Environmental Pollution 278 (2021) 116762

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Ostracods as pollution indicators in Lap An Lagoon, central Vietnam☆

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ARTICLE INFO

Article history:
Received 11 February 2020
Received in revised form
28 January 2021
Accepted 14 February 2021
Available online 16 February 2021

Keywords:
Bioindicators
Heavy metals
Organic matter
Pollution
Proxy
Lagoon
Vietnam

ABSTRACT

Southeast Asia is particularly susceptible to the negative impacts of increasing coastal pollution as coastal populations and cities grow at unprecedented rates. Although water chemistry can be monitored, there are greater advantages in using bioindicators as reflectors of the combined effect of multiple pollution types on coastal ecosystem health and for early detection of the negative impacts of pollutants on biotic systems. This study explores the utility and application of ostracods as pollution bioindicators and examines the response of ostracod assemblages to variable pollution in Lap An Lagoon, central Vietnam. From 14 sites within the lagoon, 79 species of 46 genera were identified and sediment grain size, total organic carbon, organic matter and heavy metal concentration were measured. Cluster analysis, detrended correspondence analysis and canonical correspondence analysis identified four distinct ostracod biofacies that were highly correlated to the physical environmental variables (salinity, depth, sediment type, heavy metal concentrations, total organic carbon and organic matter) and are shown to be the main factors controlling ostracod biofacies. Low ostracod diversities were found in silty sediments with heavy metal concentrations likely toxic. *Sinocytheridea impressa* was indicative of a marginally polluted environment within the lagoon. This study provides evidence for the potential for Southeast Asian ostracods to be used in water quality assessments and the data collected can be used as a baseline for future pollution monitoring.

1. Introduction

Coastal lagoons are rich in biodiversity, highly productive and provide important ecosystem services such as recreation, tourism and aquaculture (Cutrim et al., 2019; Kennish and Paerl, 2010), but are increasingly threatened by rising sea level, intensifying storms, floods and anthropogenic activities (Bird, 2008; IPCC, 2019; Kjerfve, 1994).

Currently, coastal ecosystems in Southeast Asia (SEA) are some of the most degraded in the world (Tiquio et al., 2017) and are expected to degrade further under the pressures of increased coastal landuse and higher populations (e.g. Neumann et al., 2015; Todd et al., 2010). Anthropogenic pressures on coastal ecosystems include excessive agricultural fertiliser run-off, untreated urban sewage, metal leachate from mining and industrial activities, and pesticides, which can result in harmful algal blooms and heavy metal accumulations in sediments (e.g. Martínez-Colon et al., 2009; Tapia González et al., 2008). Consequently, large scale fish deaths, heavy metal bioaccumulation and poisoning may occur. The detrimental impacts arising from eutrophication and heavy metal pollution on both marine life and human health signal an urgent need to prevent and reduce pollution in SEA (e.g. Anderson et al., 2002; Klake et al., 2012).

Globally, there is an increasing interest in using bioindicators for water quality monitoring (e.g. Parmar et al., 2016). Information about the environmental conditions can be drawn from the analysis of the composition, abundance or age structure of the bioindicator population (Rinderhagen et al., 2000). While water quality analysis is one of the measures for monitoring pollution, not all pollutants and early stages of negative biological impacts can be detected (e.g. Ghetti, 1980; Parmar et al., 2016). Thus, the use of bioindicators can

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☆ This paper has been recommended for acceptance by Maria Cristina Fossi.
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https://doi.org/10.1016/j.envpol.2021.116762
0269-7491/

be advantageous for examining the impacts of pollution on the biota.

Ostracods are highly sensitive to environmental changes and are increasingly recognised as bioindicators of pollution gradients (e.g. Yasuhara et al., 2012, Table 1). For example, *Cyprideis torosa* was found to dominate ostracod populations in areas polluted by untreated industrial and domestic waste in Melah Lagoon, Egypt (Ruiz et al., 2006b). Similarly, a single ostracod species (*Cyprideis salebrosa*) was found in a Brazil lagoon polluted by untreated urban and domestic sewage containing heavy metals (Vilela et al., 2011). In contrast, opportunistic ostracod species, such as *Loxoconcha elliptica*, were absent from the highly polluted Odiel Estuary in Spain (e.g. Ruiz et al., 2004). Although there are documented variations in the response of ostracods to pollution, low ostracod abundance and diversity are commonly observed in polluted environments (Ruiz et al., 2005).

Despite the potential for ostracods to monitor pollution in degraded environments, there are few comprehensive data sets linking ostracod biofacies to anthropogenic pollutants in SEA. To address this gap and test the applicability of ostracods as pollution bioindicators in SEA lagoons, we examine the ostracod communities and anthropogenic pollution in Lap An Lagoon, central Vietnam.

2. Study area

The Lap An Lagoon, is a relatively small (16 km^2), shallow (< 3.1 m) semi-enclosed lagoon. It is adjacent to the burgeoning tourist town of Lang Co which is built upon the 6 km long sandy spit forming the lagoon's eastern margin (Fig. 1). The lagoon connects to the South China Sea via a narrow inlet in the southeast and is bordered by the forested Bach Ma mountain range. Lap An Lagoon is a relatively simple system compared to the much larger, hydrodynamically and geomorphologically complex coastal lagoons to the north and south (e.g. Romano et al., 2012). Moreover, the lagoon faces anthropogenic pressures from development in adjacent areas (Dũng, 2012; Gouramanis et al., 2020). Untreated aquaculture waste from shrimp and oyster farming is directly released into lagoon (Chat, 2020; Thang et al., 2012) and numerous resorts have been constructed to support the growing tourism industry (Nhơn, 2008). Thus, it is an excellent site to test the applicability of ostracods as pollution bioindicators in SEA.

Lap An Lagoon experiences monsoonal climate, with the wet season occurring from September to December, and the dry season from April to August (Dũng, 2014). Interseasonal variations in water temperature (28.8 to 33.7°C), salinity (24 to 32 ppt) and pH (8.23 to 8.38) are larger than the within lagoon variability at the time of sampling (Nhơn, 2008; Dũng, 2014). The lagoon receives freshwater input directly from precipitation and from three small rivers flowing from the southwest (Fig. 1). The hydrochemistry and water level is controlled by the *ca*. 70 cm semi-diurnal microtidal regime (Nhơn, 2008).

