Calibration of Mg/Ca and Sr/Ca in coastal marine ostracods as proxy of temperature

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Abstract. The Mg/Ca and Sr/Ca ratio of marine shells have been widely used in environmental paleoreconstructions to understand past marine conditions. Temperature calibrations to ostracod Mg/Ca ratios are known to be species-specific but only available for a few species, despite the large number of known ostracod species. Here, we develop temperature calibrations for two shallow marine ostracods, *Sinocytheridea impressa* and *Neomonoceratina delicata*, using modern sediment samples. Our results show that adult specimens of these two species might be useful as a paleothermometer. We observed significant correlations using the Mg/Ca ratios of both species to the annual ($Mg/Ca_{S.\ impressa}=3.7 \bullet T-62.7$, $Mg/Ca_{N.\ delicata}=1.6 \bullet T-16.8$) and April ($Mg/Ca_{S.\ impressa}=2.7 \bullet T-37.2$, $Mg/Ca_{N.\ delicata}=1.6 \bullet T-15.7$) temperatures. The correlation of temperature to the Mg/Ca ratio of *S. impressa* is more significant, and therefore should be preferred for paleoreconstructions. Re-analysis from satellite data allows us to validate our temperature calibration to an extended area around the Pearl River Estuary. Our results show that Mg/Ca of *S. impressa* and *N. delicata* ostracods can be used to reconstruct water temperature at a regional scale, which provides information on the oceanic circulation in coastal areas of the South China Seas. Sr/Ca ratios of both species do not correlate with any of the 24 water parameters recorded by the Environmental Protection Department of Hong Kong, including temperature (21.7–24.1 °C), salinity (23.8–33.7 PSU), dissolved oxygen (4.3–7.1 mg L^{-1}), suspended solids (1.9–35.4 mg L^{-1}) and pH (7.7–8.2).

1 Introduction

Element/Calcium ratios (E/Ca) of secreted biogenic calcium carbonate by marine organisms, such as foraminifera and corals, have been used as proxies of past environmental marine parameters (Hendy et al., 2002; Lea, 2003; Lea and Boyle, 1989; Linsley et al., 2000; Martin et al., 2002). Mg/Ca in foraminifera and Sr/Ca in corals have been frequently used to reconstruct water temperatures (Beck et al., 1992; Cohen et al., 2001, 2002; Lea, 2003; Rosenthal et al., 2006; Sinclair et al., 1998; Yu and Elderfield, 2008). In addition, Mg/Ca of ostracod shells have also provided valuable information about water temperatures (Chivas et al., 1983, 1986a; De Deckker and Forester, 1988; Dwyer et al., 2002). The thermodependency of the Mg incorporation into calcite has been observed in natural environment (Cadot and Kaesler, 1977; Corrège and De Deckker, 1997; Cronin et al., 2005a; Dwyer et al., 1995) and culture experiments (Chivas et al., 1986b; Kondo et al., 2005). Several studies have focused their efforts on the development of calibrations for deep water genera, such as *Krithe*, which are found in the Pacific, Atlantic, and Arctic oceans (Corrège and De Deckker, 1997; Cronin et al., 1996; Dwyer et al., 1995, 2002; Elmore et al., 2012; Farmer et al., 2011, 2012; Rodriguez et al., 2020). Other studies have investigated species found in shallow marine environments, such as those from the genus *Loxoconcha* (Cronin et al., 2003, 2005a) and *Cyprideis* (Holmes and De Deckker, 2016; Roberts et al., 2020). However, there is uncertainty in temperature calibrations to Mg/Ca of marine ostracods due to the low number of calibrations developed in comparison to the number of known species (Holmes and De Deckker, 2012) and the contrasting calibration slopes observed in different species (Yamada et al., 2014). Developing calibrations for new ostracod species can enhance our understanding of the processes controlling ostracod Mg/Ca uptake and broaden its use to other areas of the globe (Holmes and De Deckker, 2012; Lea, 2003). The comparison of Mg/Ca and Sr/Ca with multiple water parameters can also improve our understanding of the variables controlling the incorporation of trace-elements into the ostracod carapace. Mg/Ca and Sr/Ca of ostracod shells have also been used as a proxy of salinity in enclosed water bodies (Chivas et al., 1983) based on two main assumptions: 1) Sr/Ca_{water} and Mg/Ca_{water} exert a control on Sr/Ca_{shell} and Mg/Ca_{shell} (Dettman and Dwyer, 2012) and 2) salinity increases simultaneously with the removal of Ca by low-Mg calcite precipitation during evaporation, increasing the water content of Mg relative to Ca (Ito et al., 2003). However, there are several variables that limit their applicability, such as groundwater inputs, non-equilibrium state of calcite precipitation, among others (Ito et al., 2003). A weak control of water temperature and alkalinity on Sr/Ca ratios has also been documented in lacustrine species (De Deckker et al., 1999; Gouramanis and De Deckker, 2010). In spite of this use in lacustrine systems, Sr/Ca of ostracod shells do not seem to be related to salinity or temperature in shallow marine environments (Dettman and Dwyer, 2012; Gouramanis and De Deckker, 2010; Ingram, 1998; Roberts et al., 2020). Here, we develop parametric calibrations for Mg/Ca and Sr/Ca of two geochemically unknown species, *Sinocytheridea impressa* and *Neomonoceratina delicata*.

Sinocytheridea impressa and *N. delicata* are shallow marine ostracods from the superfamily Cytheroidea (Brandão and Karanovic, 2020), which are mainly distributed in Asian waters (Hong et al., 2019; Tanaka et al., 2011). Species of *S. impressa* and *N. delicata* have been reported in sediment records from Miocene and Pleistocene respectively (Irizuki et al., 2005, 2009). The abundance of both species has been used as an indicator of paleoenvironmental response to sea-level transgressions (Chunlian et al., 2013). A high abundance of both species rarely occurs simultaneously as *S. impressa* is commonly found in hypoxic environments with low salinity and high turbidity, while *N. delicata* is common in well-ventilated, polyhaline bays (Hong et al., 2019; Irizuki et al., 2005). Paleoenvironmental investigations have focused on the study of assemblages of these two species, but not the elemental composition of their shells as indicator of environmental marine variables, such as temperature or salinity. The calibration of E/Ca ratios of their shells with ocean parameters in an estuary-dominated system may be used to reconstruct shallow marine environments in Asia and complement previous studies on the paleoenvironmental response to sea-level transgressions (Chunlian et al., 2013).

Estuaries are ecosystems with high productivity and biodiversity (Day et al., 2013). Water temperature and salinity are important physical properties of these ecosystems. These parameters are controlled by the combined effects of oceanic currents, upwelling waters, river discharge and atmospheric forcings, such as winds and precipitation. The study of water temperature and salinity can help us understand the evolution of marine currents and global atmospheric patterns, which can improve our understanding of glaciation cycles and sea-level transgressions. For example, Mg/Ca of *Loxoconcha* specimens from Chesapeake Bay has been used as a temperature proxy to evaluate anthropogenic and North Atlantic Oscillation (NAO) impacts on past and present climates (Cronin et al., 2005b). Hong Kong (HK) and the surrounding waters of the Pearl River Estuary (PRE) are similar. The local waters are affected by multi-annual oceanic and atmospheric patterns such as El Niño-Southern Oscillation (Niu, 2013; Zhang et al., 2013). The development of a new water temperature and salinity proxy will give us a tool to improve our understanding of past marine conditions as well as a better knowledge of oceanic and atmospheric regional patterns. However, the calibration of marine shells with ocean parameters in estuarine systems are challenging due to the highly dynamic chemical and physical variabilities (Snedden et al., 2013), and the combined presence of ostracod populations from different years. Here, we investigate the applicability of ostracod Mg/Ca and Sr/Ca ratios as proxies of temperature in a freshwater-influenced marine system and several factors that control the robustness of the parametric calibrations including: 1) number of shells per site, 2) seasonal variability of ocean parameters, 3) ostracod molting time and 4) the life stage of the ostracods.

