringicula sinensis*1)	1	14
		Cerithidea largillierli	2	–
		Elachisina sp. 1)	236	7
		*Cerithidea sinensis*1)	4	11
		Nerita polita L. 2)	1	–
		*Lunatia gilva*2)	1	–
		Assiminea sp.	–	269
		*Neritina violacea*2)	–	1
	Bivalvia	*Corbicula fluminea*1)	1 186	24
		*Glauconome chinensis*1)	232	28
		*Sinonovacula constricta*1)	29	2
		*Moerella iridescens*2)	5	–
Arthropoda	Crustacea	*Corophium sinensis*1)	6	6
		*Gonrimosphaeroma rayi*1)	20	13
		*Orchestia platensis*1)	64	4
		*Ilyoplax deschampsi*1)	292	255
		*Chiromantes dehaani*1)	34	8
		*Helice tientsinensis*1)	7	2
	Insecta	*Insecta* sp. 1)	40	25
	Nemertinea	*Nemertinea* sp. 1)	5	2
		Total species number	24	22

Note: 1) Species were observed in two sampling methods; 2) the rare species.

biased at all levels of fixed intervals. As anticipated, the estimation performed the best at 1-m intervals for the three habitats. As the sampling effort declined, the bias tended to increase and the precision decreased. Even with that, for the segments in the salt marsh vegetation and tidal flat habitats, data from the 10-m interval sampling method performed in a manner very similar to the 1-m fixed interval sampling. The estimates of macrofaunal species were underestimated by 4.16% and 14.48% at 10-m intervals (at 1-m intervals: 3.78% and 13.43%) for the salt marsh vegetation and tidal flat habitat, respectively (Fig. 4). This indicated that the estimates from the 10-m intervals could be in place of the 1-m interval sampling as the effective sampling effort for the salt marsh vegetation and tidal flat habitats. On the other hand, these biases were significantly large at the 20-m interval sampling for both transects. This indicated that the number of species was underestimated at low levels of sampling effort because species aggregation decreased at large intervals (>10 m). It was noted that, at segments of the tidal creek, all estimates except for those at 1-m intervals were ineffectual in terms of providing data to estimate species richness, which was underestimated by more than 15% (Fig. 4c). This indicated that the aggregation was not obvious in tidal creek habitats and only the 1-m sampling interval was an effective sampling effort.

3.3 *Species–area curves*

The species–area curves illustrated that all segments accumulated species in a fairly conventional manner as the area sampled increased. The plateau value was reached at all sample interval levels in the salt marsh vegetation and at the 1-m, 5-m, and 10-m intervals in the tidal flat, but it was not reached at any interval in the tidal creek (Fig. 5). Relating to the interval analysis, only less sampling effort of 1.8 m^2 was needed at 1 m or 10 m (as the effective sample intervals) to acquire more than 90% of the species in the salt marsh vegetation habitat. For the tidal flat, the minimum sampling effort should be 2 m^2, which would allow more than 90% of the species to be sampled. For the tidal creek, however, the sampling effort must exceed 3 m^2 in order to be confident of obtaining more than 90% of the species on the transect (Fig. 5).

Combined with the results of sampling interval, in the salt marsh vegetation, the 10-m interval and least amount of sampling effort (1.8 m^2) can satisfy the sampling requirement. In the tidal flat, the 10-m interval and sampling effort of 2.0 m^2 can satisfy the sampling requirement. In the tidal creek, the 1-m interval and the most amount of sampling effort (>3.0 m^2) can satisfy the sampling requirement.

4 Discussion

4.1 *The performance of within-transect stratification sampling*

The comparisons of species counted between the within-transect sampling and random sampling showed that the species observed from the within-transect sampling can account for up to 81% of the total species in the random sampling. In other words, this result verified that stratification was an effective op-