3. Methods

3.1. Sample collection

Fourteen sites were sampled along a north-south transect in June 2011 (Fig. 1). At each site, a Horiba U52g sonde measured dissolved oxygen (DO), depth, pH, salinity, temperature, turbidity above the sediment-water interface at each site (Table S1) and a Van Veen grab sampler was used to collect sediment and faunal samples. The shallow depth of the lagoon and slow raising of the grab sampler ensured that very little material was displaced or lost from the sediment-water interface. The top 1 cm of sediment was collected representing approximately one year of time-averaged lagoonal sediment and fauna. Below 2 cm, an 11-cm thick, clean, sandy unit was deposited by Typhoon Ketsana in late September 2009 (Unpublished data). The sediments and microfossils were preserved in 100% ethanol, refrigerated at <4 °C and transported to Singapore for analysis.

3.2. Laboratory analysis

Grain size analysis was performed on a Malvern Mastersizer 2000 following the protocols of Switzer and Pile (2015), analysed

Table 1
Examples describing impact of pollution on marine and brackish water ostracod abundance and diversity.

Location	Sources of pollution	Ostracod response to environmental conditions	References
Louisiana shelf, USA	Anthropogenic nutrient loading	Hypoxic and eutrophic waters: Dominance of *Loxoconcha* spp.	Alvarez Zarikian et al. (2000)
Manzalah lagoon, Egypt	Industrial and domestic wastes	One species, *Cyprideis torosa*, congregated near pollution source	Samir (2000)
Mae Khlong river mouth, Thailand	Urban and domestic waste	Areas of lowest salinity, dissolved oxygen and pH: low ostracod abundance and diversity	Montenegro et al. (2004)
Odiel estuary, Spain	Industrial acidic waste and mining	Highly polluted areas (Pb (400 to 1300 ppm), Zn (300 to 4800 ppm) and Cu (300 to 1400 ppm)): Absence of opportunistic species, e.g. *Loxoconcha elliptica*	Ruiz et al. (2004)
Nador lagoon, Morocco	Fecal water effluent and mining	Areas near fecal water effluent: abundant and monospecific populations of *C. torosa* (> 10 individuals/g of sediment)	Ruiz et al. (2006a)
El Melah lagoon, Tunisia	Industrial and domestic waste	Solid waste disposal site with high As and Cr concentrations: no ostracods	Ruiz et al. (2006b)
Osaka Bay, Japan	Urban wastewater	Eutrophic waters: increase abundance at middle bay due to more food supply; decrease abundance at inner bay due to low oxygen levels	Yasuhara et al. (2007)
Rodrigo de Freitas Lagoon, Brazil	Untreated urban and domestic sewage containing heavy metals	Highly polluted areas: Only *Cyprideis salebrosa* found	Vilela et al. (2011)
Kasado Bay, Japan	Industrial waste	Abundances of *Bicornucythere bisanensis* and *Loxoconcha viva* increased with TOC content that was below 2%	Irizuki et al. (2015)
Gulf of Saros, Turkey	Sewage outfall and agricultural discharges	Total petroleum hydrocarbon content > 10 mg/kg: Low ostracod abundance and diversity	Barut et al. (2015)
Seto Inland Sea, Japan	Industrial and domestic waste	Ostracods unable to survive in sediments with TOC and COD content > 20 mg/g	Irizuki et al. (2018)
Marginal marine waters off Hong Kong	Urban sewage discharge and land reclamation	Relative abundance of *Sinocytheridea impressa* is positively correlated with eutrophication and bottom-water hypoxia Relative abundance of *Neomonoceratina delicata* is positively correlated with Zn and turbidity	Hong et al. (2019)

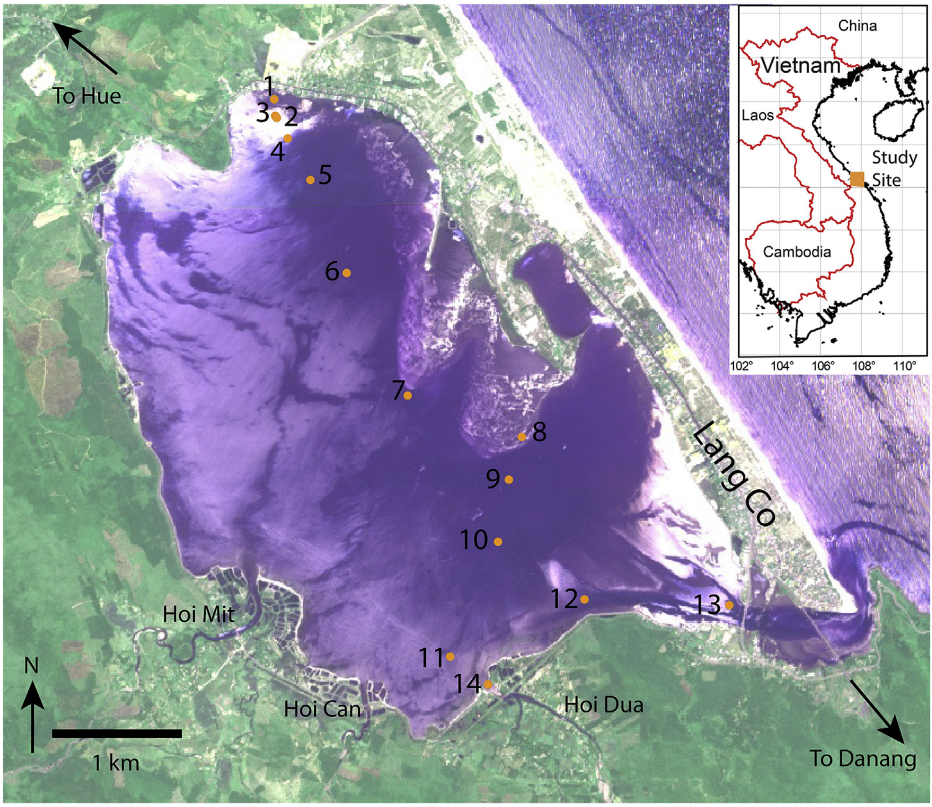

Fig. 1. Lap An Lagoon in central Vietnam showing the location and distribution of grab sample sites (black numbers) representative of the sub-environments within the lagoon. RapidEye satellite image collected on April 23, 2012.

using GRADISTAT (Blott and Pye 2001) and results reported using Folk (1974)'s terminology.