2 Methodology

2.1 Water parameters

2.1.1 Marine stations

The main dataset consists of measurements of water parameters around the PRE and Hong Kong waters. Water parameters from Hong Kong were obtained from the Environmental Protection Department of Hong Kong (EPD), which records 24 parameters of water quality, including temperature, salinity, pH, and dissolved oxygen around HK (Fig. 1). Monthly single measurements are performed in 8 water control zones: Tolo Harbour, Southern Waters, Port Shelter, Junk Bay, Deep Bay, North Western, Mirs Bay, Western Buffer, Eastern Buffer and Victoria Harbour (Fig. S1). The records correspond to one or two daily values per month from 1986 to the present date (EPD, 2018). The sedimentation rate in Hong Kong varies from 0.2 to 5 cm yr^{-1} (Owen and Lee, 2004; Tanner et al., 2000), which suggests the presence of specimens from different years. For the calibration, we calculated monthly mean values using the last 20 years of data from the collection time of our sediment samples (i.e. 2012) in order to determine a robust monthly mean value with the maximum amount of available data. Extreme values with a probability of exceedance higher than 99% and lower than 1% were removed from each parameter, in order to consider the most probable values. This was calculated by organizing the historical dataset in descending order and estimating the probability of occurrence of each value regarding the whole dataset. Seawater Mg, Sr and Ca concentrations are available in the Environmental Impact Assessment (EIA) study of the desalination plant in Tseung Kwan O submitted to the EPD in 2013 (Black & Veatch Hong Kong Limited and Water Supplies Department, 2013).

Additionally, seawater surface temperatures at 0.5 m depth were retrieved from Tanoura buoy (田浦) to estimate water temperatures at the site of ostracod collection in the Yatsushiro sea. This data is available in the webpage of the Kumamoto Prefectural Fisheries Research Center (2021).

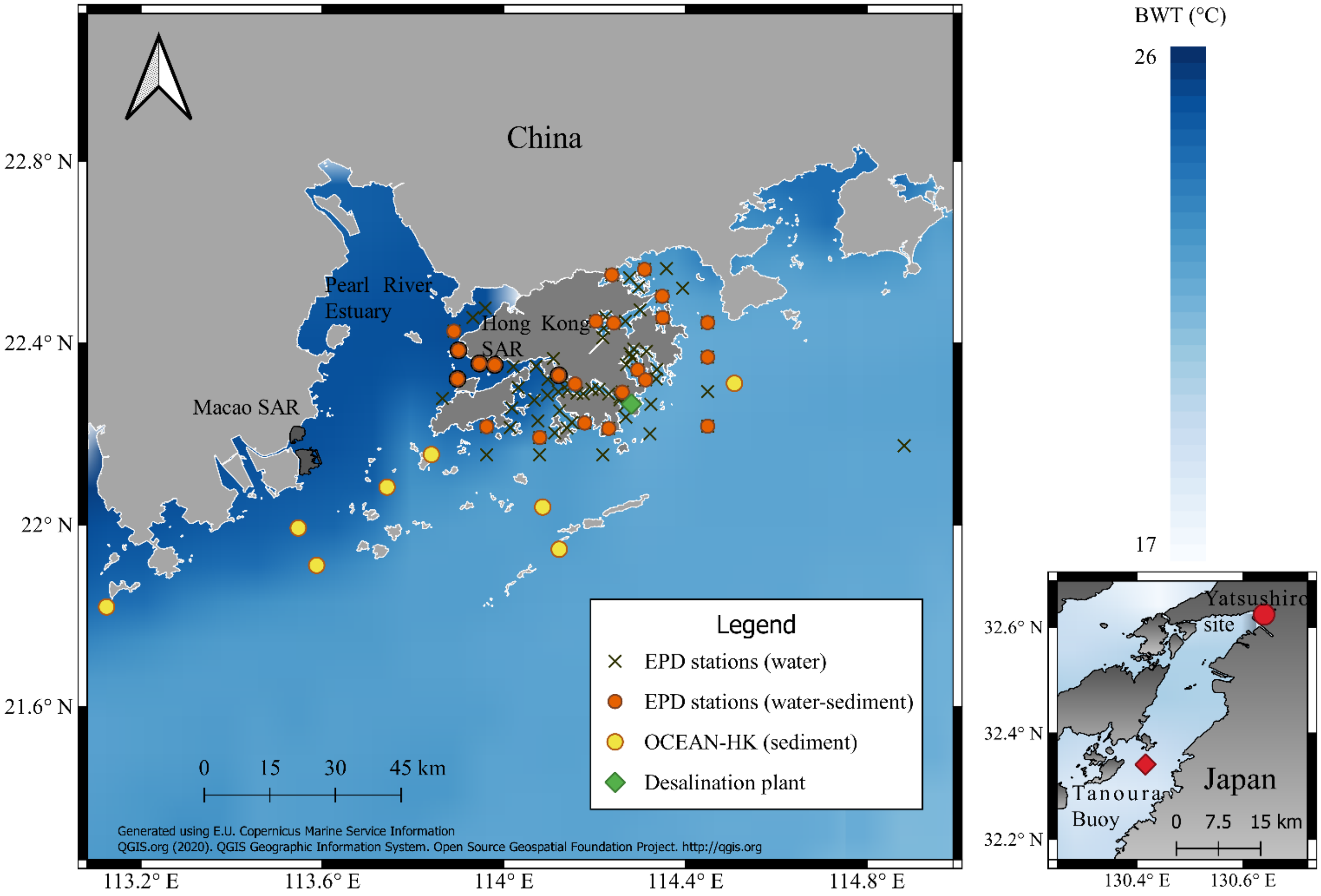


Figure 1 Sampling sites around the Pearl River Estuary (PRE) and the Yatsushiro bay in Japan. Ostracods were collected from sediment samples obtained by the Environmental Protection Department of Hong Kong (EPD) and OCEAN-HK in Hong Kong. Monthly water quality data is obtained from different sampling locations across Hong Kong (EPD stations). Mean bottom water temperature (BWT) for the year 2011 was obtained from the satellite product of the Copernicus Marine Environment Monitoring Service (CMEMS).

2.1.2 Copernicus products

Worldwide potential bottom water temperature (BWT), provided by the Copernicus Marine Environment Monitoring Service (CMEMS) of the European Union, is available from 1993 to 2018 at a spatial resolution of 1/12° grid. Potential BWT is a product calculated from re-analysis, which are intended to be as close as possible to real observations (Drévillon et al., 2018). The product is obtained after the assimilation of satellite observations through real-time marine observations and the modelling of atmospheric and oceanic variables, such as tidal and heat fluxes, among others (CMEMS, 2020). The product has low biases at regional and global scales (<0.4° C and <0.1° C respectively), but higher errors are present in coastal regions due to land cover and river inputs. We calculated the bias and correction factors between the BWT from the CMEMS and EPD in areas where the data overlaps (Fig. S1). These factors were applied to correct the potential BWT in areas where we do not have direct measurements such as in the sampling locations of OCEAN-HK.