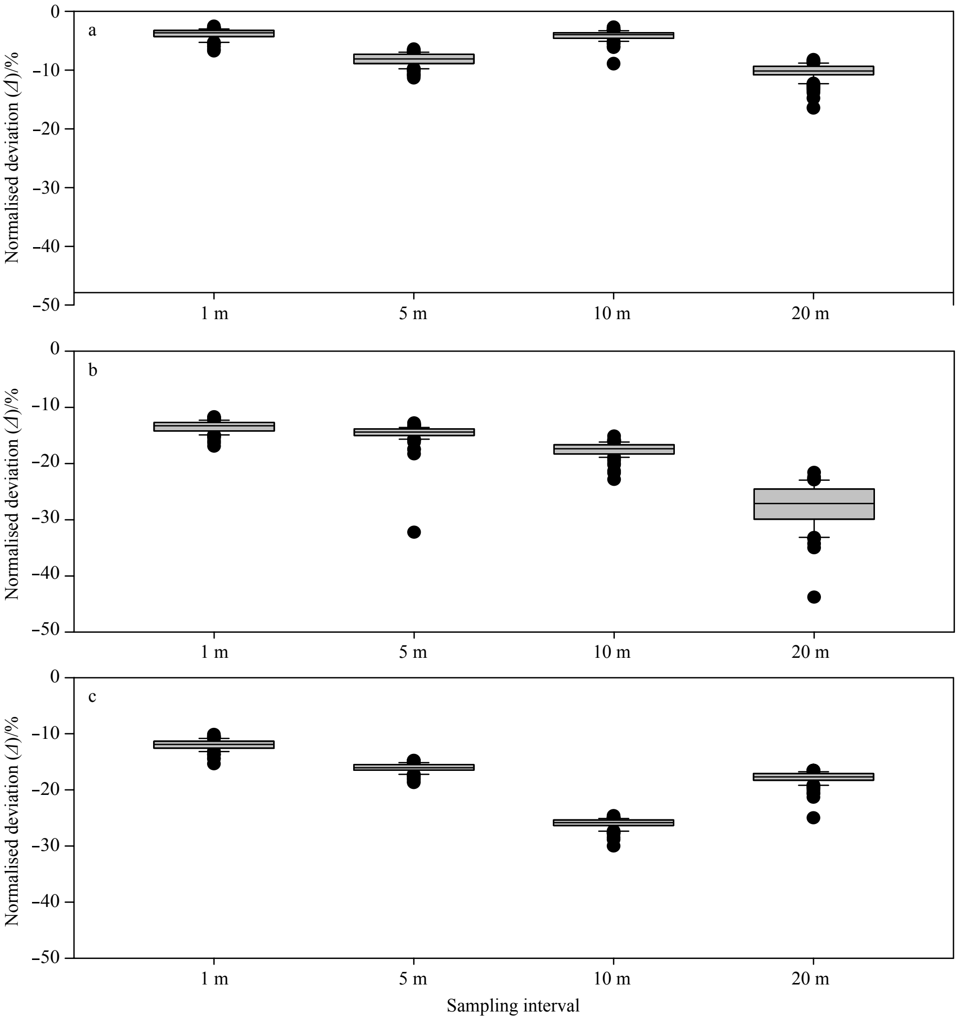

Fig. 4. Graphical summary of the normalized estimates (*D*) of macrobenthic species richness from Monte Carlo sampling simulations for 1-, 5-, 10- and 20-m intervals at three habitat types. a. Salt marsh, b. tidal flat, and c. tidal creek. The solid horizontal line represents the median; the box outlines the interquartile region; the whiskers indicate the 10th and 90th percentiles and the circles mark the fifth and 95th percentiles.

timization in transect sampling that required fewer samples but encompassed most of the representative species in the marsh wetland. The reason that stratification was so effective could be the macrobenthos have a pronounced preference for three habitats (Quan et al., 2016). This allowed using the relationship as prior knowledge to optimize the sampling and estimate the minimum sampling effort by stratifying different habitat types within the transect. The good performance in the sampling effort and precision was also supported by Skibo (2005), who used within-transect stratification sampling according to the substrate types as the strata to estimate the red sea urchins' richness. It should be noted that the bias could be very large when comparing the species richness if only sampling one transect. When more transects are chosen, the bias is reduced as species distribution is closer to the actual number of species. In particular, strata classification should be carefully treated within a transect according to prior knowledge. The strata type should be most related to the distribution of the dominant species. Moreover, the strata types should be clearly differentiated from each other in the field sampling, such as vegetation types and the distance from the sea shoreline (Beukema and Dekker, 2012).