Approximately 25 mg of dried and ground sediment was acid fumed in 100 ml of 12M HCl for 6 h to remove carbonates and the total organic carbon (TOC) was determined using an Elementar vario TOC tube carbon analyser (Harris et al., 2001).

The USEPA Method 3051A (USEPA, 2007) was used to extract heavy metals (Cu, Fe, Mn, Ni, Pb and Zn) from the sediments. Approximately 5 g of dry, ground sediment was digested in 3 HNO_3:1 HCl solution and microwave digested in a Milestone START D for 60 min. The supernatant was analysed for Cu (327.393 nm), Fe (238.204 nm), Mn (257.61 nm), Ni (231.604 nm), Pb (220.353 nm) and Zn (206.2 nm) using the PerkinElmer Optima 8300 inductively coupled plasma-optical emission spectrometry (ICP-OES). Six standard stock solutions of 0.1 mg/kg, 1 mg/kg, 5 mg/kg, 10 mg/kg, 25 mg/kg and 40 mg/kg were prepared in 2% HNO_3 for calibration. The coefficient of determination (R^2) for the calibration curves were $\geq$0.997. The accuracy of the analyses was validated using the SRM 2711a (standard reference material). The recovery efficiency of heavy metals was within the acceptable range of ±20% (USEPA, 2007; Table S2).

We calculated the Enrichment Factor (EF) to determine if heavy metals were anthropogenically enriched:

$$EF = \frac{C_{sample}}{C_{Fe}} \div \frac{C_{standard}}{C_{Fe\ standard}}$$

where, C_{sample} = concentration of the metal in the sample and C_{Fe} = Fe concentration in the sample (Müller, 1969). Fe was used as the normalising factor to reduce the effect of grain size and mineralogy on sediment metal concentrations (Schiff and Weisberg, 1999). Metal concentrations from the lagoon in 2005 (Romano et al., 2012) were used as the standard. An EF < 1 indicates no enrichment and 1 < EF < 3 indicates minor enrichment (Sakan et al., 2009).

To measure the degree of heavy metal contamination (Müller, 1969), the index of geoaccumulation (Igeo) was calculated:

$$Igeo = \log_2\left(\frac{C_n}{1.5B_n}\right)$$

where, C_n = concentration of metal n in the sediment, and B_n = background concentration of the metal n. The metal concentrations reported in Romano et al. (2012) were used as the background concentrations.

3.3. Ostracod analysis

Approximately 8 g of sediment was wet sieved through 106 μm stainless steel mesh and oven dried at 50°C for 2h. Adhering particles were removed with 2% H_2O_2. Ostracods were dry-picked under a binocular stereomicroscope using a 000 sable hair brush. A microsplitter subdivided samples with high faunal densities to obtain approximately 200 valves (Ishizaki, 1978). Otherwise, all valves were counted. One specimen refers to a single valve and a carapace is considered as two valves. Internal and external valve characteristics were used to identify ostracods from the taxonomic literature (Dewi, 1993; Dung and Tsukagoshi, 2018; Hu and Tao, 2008; Keij, 1964; Mostafawi, 1992, 2003; Munef et al., 2012;

Niiyama et al., 2019; Tanaka et al., 2009, 2011; Wang and Zhao, 1985; Whatley and Zhao, 1987, 1988; Wouters, 2005; Zhao and Wang, 1988; Zhao and Whatley, 1989; Zhou, 1995).

3.4. Data analysis

The ostracod density was determined by dividing the total number of individuals with the dry sample sediment weight (Hayek and Buzas, 1997). The Shannon exponential index (exp(H)) describes the species richness and abundance of the samples (Jost, 2006):

$$exp(H) = \exp\left(- \sum_{i=1}^{s} p_i \times ln\ (p_i) \right)$$

where, p_i = proportion of species in the sample. Q-mode cluster analysis using Ward's method and Morisita-Horn overlap index, identified ostracod biofacies within the lagoon (Horn, 1966). Morisita-Horn overlap index is independent of sample size (Wolda, 1981) and appropriate for our variable sample sizes. Samples of similar distances were grouped and re-evaluated until all the samples were included in a cluster. The relative abundance of ostracods within the clusters was calculated to identify representative species within a biofacies. Only samples with more than 32 individuals were used in the analysis since a lower number involves a loss of statistical significance (Martínez-García et al., 2013). Additionally, only species with at least three specimens in at least one sample were analysed. Permutational multivariate analysis of variance (PERMANOVA) compared the distances between clusters to determine if the clusters differed in terms of composition (Anderson, 2017). The clusters were considered statistically distinct when $p < 0.05$. The index value (INDVAL) was calculated to determine the association between a species and its cluster (Dufrene and Legendre, 1997).

Principal Component Analysis (PCA) was performed to examine the relationship between variables and sites using all of the variables (Fig. S1). Based on the results of the PCA with all variables included, Cu, Fe, Ni, Pb and Zn were removed as these were highly correlated due to the large proportion of below detection limit (BDL) measurements returned and highly correlated to Mn, which had a full complement of analyses. Mn was also selected because it is likely anthropogenically enriched (EF > 1, see section 3.1). A PCA lacking Cu, Fe, Ni, Pb and Zn was subsequently performed. Detrended correspondence analysis (DCA – Fig. S2) was used to decide on the ordination method to examine the relationship between sampling sites, species composition and environmental parameters (Smilauer and Lepš, 2014). As the length of DCA axis 1 was 3.71, canonical correspondence analysis (CCA) was conducted to extract the major environmental gradients from the ecological data (Braak and Verdonschot, 1995; Smilauer and Lepš, 2014). Prior to performing CCA, temperature was removed as a variable as it was heavily influenced by the time of day at which it was measured; pH was removed as there was very little variability across all sample sites; and, clay was removed as clay was very highly correlated with the silt fraction of the sediments across the entire lagoon. Preliminary geochemical analysis indicated that the pollutant variables may be significant in defining ostracod biofacies and this *a priori* information was utilised and tested using backward selection of variables to the reduced dataset. The removal of variables stopped when variables in the model had a p-value < 0.05 or the cumulative adjusted R^2 of the model exceeded that of the global model. Holm's correction was applied to reduce the error from multiple testing using backward selection.