2.2 Ostracod samples

We investigated adult specimens of *S. impressa* (Brady, 1869; Whatley and Zhao, 1988) and *N. delicata* (Ishizaki and Kato, 1976) from the uppermost 1 cm sediment layer collected in HK by the EPD in January and July 2012 (Hong et al., 2019; Rodriguez, 2021). The temperature calibration was developed using only samples from HK waters. We also used samples collected around the PRE by OCEAN-HK in July 2017 to validate the calibrations. Ostracods were collected from sediment samples sieved in a 150 µm mesh. Most of the specimens collected from HK and PRE consist of single valves without animal appendages (Fig. 1). For *S. impressa*, we distinguished adult ostracods as those larger than 600 µm (Irizuki et al., 2005) and with a well-developed inner lamella (Fig. S2). For *N. delicata*, we distinguished adult specimens by size (>450 µm) based on Wang et al. (2018) and Fig. S2. We additionally included *S. impressa* specimens from the Yatsushiro sea collected on June 7th and November 7th, 2020 from the intertidal zone (32.624° N, 130.640° E, 0.5 m depth, Fig. 1) in order to test the calibration developed. These specimens present the animal appendages and intact right and left valves. The size of these ostracods ranged between 550 µm and 650 µm and had a well-developed inner lamella.

2.3 Trace-element analyses

Ostracods shells were sonicated in a methanol bath, rinsed twice with Milli-Q water, bleached with 5% sodium hypochlorite for 12-24 hours and rinsed twice again with Milli-Q water to limit potential contamination affecting the carbonate Mg/Ca and Sr/Ca (Rodriguez, 2021). Then, elemental concentrations on individual shells were measured by ICP-MS Agilent 7900 in the School of Biological Sciences at The University of Hong Kong. We measured ^{48}Ca, ^{43}Ca, ^{24}Mg, ^{25}Mg, ^{86}Sr and ^{88}Sr. In addition, we measured ^{27}Al and ^{56}Fe to control for potential contamination in our samples. The data was corrected by a blank (2% HNO_3) measured every third sample and a multi-element standard measured every sixth sample, which was prepared from individual pure elemental solutions (MES1, Mg=28.1 ppb, Sr=29.6 ppb, Al=28.1 ppb, Fe=29.4 ppb, Ca=1830 ppb). A multi-element standard prepared from a pure multi-element solution (MES2, Mg=28.6 ppb, Sr=28.6 ppb, Al=28.6 ppb, Fe = 28.6 ppb and Ca=1861 ppb) and a carbonate standard JCp-1 (Hathorne et al., 2013; Inoue et al., 2004; Okai et al., 2002) were used to check the quality of the analysis. Our precision and accuracy improved using ^{48}Ca and ^{24}Mg. The accuracy and precision (RSD) of the analysis for JCp-1 and MES2 standards are shown in Table 1 (n=90). The detection limit of the concentrations was initially estimated using the blank as 3σ, resulting in a value lower than 0.3 ppb for Mg, Sr, Al and Fe. Then, the detection limit of the ratios was calculated as 3σ of a solution with a concentration of 0.1 ppb for Mg, Sr, Al and Fe and 990 ppb of Ca (Yu et al., 2005). The precision and accuracy for Al and Fe could not be determined for JCp-1 because of the low ratios within the standard and contamination associated with our analytical procedure.

Table 1 Detection limit (DL), accuracy (acc.), and precision (RSD) of the standards used to assess the analytical quality of the measurements.

Ratios	DL (mmol mol^{-1})	JCp-1 ratio (mmol mol^{-1})	JCp-1 acc. (%)	JCp-1 RSD (%)	MES2 ratio (mmol mol^{-1})	MES2 acc. (%)	MES2 RSD (%)
Mg/Ca	0.7	4.2	0.2	3.7	25.4	-2.7	2.7
Sr/Ca	0.1	8.7	-2.5	2.9	7	-2.5	3.3
Al/Ca	1.4	1.9	>20%*	>20%*	22.9	-2.2	3.0
Fe/Ca	1.0	0.05	Below DL	Below DL	11	-2.2	3.2

*The poor accuracy and precision for Al/Ca ratios in JCp-1 are the result of the low ratios of the standard and external contamination in the analytical procedure.

3 Results

3.1 Marine waters

Considering Hong Kong sampling sites, annual mean BWT ranges between 21.8° C and 23.9° C (Fig. 1). The maximum monthly temperature has been recorded close to the PRE (28.6° C) in August, while the minimum monthly temperature has been recorded eastward Hong Kong Island (16.2° C) in February. Mean annual salinity ranges from 24.7 PSU to 33.7 PSU. The maximum monthly salinity was measured eastward Hong Kong Island in August (34.3 PSU), while the minimum salinity was recorded close to the Pearl River in July (15.6 PSU). A negative linear correlation is observed between the annual temperature and salinity considering all EPD stations in Hong Kong waters (R^2=-0.67, Fig. S4). BWT and salinities from the EPD grouped by water control zones are shown in Table 2. A summary of annual mean values of other parameters such as dissolved oxygen and suspended solids can be found in Table S1.

Average surface water temperatures at the Tanoura buoy recorded in the months of ostracod collection were 23° C and 20.7° C for June and November, respectively. Satellite images reveal a temperature difference lower than 1° C between the surface temperature in the Tanoura buoy and the sampling site in the Yatsushiro sea. Therefore, we considered surface water temperatures from Tanoura buoy (0.5m depth) as representative of the water temperature collected in the sampling site in the Yatsushiro sea.

Table 2 Mean and standard deviation (1 sd) of Mg/Ca and Sr/Ca ratios of adult *S. impressa* and *N. delicata*. The number of shells for Mg/Ca and Sr/Ca are the same. Last two columns show mean and standard deviation (1 sd) of annual bottom water salinity and temperature by water control zone of HK.

Zone	Mg/Ca mmol mol^{-1} (*S. impressa*)	Mg/Ca mmol mol^{-1} (*N. delicata*)	Sr/Ca mmol mol^{-1} (*S. impressa*)	Sr/Ca mmol mol^{-1} (*N. delicata*)	Salinity (PSU)	BWT (°C)
Deep Bay	17.8 (n=1)	19.7±4.4 (n=9)	3.3	3.7±0.5	26.5±2.5	23.7±0.3
Junk Bay	19.5±4.7 (n=6)	No samples	3.5±0.1	No samples	32.9±0.2	22.5±0.2
Mirs Bay	19.8±3.7 (n=48)	18.3±3.3 (n=36)	3.6±0.2	3.4±0.4	33.1±0.5	22.2±0.4
Northern Waters	23.6±3.8 (n=49)	19.9±3.2 (n=21)	3.6±0.3	3.5±0.5	30.3±1.1	23.1±0.2
Port Shelter	18.3±3.2 (n=13)	17.5±3.2 (n=7)	3.6±0.1	3.4±0.3	32.9±0.3	22.5±0.5
Southern Waters	20.9±4.4 (n=27)	17.2±3.9 (n=7)	3.5±0.2	3.2±0.4	32.3±1.0	22.9±0.4
Tolo Harbour	19.4±2.6 (n=8)	No samples	3.6±0.1	No samples	32.0±0.7	22.8±0.7
Victoria Harbour	21.7±4.0 (n=18)	No samples	3.3±0.2	No samples	31.9±0.6	22.9±0.2
HK Average	21.1±4.2 (n=170)	18.7±3.5 (n=80)	3.5±0.2	3.5±0.4	32.2±1.4	22.7±0.5

3.2 Ostracod ratios

Mg/Ca mean values of adult *S. impressa* and *N. delicata* ostracods from EPD samples are 21.1±4.2 mmol mol^{-1} (n=170, min=13.6 mmol mol^{-1}, max=34.8 mmol mol^{-1}) and 18.7±3.5 mmol mol^{-1} (n=80, min=10.3 mmol mol^{-1}, max=28.1 mmol mol^{-1}) respectively. Mg/Ca mean values of adult *S. impressa* and *N. delicata* ostracods from OCEAN-HK are 20.1±3.7 mmol mol^{-1} (n=51, min=14.4 mmol mol^{-1}, max=35.9 mmol mol^{-1}) and 19 mmol mol^{-1} (n=30, 14.1 mmol mol^{-1} to 24.7±2.8 mmol mol^{-1}) respectively. Mg/Ca mean values in each EPD water control zone are in Table 2. Mg/Ca mean values of *S. impressa* specimens from the Yatsushiro sea were 21.6±3.8 mmol mol^{-1} (n=14, min=15.3 mmol mol^{-1}, max=28 mmol mol^{-1}) and 18.3±5 mmol mol^{-1} (n=9, min=12.1 mmol mol^{-1}, max=26.1 mmol mol^{-1}) for June and November, respectively.