4.2 *Advantages of the numerical technical application*

The Monte Carlo simulation and species accumulative curves were herein convenient and robust methods to statistically and numerically analyze the optimal sampling interval and minimum sampling effort. For convenience, the software resources for the two approaches were both readily available for access. From a statistically explicit perspective, Monte Carlo simulation was conducted to repeat the sampling procedure and ensure that the results were not due to some by chance selection of a small number of sampling units. The applications of Monte Carlo simulation have proven to be useful for kinds of situations in sandy beaches, such as simulating different survey types (Skibo, 2005), simulating different sampling intervals and sampling efforts (Schoeman et al., 2003), and simulating large samples (Schoeman et al., 2008). On the other hand, the extrapolation of species accumulation curves has been considered to provide an accurate estimation of species richness using existing data (Colwell, 1997). The species accumulation curves are generally accepted for species richness estimation and are an effective way to determine the minimum species effort by the value at the plateau (Beukema and Dekker, 2012; Schoeman et al., 2003). It is noted

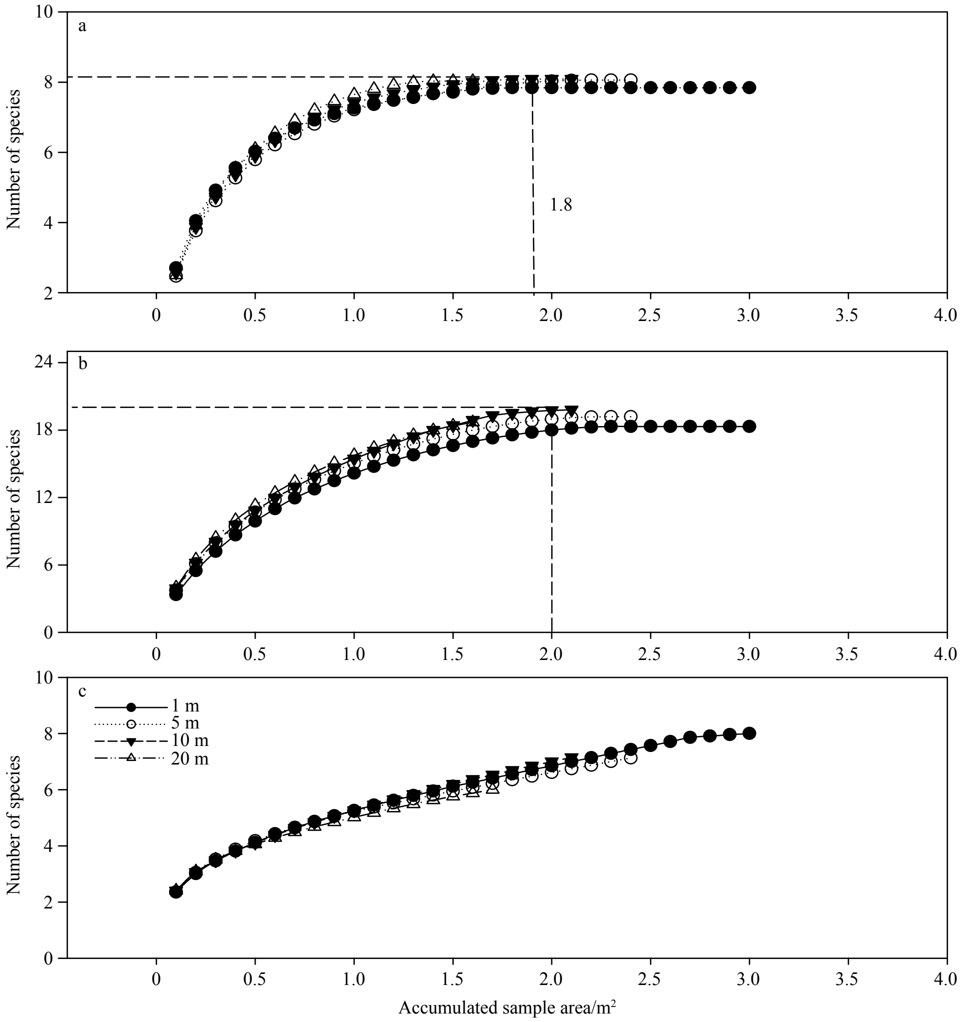

Fig. 5. Species-accumulative curves of macrobentic species at three habitat types. a. Salt marsh vegetation, b. tidal flat, and c. tidal creek. Filled, hollow circle and triangle line represent the specie accumulative curve at 1-, 5-, 10- and 20-m intervals, respectively. Dashed vertical lines mark the minimum sampling effort level at the optimum interval for each habitat types.

that the functions and the variables should be carefully treated and rigorously followed by definition.