4. Results

4.1. Sediments

The sand, silt and clay fraction of the sediments ranged from 1.4 to 98.3% sand, 1.3 to 76.6% silt and 0.4 to 31.4% clay, respectively (Table 2). The mean grain size of the sediments ranged from 1.2 to 7.3 Φ, averaging $4.4 \pm 2.6\ \Phi$, indicating that most of the samples were silt (Table 2).

OM and TOC content of the sediments ranged from 0.5 to 14.9% and 0.05 to 3.2%, respectively (Table 2). Site 14 had the highest OM and TOC content.

Concentrations of Cu, Ni, Pb and Zn in sediments from sites 1, 2, 4, 8 and 13 were BDL (Table 3). The concentrations of Cu (BDL to 34.3 mg/kg), Fe (723 to 45,800 mg/kg), Mn (20.6 to 994 mg/kg), Ni (BDL to 51.6 mg/kg), Pb (BDL to 51.4 mg/kg) and Zn (BDL to 115 mg/kg) showed similar patterns of increasing concentrations from site 3 to 5 and decreasing concentrations towards the lagoon outlet (Table 3).

The EFs of Cu, Mn, and Zn at most of the sites were greater than 1, indicating enrichment from anthropogenic activities between 2005 and 2011 (Table S3). Ni showed a slight enrichment at site 5 and Pb showed a slight enrichment at sites 7, 12 and 14, respectively.

Igeo measures the degree of pollution at each site within the lagoon since 2005. The majority of the metals had negative Igeo values suggesting that the lagoon was largely unpolluted (Table S4). The EF and Igeo values suggest that heavy metals were anthropogenically enriched, but had not crossed QCVN's standards for heavy metal pollution.

4.2. Ecology of ostracod biofacies

A total of 2140 valves were picked and identified, representing 79 species (three species could not be identified) from 46 genera (Table S5; Fig. 2). All of the species are brackish-marine and marine taxa. Adult and juvenile valves were found at many sites, except for sites near the lagoon outlet that only contained adults. Most valves were highly calcified and well preserved with no morphological abnormalities observed. Ostracods were absent from site 11 and only two valves were found at site 14. Exp(H) values ranged from 0.03 to 3.41 (Table S5). Nearshore sampling sites (1 to 4) had similar exp(H) values. The exp(H) values decreased and plateaued at sampling sites 4 to 8 before peaking at outer lagoon sites (10 to 13). An opposite trend was observed for ostracod density.

The Q-mode cluster analysis divided the ostracods into four distinct biofacies ($p < 0.05$; Fig. 3). Biofacies I (sites 1 and 8), were characterised by shallow depths (< 0.4 m), sand (85%) and the three most abundant species were *Caudites huyeni*, *Spinileberis quadriaculeata* and *Tanella gracilis*. Biofacies II (sites 2 and 4), were composed of silty sand and dominated by *Xestoleberis* spp. Biofacies III (sites 12 and 13 and located near the lagoon outlet) were sandy (> 78%) and contained a mixed brackish (e.g. *Xestoleberis* spp. and *Keijella gonia*) and marine ostracod fauna (e.g. *Cytherelloidea* sp. aff. *C. leroyi* and *Keijella* sp. aff. *K. karwarensis*). Biofacies IV (sites 3, 5, 6, 7, 9 and 10) were composed of silty sediments (61 to 76%) and dominated by *Sinocytheridea impressa*. Additionally, INDVAL analysis identified *Neomonoceratina iniqua* (assuming the synonymisation of *Neomonoceratina delicata* as a junior synonym of *N. iniqua* by Mostafawi (2003)) and *Bicornucythere bisanensis* as cosmopolitan species occurring in all biofacies.

The PCA and Broken stick model (Fig. 4) shows that only two Principal Components (PCs) are required to explain 79% of the total variance with PC1 explaining 51% and PC2 explaining 28% of the total variance. PC1 indicates that the pollutants Mn (as a proxy for

Table 2
Grain size, sediment fraction, organic matter (OM) and total organic carbon (TOC) of bottom sediments for each site in Lap An Lagoon. Avg = average. Std = standard deviation.

Site no.	Mean Grain size (Φ)	Sand (%)	Silt (%)	Clay (%)	OM (%)	TOC (%)	Folk's classification
1	1.5	98.3	1.30	0.40	0.45	0.07	sand
2	1.4	83.6	11.5	4.9	1.41	0.13	silty sand
3	7.3	7.60	61.1	31.4	10.04	1.08	mud
4	1.6	80.6	14.1	5.40	0.51	0.05	silty sand
5	7.1	1.40	76.2	22.5	9.89	1.20	silt
6	7.1	1.60	76.4	22.1	9.53	1.21	silt
7	6.7	6.30	76.6	17.1	9.10	1.13	silt
8	1.2	86.7	10.0	3.30	0.70	0.13	silty sand
9	6.8	6.00	75.8	18.2	8.44	1.11	silt
10	6.5	12.0	71.1	16.9	6.52	0.84	sandy silt
11	2.9	76.8	20.6	2.60	3.77	0.41	silty sand
12	3.1	78.3	16.7	5.10	3.15	0.29	silty sand
13	2.7	94.3	4.70	1.00	1.34	0.10	sand
14	6.3	10.8	74.7	14.5	14.95	3.22	sandy silt
Min	1.2	0.40	1.40	1.30	0.45	0.05	–
Max	7.3	31.4	98.3	76.6	14.95	3.22	–
Avg	4.4	46.0	42.2	11.8	5.70	0.78	–
Std	2.6	41.5	32.6	9.8	4.67	0.85	–

Table 3
Heavy metal concentrations (mg/kg) for sediments of each site in Lap An Lagoon and the toxicity criteria for sediments. BDL = below detection limit. TEL = threshold effect level. PEL = probable effect level. ERL = effect-range low. ERM = effect-range median. Values bolded are >TEL. Values bolded and italicised are >PEL.