Sr/Ca mean values of adult *S. impressa* and *N. delicata* ostracods from EPD samples are 3.5±0.2 mmol mol^{-1} (n=170, min=2.9 mmol mol^{-1}, max=4.3 mmol mol^{-1}) and 3.5±0.4 mmol mol^{-1} (n=80, min=2.3 mmol mol^{-1}, max=4.7 mmol mol^{-1}) respectively, while for OCEAN-HK samples are 3.6±0.2 mmol mol^{-1} (n=51, min=2.9 mmol mol^{-1}, max=4 mmol mol^{-1}) and 3.5±0.4 mmol mol^{-1} (n=30, min=2.6 mmol mol^{-1}, max=4.4 mmol mol^{-1}) respectively. Sr/Ca mean values in each EPD water control area are in Table 2. Sr/Ca mean values of *S. impressa* specimens from the Yatsushiro sea were 3.9±0.6 mmol mol^{-1} (n=14, min=2.8 mmol mol^{-1}, max=4.5 mmol mol^{-1}) and 3.3±0.5 mmol mol^{-1} (n=9, min=2.7 mmol mol^{-1}, max=4.4 mmol mol^{-1}) for June and November, respectively.

Al/Ca mean values of adult *S. impressa* and *N. delicata* from EPD samples are 1±1.3 mmol mol^{-1} (n=160, min=0 mmol mol^{-1}, max=11.7 mmol mol^{-1}) and 1.8±1.2 mmol mol^{-1} (n=76, min=0 mmol mol^{-1}, max=5.5 mmol mol^{-1}) respectively. Al/Ca mean values of adult *S. impressa* and *N. delicata* from OCEAN-HK samples are 1.2±0.7 mmol mol^{-1} (n=51, min=0 mmol mol^{-1}, max=3.4 mmol mol^{-1}) and 1.5±1.7 (n=28, min=0 mmol mol^{-1}, max=7 mmol mol^{-1}) respectively. Mg/Ca and Al/Ca ratios of *S. impressa* and *N. delicata* specimens do not correlate (Fig. S3). The low Al/Ca ratios indicate the absence of clays in the ostracods. Al/Ca mean values of *S. impressa* specimens from the Yatsushiro sea were 2.3±0.8 mmol mol^{-1} (n=14, min=1 mmol mol^{-1}, max=3.6 mmol mol^{-1}) and 3.3±1.4 mmol mol^{-1} (n=9, min=1.5 mmol mol^{-1}, max=6 mmol mol^{-1}) for June and November, respectively.

Fe/Ca mean values of adult *S. impressa* and *N. delicata* from EPD samples are 1.1±1.8 mmol mol^{-1} (n=160, min=0 mmol mol^{-1}, max=11.9 mmol mol^{-1}) and 1.5±1.4 mmol mol^{-1} (n=67, min=0 mmol mol^{-1}, max=6.1 mmol mol^{-1}) respectively. Fe/Ca mean values of adult *S. impressa* and *N. delicata* from OCEAN-HK samples are 0.6±0.4 mmol mol^{-1} (n=47, min=0 mmol mol^{-1}, max=2.1 mmol mol^{-1}) and 1.3±1.4 mmol mol^{-1} (n=26, min=0 mmol mol^{-1}, max=6.5 mmol mol^{-1}) respectively. The low Fe/Ca values indicate the absence Mn-Fe oxides in the ostracods. Fe/Ca mean values of *S. impressa* specimens from the Yatsushiro sea were 0.2±0.2 mmol mol^{-1} (n=14, min=0 mmol mol^{-1}, max=0.8 mmol mol^{-1}) and 1±0.6 mmol mol^{-1} (n=9, min=0.4 mmol mol^{-1}, max=2.1 mmol mol^{-1}) for June and November, respectively.

3.3 E/Ca calibrations to water parameters

Mg/Ca ratios of *S. impressa* significantly correlate to annual water temperatures in HK waters ($R^2_{S.\ impressa}$ = 0.32, $p_{S.\ impressa}$=0.007), but the Mg/Ca ratios of *N. delicata* ostracods do not correlate if the full data set is used (Table 3). However, the removal of sampling sites with only one shell allow us to produce significant temperature calibrations for both species (Table 3). The highest R^2 at a 95% significance for the temperature calibration to Mg/Ca ratios for *S. impressa* and *N. delicata* was obtained considering a minimum number (η) of 11 and 3 shells per sampling site respectively (Table 3). The highest R^2 at a 99.9% significance for *S. impressa* was obtained considering a minimum number of 7 shells per sampling site, while temperature calibrations for *N. delicata* did not reach this level of significance. Juvenile ostracods from both species do not correlate with temperature. Juvenile ostracods of *S. impressa* obtained from samples from the eastern side of HK have similar Mg/Ca ratios to adults, but juveniles close to the PRE have lower Mg/Ca ratios than adults (Fig. 2).

Table 3 Correlation fit and significance of temperature calibrations using different number of shells per sampling site to calculate the mean value of ostracod Mg/Ca ratios for *S. impressa* and *N. delicata*.

Minimum number of shells per sampling site (η)	*S. impressa*	*N. delicata*
	R^2 (p-value, sampling sites)	R^2 (p-value, sampling sites)
1	0.32 (0.007, 22)	0 (0.86, 15)
2	0.51 (<0.001, 21)	0.37 (0.036, 12)
3	0.47 (0.001, 20)	0.4 (0.05, 10)
4	0.57 (<0.001,17)	0.41 (0.063, 9)
5	0.64 (0.001, 13)	0.41 (0.063, 9)
6	0.69 (0.001, 12)	0.41 (0.063, 9)
7	0.7 (0.001, 11)	0.24 (0.408, 5)
8	0.73 (0.002, 10)	0.16 (0.596, 4)
9	0.73 (0.002, 10)	0.02 (0.906, 3)
10	0.76 (0.002, 9)	0.02 (0.906, 3)
11	0.85 (0.009, 6)	0 (No applicable, 1)

Mg/Ca ratio of *S. impressa* and *N. delicata* also correlates with other water parameters (from Fig. S5 to Fig. S12) such as volatile suspended solids ($R^2_{\mathit{S.\ impressa}} = 0.60$, $p_{\mathit{S.\ impressa}}<0.001$, $R^2_{\mathit{N.\ delicata}} = 0.49$, $p_{\mathit{N.delicata}}=0.037$), turbidity ($R^2_{\mathit{S.\ impressa}} = 0.58$, $p_{\mathit{S.\ impressa}}<0.001$, $R^2_{\mathit{N.\ delicata}} = 0.5$, $p_{\mathit{N.delicata}}=0.033$), suspended solids ($R^2_{\mathit{S.\ impressa}} = 0.56$, $p_{\mathit{S.\ impressa}}=0.001$, $R^2_{\mathit{N.\ delicata}} = 0.50$, $p_{\mathit{N.delicata}}=0.034$), silica ($R^2_{\mathit{S.\ impressa}} = 0.48$, $p_{\mathit{S.\ impressa}}=0.002$, $R^2_{\mathit{N.\ delicata}} = 0.47$, $p_{\mathit{N.delicata}}=0.041$), salinity ($R^2_{\mathit{S.\ impressa}} = 0.64$, $p_{\mathit{S.\ impressa}}<0.001$, $R^2_{\mathit{N.\ delicata}} = 0.49$, $p_{\mathit{N.delicata}}=0.037$), nitrite ($R^2_{\mathit{S.\ impressa}} = 0.60$, $p_{\mathit{S.\ impressa}}<0.001$, $R^2_{\mathit{N.\ delicata}} = 0.51$, $p_{\mathit{N.delicata}}=0.031$) and nitrate ($R^2_{\mathit{S.\ impressa}} = 0.62$, $p_{\mathit{S.\ impressa}}<0.001$, $R^2_{\mathit{N.\ delicata}} = 0.56$, $p_{\mathit{N.delicata}}=0.02$).