4.3 *Sampling interval determination*

The determination of the optimal sampling interval by Monte Carlo simulations (Fig. 4) demonstrated that the bias of estimates for macrobenthic species tended to increase as sampling effort decreased. All estimations were calculated the best at 1-m intervals for the three habitat types, but the accuracy decreased with decreasing sampling effort, especially at levels of very low sampling effort (20 m). This is a distance which is even smaller than what is most commonly reported in the literature (Chao et al., 2012; Chen et al., 2009; Liu, 2007; Lv et al., 2016; Shou et al., 2013). Moreover, this indicated that the macrobenthic communities were patchily distributed at a small scale (Moodley et al., 1998) and that the sampling interval should be less than 20 m in the marsh wetland; otherwise, the risk of missing species would be much greater in the sampling.

On the other hand, the optimal and effective interval (10 m) was found in the salt marsh vegetation and tidal flat, which could be used as an alternative for the 1-m interval. With this result, an assumption could be raised that the range of 10 m may be a common mobile or active range for the dominant macrobenthic species of both habitats. To prove this assumption, the macrobenthic abundance in the three habitats was investigated further to determine the dominant species (Table 2). As shown in Table 2, the dominant species in the salt marsh and tidal creek were *Ilyoplax deschampsi* and *Stenothyra glabra*, and in the tidal flat, they were *Ilyoplax deschampsi*, *Stenothyra glabra*, *Assimima violacea*. Obviously, the mobility of the crustacean *Ilyoplax deschampsi* is larger than that of the gastropods *Stenothyra glabra* and *Assimima violacea*. Thus, the results that the effective interval was 10 m in the salt marsh vegetation and tidal flat were reasonable from this aspect. However, this alternative sampling interval did not exist in the tidal creek habitat. The only effective sampling interval for the tidal creek was 1 m. These results may be affected by the physical differences between two habitats (e.g., turbulent tidal water, substrate and vegetation cover), as the dominant species were the same with the ones in the salt marsh vegetation. For example, the effect of the tidal water was mitigated by the vegetation in the salt marsh vegetation, allowing the species community to be aggregated at a common range. Additionally, the tidal water force in the tidal flat was not as large as that in the tidal creek because the tidal flat was broad, wide and flat while

Table 2. Composition and abundance of macrobenthos in the sampling sites

	Salt marsh vegetation	Tidal flat	Tidal creek
Annelida			
Polychaeta			
Dentinephtys glabra	–	+	–
Potamilla torelli	–	+	–
Heteromastus filiformis	–	+	–
Mollusca			
Gastropoda			
Assimima violacea$^{1)}$	++	+++	–
Assimima latericea	++	+	+
Stenothyra glabra$^{1)}$	+++	+++	+++
Pseudoringicula sinensis	–	+	–
Elachisina sp.	–	+	–
Cerithidea sinensis	+	–	+
Assiminea sp.	++	+	++
Neritina violacea	–	+	–
Bivalvia			
Corbicula fluminea	+	++	+
Glauconome chinensis	–	++	–
Sinonovacula constricta	–	+	–
Arthropoda			
Crustacea			
Corophium sinensis	+	+	–
Gonrimosphaeroma rayi	–	++	+
Orchestia platensis	–	–	–
Ilyoplax deschampsi$^{1)}$	+++	+++	+++
Chiromantes dehaani	+	–	–
Helice tientsinensis	–	–	+
Insecta			
Insecta sp.	–	+	+
Nemertinea			
Nemertinea sp.	–	+	–

Note: –, +, ++, +++ indicated the abundance of 0, <10%, between 10% and 70%, >70% of the total numbers, respectively. $^{1)}$ The dominant species (+++) in three habitat types.

the tidal creek was narrow and steep (Fig. 2). The greater tidal water force in the tidal creek was not convenient for the colonization of macrobenthic species. This may be the reason for why the bias and precision were the worst in the tidal creek for all levels of sampling intervals. According to the above discussion, we inferred that the activity ranges of the dominant species were commonly affected by the mobility of the species themselves as well as the related physical characteristics of the habitat.