Site no.	Cu	Fe	Mn	Ni	Pb	Zn
1	BDL	761	24.8	BDL	BDL	BDL
2	BDL	2670	69.8	BDL	BDL	BDL
3	**27.8**	38300	853	**39.5**	26.9	102
4	BDL	723	21.7	BDL	BDL	BDL
5	**29.6**	45800	994	***51.6***	**38.8**	115
6	**29.5**	45000	910	***46.4***	**36.2**	111
7	**31.7**	43100	885	***43.3***	**51.4**	115
8	BDL	1250	20.6	BDL	BDL	BDL
9	**30.4**	42100	814	**39.8**	**41.50**	102
10	**24.3**	34300	675	**30.6**	24.1	77.7
11	BDL	27600	232	**28.1**	13.4	50.7
12	BDL	15100	393	14.2	16.4	26.2
13	BDL	6440	261	BDL	BDL	BDL
14	**34.3**	41300	499	**40.7**	**45.3**	99.6
Min	BDL	723	20.6	BDL	BDL	BDL
Max	34.3	45800	994	51.6	51.4	115
TEL[a]	18.7			15.9	30.2	124
PEL/QCVN (Vietnam's national technical regulations)[b]	108			43.0	112	271
ERL[c,c]	34.0			20.9	46.7	150
ERM[c,c]	270			51.6	218	410

[a] Burton Jr. (2002), CCME (2001).
[b] CCME (2001), QCVN (2012).
[c] National Oceanic and Atmospheric Administration (NOAA) (2012).

the other tested metals) and TOC are linked to fine grained sediments and inversely related to coarse grained sediments. PC1 also correlates central-lagoon sites (sites 6, 7, 9 and 10). Salinity and DO are positively correlated to PC2 and inverse to temperature and turbidity. Higher salinity and DO correspond to sites closer to the mouth of the lagoon and lower salinity and DO corresponding to sites further from the mouth (sites 2 to 5).

The CCA (Fig. 5) supports the results from the Q-mode cluster analysis (Fig. 3). The first two CCA axes (axis 1 = 25.4% and axis 2 = 21.5%) were significant ($p < 0.001$) and explained a 46.8% of the total variance (Fig. 5) The eight environmental variables shown (OM, TOC, Mn, sand, silt, turbidity, salinity and depth) were statistically significant (after variance partitioning, ranging from $p < 0.025$ for silt to $p < 0.005$ for Mn) in explaining the ostracod composition and distribution across Lap An Lagoon.

Mn and water depth were negatively correlated with Biofacies I and II. Salinity was positively correlated with Biofacies III. Turbidity, TOC, silt and OM were positively correlated with Biofacies IV, whereas sand was negatively correlated with Biofacies IV. Mn is also significant for site 10 in Biofacies IV.

5. Discussion

5.1. Sediments

Higher sand fractions were found at nearshore sites and outer lagoon sites due to the removal of fine grains by tidal action (Bidet and Carruesco, 1982; Ruiz et al., 2004). The low OM and TOC contents in the nearshore sites are likely due to the coarser sediments having a reduced surface area to adsorb organic matter (Hedges et al., 1993). In contrast, site 14 has high OM and TOC content due to its proximity to the Hoi Dua River outlet, which transports fine and coarse sediments and organic debris to this site (Nhơn, 2008).

The sediments were largely unpolluted by heavy metals. The low Igeo (Table S4) can be attributed to scarce industrial activities in adjacent areas to the lagoon as concentrations of heavy metals were similar to neighbouring lagoons and the seas (Table S7; Romano et al., 2012). The metals found in the sediment were likely