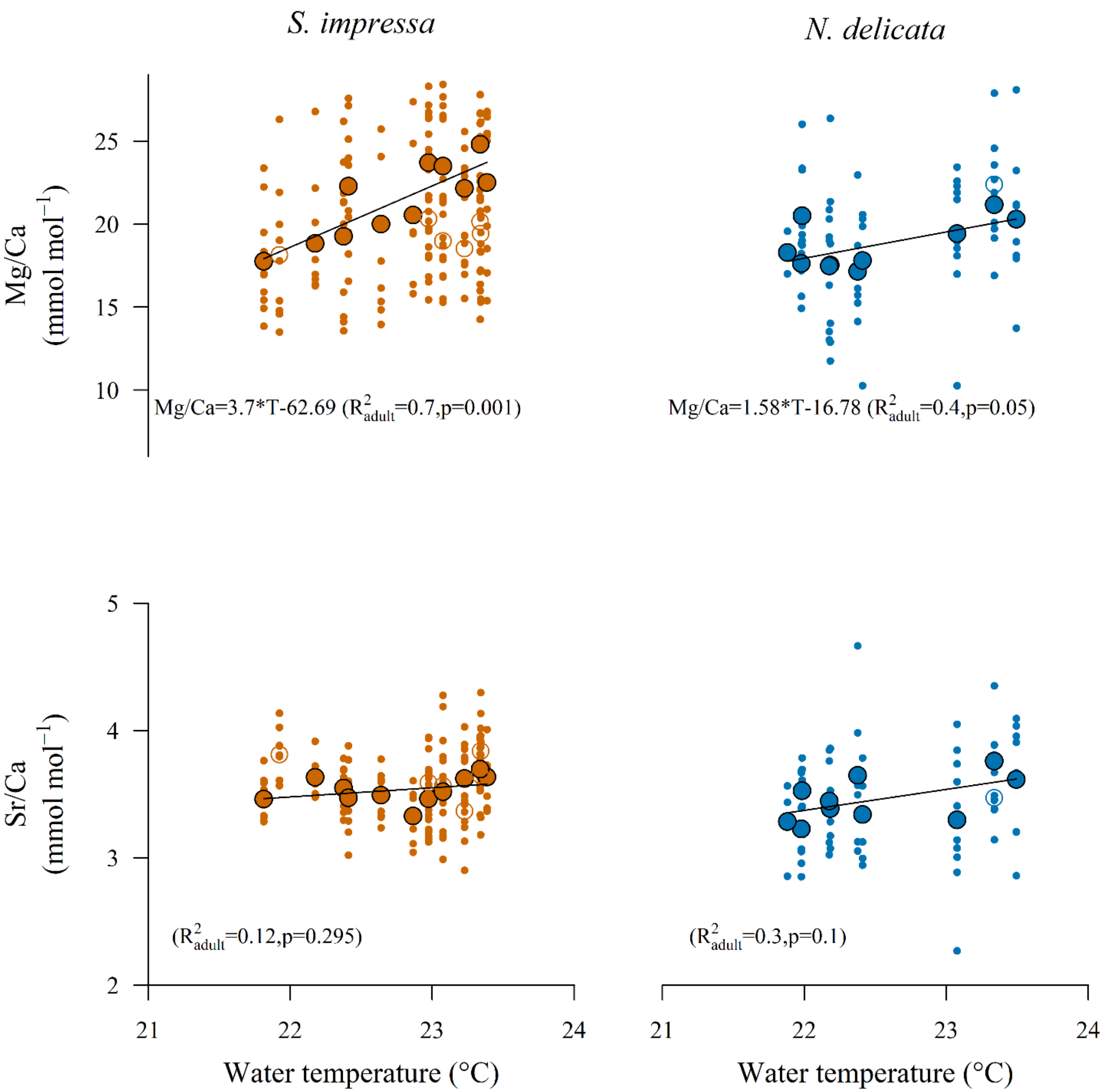


Figure 2 Relationships of Mg/Ca and Sr/Ca with annual temperature for adult (filled circles) and A-1 juvenile (open circles) ostracod samples of *S. impressa* (η=7) and *N. delicata* (η=3). Single-shell samples are shown in light colors. Solid lines show the linear regressions for adult specimens.

The temperature calibration to Mg/Ca is more significant in spring-summer months for both species (Fig. 3) when the ocean temperature increases. The highest correlation occurred in April for *S. impressa* and *N. delicata* ($\eta_{S.\ impressa}=7$, $R^2_{S.\ impressa} = 0.83$, $p_{S.\ impressa}<0.001$, $\eta_{N.\ delicata}=3$, $R^2_{N.\ delicata} = 0.55$, $p_{N.delicata}=0.015$).

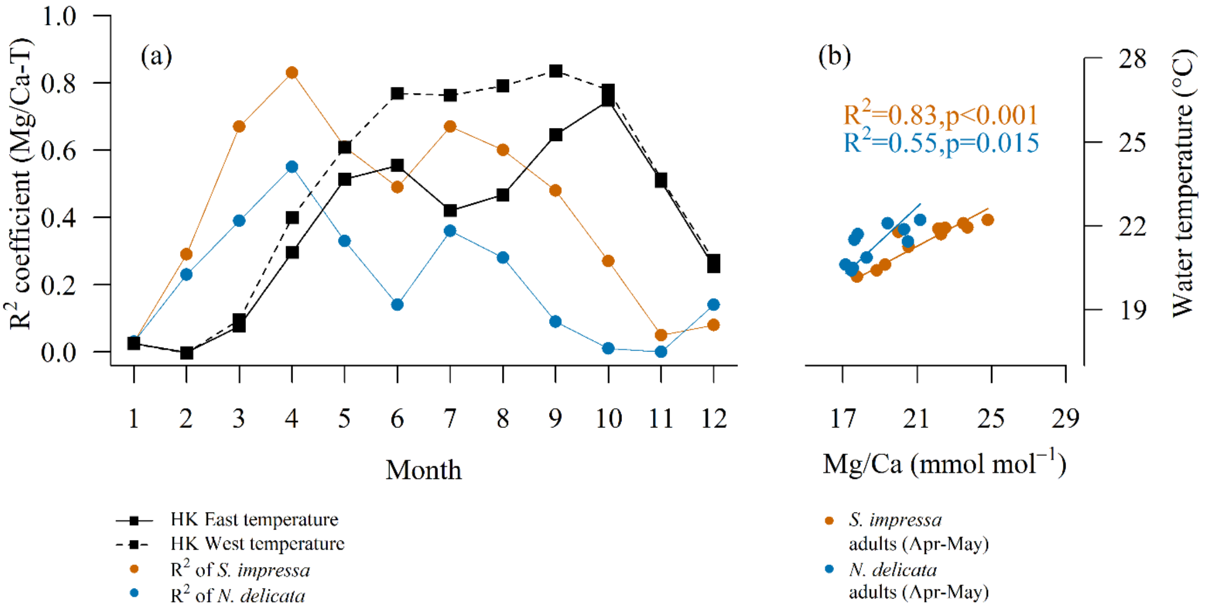


Figure 3 (a) Monthly temperature calibrations to Mg/Ca of *S. impressa* (η=7) and *N. delicata* (η=3) for adult ostracod. (b) The calibrations for *S. impressa* and *N. delicata* for April are $Mg/Ca_{S.\ impressa}=2.7\bullet T-37.2$ and $Mg/Ca_{N.\ delicata}=1.6\bullet T-15.7$.