4.4 *The significance of minimum sampling effort determined in three habitats*

The species accumulation curves reached a plateau in the salt marsh vegetation at all intervals and at 1-, 5-, and 10-m intervals in the tidal flat, but a plateau was not reached at any interval in the tidal creek. This indicated that the species composition was stable in the salt marsh vegetation, which only needed a few quadrats (18 quadrats) or the least amount of sampling effort, but it was less stable in the tidal flat and the most variable in the tidal creek, which needed more sampling quadrats (>30) to reach the plateau. The stability may be related to systematic stability in the salt marsh vegetation, which has higher productivity, less disturbance, more mature species and individuals in communities, a stable food chain, etc. In contrast, the differences of these variables could lead to the variability of species composition in the tidal flat and tidal creek. These inferences certainly need to be researched further and need to be proven with quantitative results.

Except for the tidal creek, the minimum sampling effort was no more than 2 m^2 for the salt marsh vegetation and tidal flat. This is a practical reference value in field sampling if there were only three or four researchers in one team. For the studies simulated for a sandy beach, the minimum sampling effort was 4 m^2, as stated by Jaramillo et al. (1995). In addition, a sampling effort of over 12 m^2 was suggested by Schoeman et al. (2003), by which the sampling can confidently obtain more than 90% of the species on any given transect of a sandy beach. It seemed that substantially larger samples were apparently needed with transect sampling. Not accounting for the differences between these two ecosystems (estuarine wetland and sandy beach), the minimum sampling effort determined to be needed in this study was largely reduced. This improvement could be attributed to the stratification according to habitat types within the transect sampling, which were closely related to the aggregation of the macrobenthic communities. Moreover, the flexible optimal sampling intervals could grasp the patchiness and aggregation characteristics of the distribution of the macrobenthic community. From this aspect, the minimum sampling effort also verified the advantage of within-transect stratification sampling.

5 Conclusions

There is no doubt that monitoring macrobenthic communities and habitats has played an important role in grasping the ecological characteristics for habitat management or ecosystem assessment. The explicit estimation of macrobenthic species richness is difficult in the heterogeneous intertidal zones of estuaries and needs a comprehensive and operational sampling design to be an optimal strategy that balances the statistical requirements and the logistical constraints in field sampling. In this study, through focusing on the three main aspects of a sampling design, the sampling process was optimized effectively. For example, the selected within-transect stratification sampling method was proven to allow explicit estimation of the species richness with less labor according to the habitat types. The Monte Carlo simulation and species accumulative curves were shown to be convenient and statistically robust methods to determine the optimal sampling interval and minimum sampling effort. Prior knowledge or the recommendations derived from the simulation results, such as the sampling effort must exceed 1.8 m^2 by 10-m intervals in the salt marsh vegetation, 2 m^2 by 10-m intervals in the tidal flat, and 3 m^2 by 1-m intervals in the tidal creek, could be used as a reference for sampling in estuarine wetlands. The determined sampling intervals and sampling efforts were shown to be reasonable and had potential ecological significances, and they were related to the aggregation ranges, the mobility of the dominant species, the stability or variability of species composition and the habitats' physical differences. Above all, the optimization of the three main aspects of the sampling design could provide a relatively full consideration of the entire sampling process, which could largely ensure good precision, reduce the sampling effort, capture the patterns of aggregated macrobenthos and utilize the related habitat characteristics. As such, this study provided guidelines or an operational sampling strategy for field sampling and monitoring for the habitat management or ecosystem assessment of estuarine wetlands. Certainly, there were no absolute rules, and this study did not consider other aspects easy to judge according to specific situations, such as sampling device (Long and Wang, 1994; Schlacher et al., 2008; Shen et al., 2012), mesh size (Aarnio et al., 2011; Schlacher and Wooldridge, 1996), sampling time (Colwell et al., 2012), sampling depth (Ferraro and Cole, 2004; Shen et al., 2012), etc. The sampling strategy here is relatively standard and complete, though, and could be easily followed in long-term monitoring programs conducted by ecologists.

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