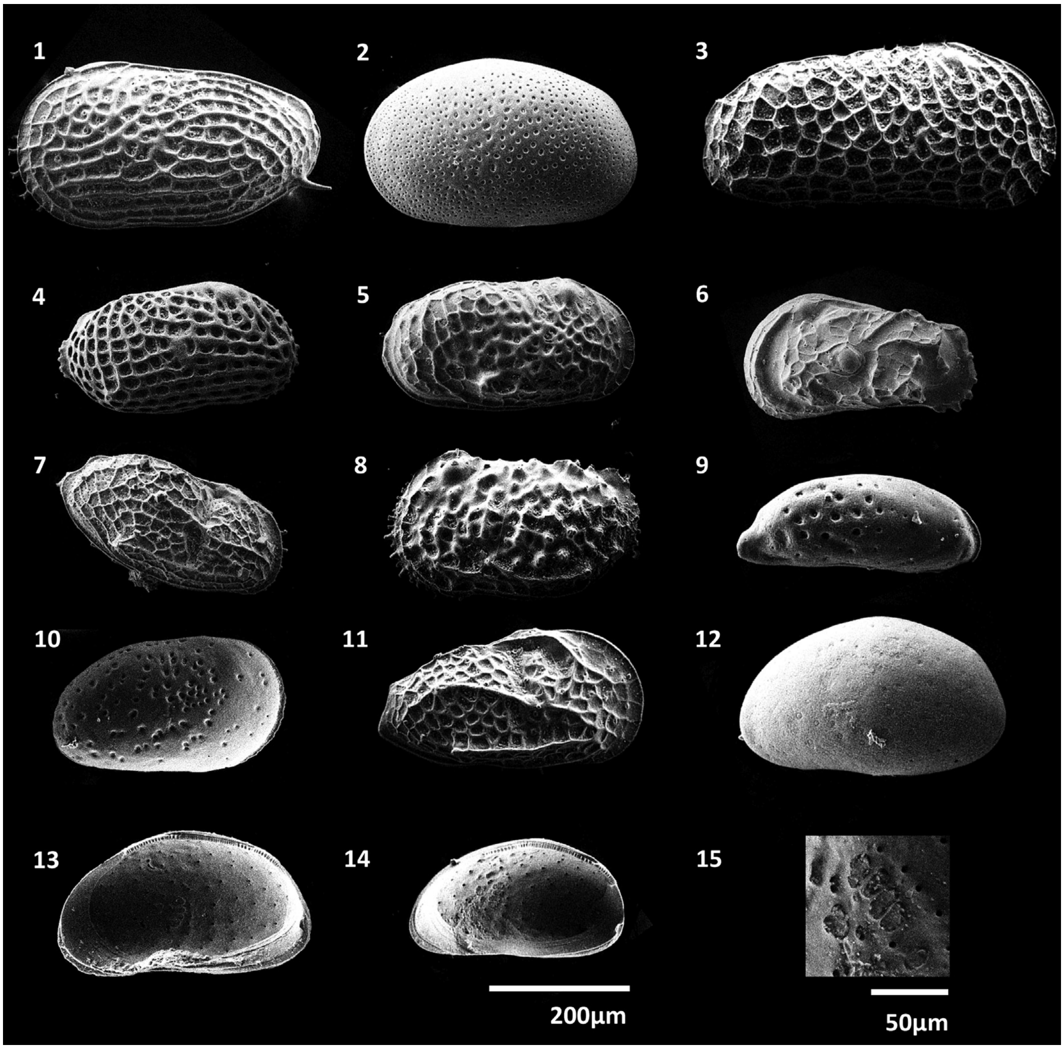

Fig. 2. Scanning electron microscope (SEM) images of dominant ostracods from Lap An Lagoon. 1: *Bicornucythere bisanensis* (Form G of Abe (1988) and Abe and Choe (1988)), left valve in exterior view, 2: *Neocyprideis agilis* (Wouters, 2005), left valve in exterior view, 3: *Hemicytheridea reticulata* (Kingma, 1948), right valve in exterior view, 4: *Keijella gonia* (Zhao and Whatley, 1989), right valve in exterior view, 5: *Loxoconcha vietnamensis* (Tanaka et al., 2009), right valve in exterior view, 6: *Cornucoquimba nodosa* (Hu and Yang, 1975), left valve in exterior view, 7: *Neomonceratina iniqua* (Brady, 1868), right valve in exterior view, 8: *Pistocythereis bradyformis* (Ishizaki, 1968), left valve in exterior view, 9: *Pontocythere mera* (Guan, 1978), right valve in exterior view, 10: *Sinocytheridea impressa* (Brady, 1869), left valve in exterior view, 11: *Spinileberis quadriculeata* (Brady, 1880), right valve in exterior view, 12: *Xestoleberis* sp., left valve in exterior view, 13: *Xestoleberis munensis* (Dung and Tsukagoshi, 2018) right valve in interior view, 14: *Xestoleberis vietnamensis*, inner valve, (Dung and Tsukagoshi, 2018), right valve in interior view, 15: *Xestoleberis vietnamensis* (Dung and Tsukagoshi, 2018), muscle scar of no. 14. Scale bar for images 1–14 is 200 µm. Scale bar for image 15 is 50 µm.

from lithogenic sources such as the weathering of calc-alkaline rocks that are ubiquitous throughout central Vietnam (Al-Shawi and Dahl, 1999; Thuy et al., 2000). However, with most sites having EF > 1, some anthropogenic sources of Cu, Mn and Zn were present. Elevated levels of Cu are likely due to pesticide run-off from nearby agricultural lands (Barut et al., 2018; Trinh et al., 2018). Mn is likely sourced from metal works and agricultural organic wastes (Li et al., 2014). Urban street runoff and domestic charge carrying Zn could have increased due to burgeoning coastal population and development near the lagoon (Dũng, 2012; Vilela et al., 2011).

Despite minor enrichment of heavy metals in the lagoon, some sites contained sediments that were likely harmful to benthic communities. A TOC $\leq$1% has a low impact on benthic communities and a TOC of 1 to 3% have intermediate negative impacts on the benthic communities (USEPA, 2002). Sites 3, 5, 6, 7 and 9 fall within the intermediate impact category.

Comparing sediment concentrations of Cu, Ni, Pb and Zn against sediment quality guidelines (Burton Jr., 2002), suggests that silty sites were potentially toxic to benthic communities. The effect range level (ERL) and threshold effect level (TEL) represent concentrations where adverse effects on benthic communities were reported around 10% and 25% of the time, respectively (CCME, 2001; NOAA, 2012). Sites 5, 6, 7 and 9 had concentrations of Cu, Ni and Pb exceeding the TEL and Ni concentrations exceeding ERL (NOAA, 2012, Table 3).

The effects range median (ERM; NOAA, 2012) and the probable effect level (PEL; CCME, 2001) represent the concentration at which toxic effects are observed at least 50% of the time. Only Site 5 had Ni levels exceeding ERM, and sites 5, 6 and 7 had Ni concentrations exceeding the PEL, suggesting that Ni concentrations were highly likely to have a negative impact on benthic communities. Hence, the TOC and heavy metal concentrations could have an adverse impact on benthic ostracods, reflecting the health of the lagoon and the commercially farmed seafood.

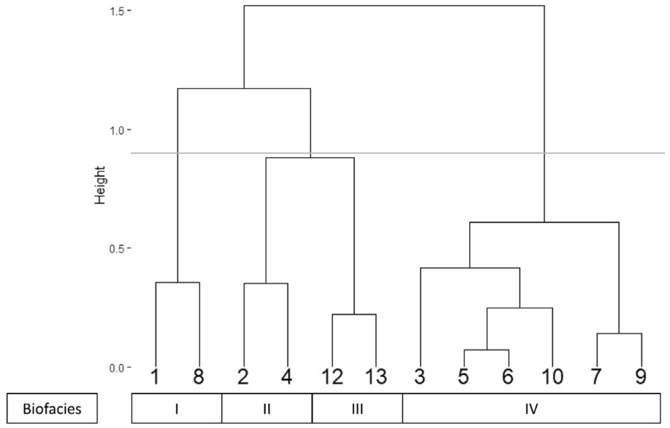

Fig. 3. Dendrogram of ostracod cluster analysis shows four different biofacies from Lap An Lagoon. The four biofacies were determined from a cut-off height of 0.9 (grey line).