A one-way ANOVA determined that Sr/Ca ratios do not show significant differences between sampling sites for *N. delicata* (p=0.16, Type II). Sr/Ca ratios were significantly different for *S. impressa* (p=0.02). However, this was only the result of samples collected from Victoria Harbour (n=18). After removal of these samples, Sr/Ca ratios of *S. impressa* were no longer significantly different between sampling sites (p=0.96). Sr/Ca ratios of both species do not correlate with any of the 24 water parameters measured by the EPD.

4 Discussion

4.1 Control on Mg/Ca and Sr/Ca ratios of *S. impressa* and *N. delicata*

The strongest linear correlation between the 24 parameters (Table S1) measured by the EPD and Mg/Ca ratio was for the annual mean temperature, which suggests that water temperature is the main control of Mg/Ca uptake in adult ostracods of *S. impressa* and *N. delicata* (Fig. 2). Correlations with other parameters are also significant but are likely caused by multicollinearity of temperature with other water parameters, such as turbidity, suspended solids, salinity, pH, and nitrite (Fig. S4). The temperature control on Mg/Ca ratio of biogenic material is well-known and has been usually described linearly for ostracods, even though inorganic calcite follows an exponential relationship (Lea, 2003). We observed a linear correlation between water temperature and Mg/Ca ratios of *S. impressa* and *N. delicata* ostracods (Fig. 2), but the significance of the linear calibration is higher for the former species, making it more suitable for temperature reconstructions.

We evaluated the sensitivity of the calibration using the natural variability of ostracod Mg/Ca ratios and seawater temperatures in Hong Kong sampling sites. The error (range) of Mg/Ca mean values in each station used for the calibration can be established at a certain confidence level (Holmes, 2008). For *S. impressa*, the error of Mg/Ca mean values in each station ranged from 1.5 mmol mol^{-1} to 4.1 mmol mol^{-1} at a 95% confidence level. For annual and spring calibrations, the temperature mean errors are 0.7° C (0.4° C to 1° C) and 1° C (0.6° C to 1.5° C), respectively. These values are lower than the temperature difference between the stations (1.6° C), which indicates that differences higher than 1° C can be estimated using *S. impressa* calibration curves. For *N. delicata*, the error of Mg/Ca mean values ranged from 1.1 mmol mol^{-1} to 5.7 mmol mol^{-1}. Annual and spring calibrations have the same slope, which produce temperature mean errors of 1.9° C (0.7° C to 3.6° C). This error is similar to the difference in temperatures between the stations. Therefore, more shells of ostracods living at different temperatures would be needed to estimate differences at 1° C for this species. We also investigated the potential impact of daily temperature fluctuations on the calibrations. We estimated daily BWT variability by using the Copernicus satellite products. Using the daily data from 1993 to 2018, we calculated the standard deviation for each month. We then determined the average variation for each month across all the years. We performed this calculation on three Hong Kong sampling stations located: a) at the lower section of the PRE, b) outside the PRE and south of Hong Kong Island and c) on the eastern side of Hong Kong island. We found the variations were 1±0.4° C, 0.8±0.5° C and 0.7±0.5° C, respectively. Therefore, daily bottom water fluctuations in Hong Kong waters are unlikely impacting the calibrations obtained.

The Mg/Ca of A-1 juvenile ostracods of *S. impressa* does not correlate to April temperature (Fig. 2). A significant weak correlation is only observed using June temperature ($R^2_{S.\ impressa} = 0.33$, $p_{S.\ impressa}=0.031$, $\eta=2$). Juvenile ostracods usually show higher Mg/Ca ratios than adults (Chivas et al., 1986b; Dwyer et al., 2002). However, juvenile specimens of *S. impressa* from sampling sites with salinity lower than 32 PSU have lower Mg/Ca ratios than adults (Fig. 4). We hypothesize that environmental factors may increase the bias between ostracod life stages. For example, strong currents can produce preferential post-mortem transport of lighter shells (Boomer et al., 2003), which may foster the mixing of ostracod shells calcified at different temperatures. Our results suggest that juvenile and adult ostracods of *S. impressa* cannot be used indistinctly to reconstruct April temperature supporting previous findings about the incompatible use of Mg/Ca ratios of different ostracod stages (Dwyer et al., 2002).

None of the 24 parameters measured by the EPD, including temperature and salinity, exert control on the Sr/Ca ratios in adult specimens of either species. The low Sr/Ca variabilities of *S. impressa* and *N. delicata* suggest that the potential control variables of this ratio, such as water Sr/Ca or vital effects, do not change considerably across different locations within the PRE. A positive correlation in Sr/Ca with chlorophyll-a and dissolved oxygen is observed for *S. impressa* specimens, but this relationship is mainly produced by specimens with low Sr/Ca from one sampling site in Victoria Harbour. Dissolved oxygen in this sampling site was particularly low, probably as a result of discharge from the nearby Stonecutters Island sewage treatment plant.

Mg/Ca ratios of both species are negatively correlated to salinity (Fig. 4). A potential control of seawater Mg/Ca on ostracod Mg/Ca would be possible if seawater Mg/Ca decreases with salinity. Marine waters have higher Mg and Ca concentrations in comparison to freshwaters (Brown et al., 1989; Bruland and Lohan, 2006). Previous studies have shown a mostly conservative behavior of Mg and Ca in estuaries and surrounding areas (Millero, 2006; Patra et al., 2012), where these concentrations increase linearly with salinity. Therefore, a higher Ca concentration over Mg concentration or a lower Mg concentration over Ca concentration toward more saline waters are unlikely in Hong Kong waters. Moreover, ostracod Sr/Ca is similar at different salinities (Fig. 4), supporting the idea that changes in seawater Ca concentrations are not the main control on Mg/Ca and Sr/Ca ostracod ratios. Measurements during 2013 and 2017 at the desalination plant in Tseung Kwan O (Fig. 1 and S13) show water Mg/Ca and Sr/Ca ranging between 4 and 6 mol mol^{-1}, and between 8 to 9 mol mol^{-1} respectively during the year. These values are mostly stable even when monthly salinity decreases to 25 PSU in some Hong Kong stations during summer, suggesting that the seawater chemistry may not be a primary control on ostracod Mg/Ca and Sr/Ca ratios of Hong Kong waters. Our dataset does not allow us to explore in more detail the potential relationship between these two variables as more data of the seawater chemical composition is needed.