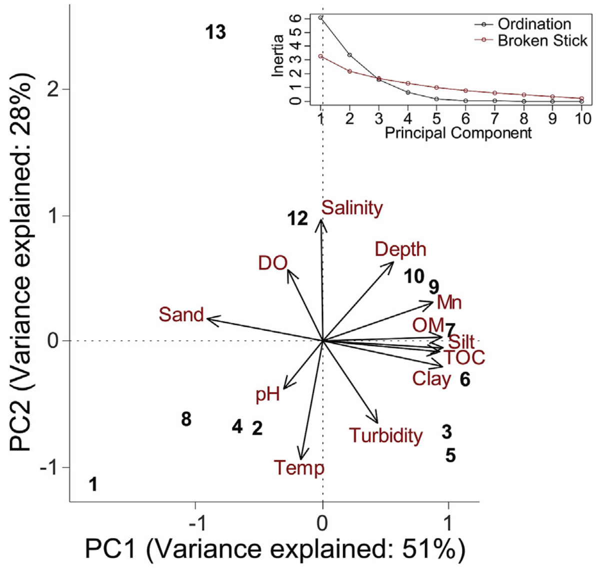

Fig. 4. PCA diagram showing the first two PCs showing the relationship between environmental variables (black arrows with red variable names) and sites (black numbers). Only variables with sufficient data for analysis are included (i.e. Zn, Pb, Ni, Fe and Cu are excluded due to the number of BDL results). Inset shows the ordination (black) against the broken stick (red) models highlighting that only two PCs are required to explain most of the variance. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

5.2. Ostracod biofacies as environmental indicators

5.2.1. Biofacies I and nearshore environments

Ostracods in Biofacies I (dominant taxa are *C. huyeni*, *T. gracilis* and *S. quadriaculeata*) prefer sandy and shallow (< 0.4 m) nearshore environments. *C. huyeni* has been found in shallow (0.1 to 0.5 m) sandy coastal environments in northern Vietnam (Tanaka et al., 2009). *T. gracilis* is also a common shallow marine species and has a wide geographic distribution, found in the coasts along India, Indonesia, Persian Gulf and Australia (Hartmann, 1978; Hussain et al., 2010). *S. quadriaculeata* prefers silty and deeper (1.5 to 12 m) aquatic environments (Irizuki et al., 2015; Nakao and Tsukagoshi, 2002; Zenina, 2009). Our findings suggest that *S. quadriaculeata* survives in a wider range of substrate types and depths.

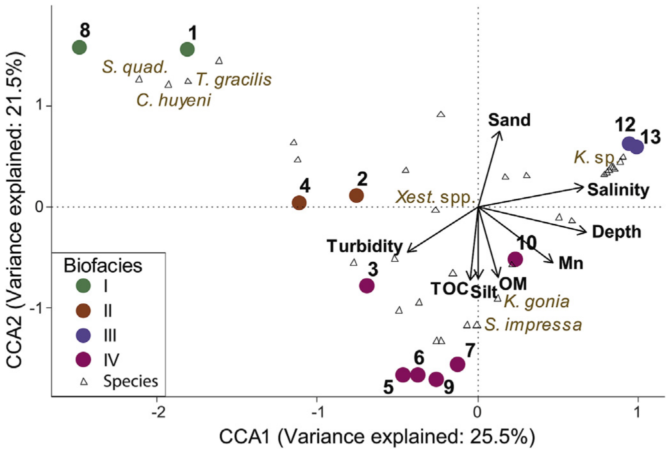

Fig. 5. CCA plot of species (represented as "△"), environmental parameters (arrows and black text) and sites (numbered and colour coded to ostracod species biofacies defined by the cluster analysis – Red = Biofacies I, Green = Biofacies II, Blue = Biofacies III and purple = Biofacies IV). Note that only a subset of the total species are named with three species names abbreviated for clarity (S. quad = *S. quadriaculeata*, K. sp. = *K.* sp. aff. *K. karwarensis* and Xest. spp. = *Xestoleberis* spp.). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

5.2.2. Biofacies II and silty sand environments

Xestoleberis spp. (likely *X. vietnamensis* and *X. munensis*) were dominant in silty sands with low TOC content consistent with Irizuki et al. (2008). *X. vietnamensis* and *X. munensis* were present in coarse sand, dead corals and algae along the Vietnam coast (Dung and Tsukagoshi, 2018). Unfortunately, due to the smooth valves of *Xestoleberis*, refining the identification of the *Xestoleberis* spp. in our samples was difficult (e.g. Sato and Kamiya, 2007).

5.2.3. Biofacies III and lagoon-shallow marine interface

Ostracod species (e.g. *Xestoleberis* spp. and *K. gonia*) in Biofacies III were associated with the transition from lagoon to shallow marine sandy environments (Dung and Tsukagoshi, 2018; Whatley and Zhao, 1987). *Xestoleberis* spp. and *K. gonia* are brackish-marine species as they were found throughout Lap An Lagoon. *K.* sp. aff. *K. karwarensis* and *C.* sp. aff. *C. leroyi* were found only in the outer lagoon, suggesting that these species likely occur in transitional shallow marine and lagoonal environments. *K. karwarensis* was found in many sediment types but dominated in muddy sand and mud over sandy environments in water depths of 2 to 80 m in the Persian Gulf (Bhatia and Kumar, 1979; Whatley and Zhao, 1988). *C. leroyi* is commonly distributed in South China Sea and inner and middle neritic waters off Brunei and Borneo (Keij, 1964).

5.2.4. Biofacies IV and marginally toxic environments

Biofacies IV was dominated by brackish water *S. impressa*, a common species along the coasts of northern Vietnam and southern China (Hong et al., 2019; Tanaka et al., 2011; Zhao and Wang, 1988). *S. impressa* prefers turbid and nutrient-rich muddy to fine sandy environments (Irizuki et al., 2006; Tanaka et al., 2009). It thrives in eutrophic and hypoxic waters of Tolo Harbour and is negatively associated with dissolved oxygen in coastal waters off Hong Kong (Hong et al., 2019; Hu et al., 2001). At Lap An Lagoon, *S. impressa* was commonly found in silty and clayey sediments with concentrations of Cu, Ni and Pb >TEL and TOC content (1 to 2%). Our findings suggest that *S. impressa* is found in marginally toxic environments and may be a useful pollution indicator in Lap An Lagoon.

5.3. Factors affecting ostracod composition and distribution

Located near the mouths of freshwater streams, sites 11 and 14 had very low ostracod abundance. Both sites had high turbidity (10 and 11 NTU, respectively (Table S1)). Additionally, site 14 had low DO (3 mg/l). Anthropogenic discharge of organic debris and waste from nearby fish farms and households (Thang et al., 2012) could lead to high decomposition of organic matter which resulted in low levels of DO. Such conditions were unfavourable to marine organisms, and thus, very low number of ostracods were found (Vaquer-Sunyer and Duarte, 2008).

Excluding sites 11 and 14 from the analysis, the CCA demonstrated that ostracod composition and distribution were controlled by salinity, depth, turbidity, Mn, sand, silt, TOC and OM. Salinity and depth were positively correlated with CCA axis 1 and Biofacies III, and negatively correlated with Biofacies II and turbidity (Fig. 5). Mn is inversely correlated with Biofacies I and III but high concentrations are observed in site 10 of Biofacies IV (Fig. 5). Sediment grain size is strongly correlated with CCA axis 2 with sand inversely correlated to silt. Silt is also highly correlated with TOC and OM, and all three variables are significant factors that define Biofacies IV (Fig. 5).

Ostracods in this study were sensitive to the narrow depth range (0 to 3.1 m) and a weak salinity gradient. Ostracods of Biofacies I were associated with very shallow depths (<0.4 m) with *T. gracilis* also recovered from shallow depths (1 to 1.5 m) in the Gulf of Thailand (Montenegro et al., 2004; Nevio et al., 2006). Most of our sampling sites had salinity levels ranging from 31.5 ppt to 32.6 ppt and were composed of brackish water ostracod species, such as *B. bisanensis* and *N. iniqua*. Sites with higher salinity levels (32.7 to 33.5 ppt) also had a higher species diversities containing both brackish and marine ostracod species.

The ostracod composition and distribution was also controlled by sediment type, TOC, OM and heavy metal concentrations. Sediments of higher silt and clay percentage supported higher ostracod densities (e.g. Biofacies IV) confirming previous ostracod studies (Armstrong and Brasier, 2013; Faiz et al., 2007, 2014; Machado et al., 2005; Mostafawi, 1992; Omar and Faiz, 2009, 2010; Whatley and Zhao, 1987, 1988). Higher TOC contents provide more food to support large benthic ostracod populations (Yasuhara et al., 2007). Correspondingly, sandy sediments, with lower OM and TOC, had low ostracod densities (Table S5). Salel et al. (2016) also observed low faunal density (0 to 2 ostracods/10 g of sediment) along sandy shorelines.

Whilst higher TOC content supports larger populations, low ostracod diversities were found, contradicting previous studies (Faiz et al., 2007, 2014; Machado et al., 2005). TOC content and heavy metals were at levels potentially harmful to benthic ostracods (Burton Jr., 2002; USEPA, 2002). Although high TOC content would likely result in high decomposition rates and low DO levels that are unfavourable to marine organisms, our daytime measured DO levels were relatively high at the sampling sites. Although we did not collect DO measurements at night, the diurnal tidal fluctuation and the large surface area to depth of the lagoon facilitated rapid exchange of gas across the water-air interface and water mixing within the lagoon. Additionally, the decomposition of organic matter under aerobic conditions releases heavy metals that were absorbed by the organic matter (Shaheen and Rinklebe, 2014). Elevated heavy metal concentrations can be toxic to ostracod communities and only a few ostracod species can tolerate such concentrations (Irizuki et al., 2018; Ruiz et al., 2005).

6. Conclusion

A diverse ostracod fauna comprising 79 species over 46 genera exists in the contemporary environments of Lap An Lagoon, central Vietnam. The spatial variability environmental parameters identified four ostracod biofacies, representative of nearshore shallow sandy, silty sand substrate, marginally toxic and a transition from lagoon to shallow marine environments. Comparing the sediment-bound TOC and heavy metal concentrations within the lagoon and the ostracod fauna, highlights the potential for particular Southeast Asian ostracods to be used as pollution bioindicators in lagoonal environments. *S. impressa* is indicative of marginally toxic environments composed of silt with TOC content between 1 and 2%. While Lap An Lagoon currently reflects conditions of low anthropogenic pollution, the lagoon is transitioning to greater pollution with sediment enrichment of heavy metal pollutants from anthropogenic sources. The ostracod data serves as a baseline for future pollution and monitoring studies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Elva Segovia for analytical support within the GeoLab of the Department of Geography at National University of Singapore and Sim Joo Huang for her assistance with the SEM at Yale-NUS College. We also extend our gratitude to Professor Karel Wouters, Professor Akira Tsukagoshi and Dr Marie-Béatrice Forel, for their help in the identification of the ostracod species. Sediments were analysed by Lee Ying Sin in the sedimentological laboratory of the Asian School of the Environment, Nanyang Technological University. CG and CTWJ acknowledge the support of Grant: R-109-000-223-133 and ADS acknowledges the support of National Research Foundation of Singapore Grant: NRF-RF2010-04 and the Singapore Ministry of Education under the Research Centres of Excellence Initiative and is EOS Contribution No 353. We thank three reviewers for their critical comments on earlier versions of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.116762.

Credit roles

CWJT; Conceptualisation, Methodology, Investigation, Formal analysis, Resources, Writing – original draft, Writing – review & editing, CG: Conceptualisation, Methodology, Investigation, Formal analysis, Resources, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition, PTD: Investigation, Formal analysis, Writing – review & editing, HDQ: Investigation, Writing – review & editing, ADS: Conceptualisation, Resources, Writing – review & editing, Supervision, Funding acquisition.

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