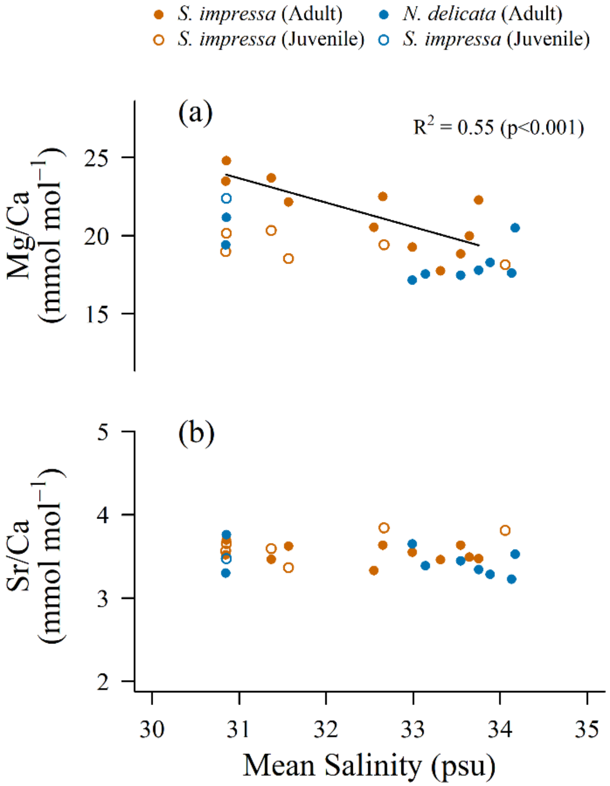


Figure 4 Mg/Ca and Sr/Ca correlation to salinity for *S. impressa* (η=7) and *N. delicata* (η=3) for salinity recorded in April considering mean values

4.2 Factors controlling the robustness of the temperature calibration to Mg/Ca

4.2.1 Number of shells per sampling site

The robustness of the annual temperature calibration to Mg/Ca with respect to R^2 and p-values depends on the number of individual shells available to calculate the mean value of each sampling site (Table 3). The removal of sampling sites with a low number of shells increases the R^2 of temperature calibrations in both species, suggesting that a low number of samples does not allow us to capture all the natural variability exerted by the temperature on ostracod Mg/Ca ratios. The most significant calibrations were obtained using at least two to four shells for *S. impressa* and at least two to three shells per sampling site for *N. delicata* respectively (Table 3). The margin of error (z•SE, z=critical value, SE= standard error) of confident intervals can be used to provide an estimation of the number of shells needed to obtain a desired level of error. Using temperature calibrations for *Bythocypris* and *Krithe* specimens, Corrège and De Deckker (1997) showed that the use of 4 shells provide an error in the temperature prediction lower than 1.4 °C at a 95% confidence. In stratigraphic studies the use of three to five shells is a common practice (Holmes and De Deckker, 2012). Holmes (2008) studied the critical sample size of ostracod specimens to keep a desired error at a 0.99 significance level in Mg/Ca and Sr/Ca from a stratigraphic sequence. The authors concluded that in modern specimens of *Cyprideis torosa* from shallow brackish environments the optimal sample size may vary from 3 to 16 shells. We can use this approach to estimate the number of shells needed per sampling site to keep the error at an acceptable level to produce a significant correlation. The use of 1, 2, 3, 4, 5, 6 and 7 shells produce margin of errors of 3.4, 2.4, 2.0, 1.7, 1.5, 1.4 and 1.3 °C at a 95% significance respectively, considering the highest standard deviation observed in our sampling sites (σ=4.7 mmol mol^{-1}, n=15) and normally distributed samples (Rodriguez, 2021). Our results suggest that in shallow marine waters the calculation of mean Mg/Ca ratios with at least four individual shells in more than ten sampling sites would likely produce a significant temperature calibration at 99% significance level ($p<0.01$), accounting for short-term temperature variations. In the following analyses, we restricted the minimum number of shells per sampling site to η=7 for *S. impressa* and η=3 for *N. delicata* as we obtained the highest R^2 at a significance of 99.9% and 95% respectively.

4.2.2 Temporal variability

Most temperature calibrations to Mg/Ca are produced with the annual mean temperature as they are intended to reflect long-term changes of paleoceanographic studies. However, ostracods molt their shells in a short period of time (usually days or weeks). Thus, it may be possible to record ocean parameters at a shorter time scale if the correct molting time is known. Our monthly analysis of linear regressions shows that the best temperature calibrations to Mg/Ca in terms of high R^2 and significant p-values, were found with the water temperature measured in April for *S. impressa* and *N. delicata* (Fig. 3). This suggests that adult ostracods of both species may inhabit HK waters mainly during spring or early summer, as has been documented for other shallow marine species (Cronin et al., 2005a; Kamiya, 1988). July temperature also correlates with Mg/Ca ratios in both species, showing a second peak (Fig. 3). Two periods of calcification has recently been shown for *C. torosa* (Roberts et al., 2020), which may also occur in *S. impressa* and *N. delicata*. The high correlation between April water temperature and Mg/Ca ratios indicates the possibility to reconstruct water temperature at a finer temporal scale, which can help to unravel coastal ocean circulation patterns. For example, the interaction of the Pearl River and Hainan, Taiwan and Kuroshio currents (Morton and Wu, 1975) determine the temperature and salinity of Hong Kong waters. The Hainan current is dominant during summer, while the Taiwan and Kuroshio currents affect Hong Kong waters during winter. *Sinocytheridea impressa* and *N. delicata* may become important tools to determine their interaction by providing information on water temperature during the transition between these currents and freshwaters from the Pearl River.

4.3 Validation of temperature calibration

April BWTs obtained from the Copernicus product are on average -1.5 ±1.8° C (2σ) below the measurements from the EPD (Fig. S1). BWT differences between EPD stations and Copernicus product range from 0 to 3° C, being the largest in stations close to the Pearl River (Fig. S1). We corrected the BWTs obtained from the Copernicus product in all OCEAN-HK sampling locations by -1.5° C.

The BWTs estimated from ostracod Mg/Ca ratios in OCEAN-HK sampling locations are in good agreement with BWTs obtained from the Copernicus product for April (Fig. 5 and Table S2). The difference between the estimated temperature by the linear regressions and the potential BWT from the Copernicus product is lower than 0.7° C in 7 out of the 8 sites for *S. impressa*. Only one sampling site shows an error of 1.8° C (Table S2). For *N. delicata*, the error was lower than 2.3° C in the three stations considered. This suggests that *S. impressa* and *N. delicata* specimens around the PRE follow the regression line developed with ostracods collected from HK waters. Our findings also suggest that calibrations may be done without direct measurements of BWT but using the potential BWT from satellite products. We highlight that the improvement in the quality of BWT products derived from satellite images may facilitate the calibration of multiple species across the world as scientists have global coverage of BWT with a high resolution (1/12°~ 9 km).

The Mg/Ca ratios of *S. impressa* ostracods from the Yatsushiro Sea are within the range of Mg/Ca ratios found in Hong Kong ostracods (Jun: 21.6±3.8 mmol mol^{-1} and Nov: 18.3±5 mmol mol^{-1}, Figure 5). Using the Hong Kong calibration developed for April, temperatures in Yatsushiro Sea were estimated to be 21.5° C in June and 20.4° C in November. We compared these temperatures with seawater temperatures recorded a) during the day of sampling (Jun: 21.6° C and Nov: 15° C, in situ), b) the mean of 10 days (Jun: 22.0° C and Nov: 21.6° C, buoy), c) 20 days (Jun: 21.5° C and Nov: 22.1° C, buoy), d) 30 days (Jun: 20.7° C and Nov: 22.6° C, buoy) and e) 60 days (Jun: 18.8° C and Nov: 23.9° C, buoy) before the specimen collection. The estimation of water temperatures using ostracod Mg/Ca ratios in November is not similar to the temperatures recorded the same day of sampling. This is likely due to the exposure of the site to freshwater inputs, which may have affected local water temperatures. In addition, the ostracod calcification may have occurred several days before its collection. The consideration of the mean temperature across the 10 days before the ostracod collection produced the greatest agreement with Mg/Ca-estimated temperatures. Thus, our results suggest that ostracods from the Yatsushiro Sea may have calcified the shells during the last few days before their collection.

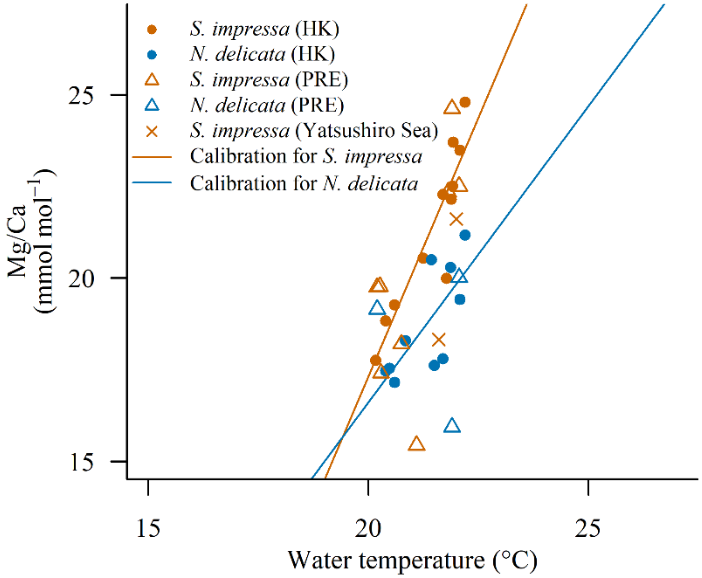
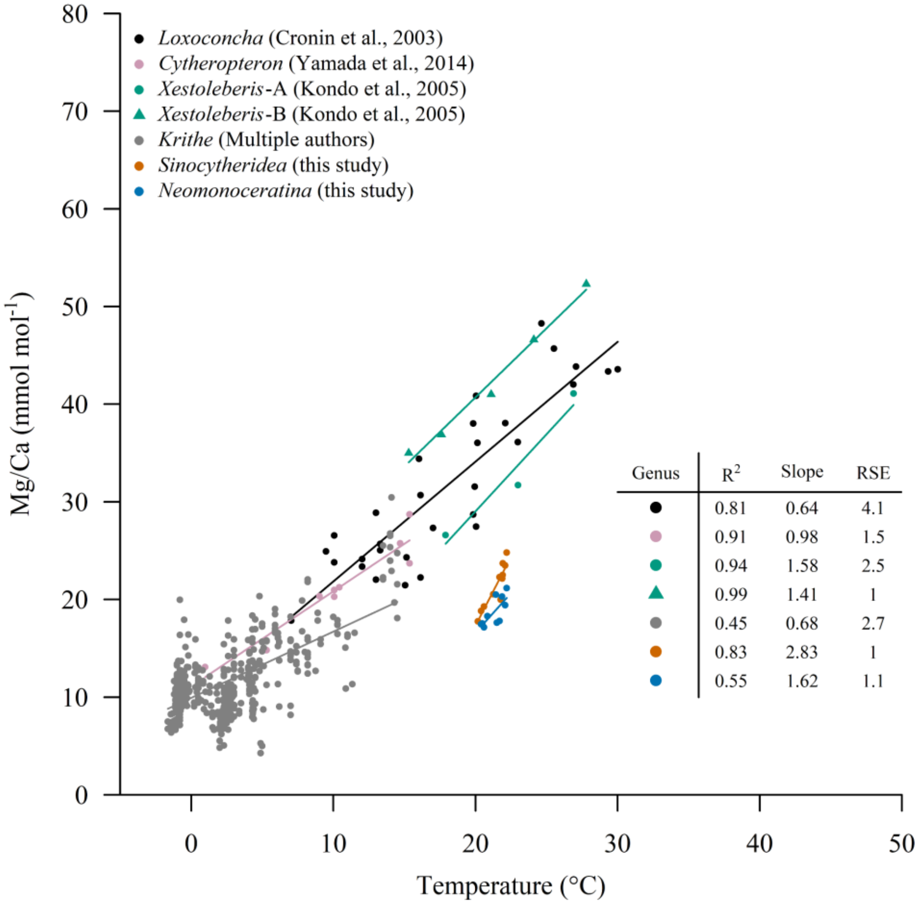


Figure 5 Mg/Ca ratios of ostracods from the PRE (OCEAN-HK) and Yatsushiro bay over the calibration for *S. impressa* ($Mg/Ca_{S.\ impressa}=2.7\cdot T-37.2$) and *N. delicata* ($Mg/Ca_{N.\ delicata}=1.6\cdot T-15.7$) performed with samples from Hong Kong (EPD). The BWT from the Copernicus product was used for OCEAN-HK samples Surface water temperature from the Tanoura buoy in the last 15 days in June (21.8° C) and November (21.9° C) were considered for the Yatsushiro sea samples.

4.4 Calibrations at a superfamily level

Sinocytheridea impressa and *N. delicata* are related at a superfamily level (Cytheroidea). A few studies have developed calibrations for ostracod species from the same superfamily. A unique calibration for ostracods from the same superfamily cannot be performed according to our results. Temperature calibrations to Mg/Ca ratios have been developed for specimens of the same superfamily, including the genus *Krithe* (Cadot and Kaesler, 1977; Corrège and De Deckker, 1997; Cronin et al., 1996; Dwyer et al., 1995, 2002; Elmore et al., 2012; Farmer et al., 2012), *Loxoconcha* (Cronin et al., 2003), *Cytheropteron* (Yamada et al., 2014) and *Xestoleberis* (Kondo et al., 2005). *Sinocytheridea impressa* and *N. delicata* show lower Mg/Ca ratios at the same temperature in comparison to other species (Fig. 6). Similar Mg/Ca ratios to *S. impressa* and *N. delicata* have been observed in *Krithe* specimens at seawater temperatures ranging between 10° C and 20° C. The residual standard errors (RSE) for the calibration of *S. impressa* and *N. delicata* are 1.1 mmol mol^{-1} for both species, showing one of the best regression fits of all calibrations for the superfamily Cytheroidea (Figure 6). The calibration of *N. delicata* has a similar slope in comparison to *Xestoleberis* specimens (Fig. 6), but *S. impressa* has the highest slope of all species from the superfamily, which shows that different species from the same superfamily do not share the same calibration slope and intercept. In addition, ostracods from the same superfamily under similar conditions of temperature and salinity may show significant differences in their calibration parameters, such as *S. impressa* and *N. delicata* specimens in HK waters or *Xestoleberis* specimens (Kondo et al., 2005). Factors such as the development of temperature calibrations from multiple species, the number of shells considered per site, variability induced after the burial of ostracods, among other reasons, might impoverish the calibration fit. Our study suggests that when species-specific calibrations are performed, the residual standard error in the temperature calibration to Mg/Ca may be around 1 mmol mol^{-1}. This happens in shells which have not been buried, are oxide-free, and were treated with non-corrosive cleaning reagents such as 5% sodium hypochlorite and methanol (Rodriguez, 2021). The Mg/Ca of ostracods are temperature sensitive at a very small temperature range and may be useful to understand variation in a localized region. *Sinocytheridea impressa* and *N. delicata* ostracods from other locations can improve the calibration shown in this study by expanding the temperature range.



Genus	R^2	Slope	RSE
●	0.81	0.64	4.1
●	0.91	0.98	1.5
●	0.94	1.58	2.5
▲	0.99	1.41	1
●	0.45	0.68	2.7
●	0.83	2.83	1
●	0.55	1.62	1.1

Figure 6 Temperature versus Mg/Ca ratios for specimens of the superfamily *Cytheroidea. Krithe* samples were retrieved from Farmer et al. (2012) **and the study of Elmore et al.** (2012) **which contains the collection of Mg/Ca and BWT from different studies including Cadot and Kaesler** (1977)**; Corrège and De Deckker** (1997)**; Dwyer et al.** (1995)**; Cronin et al.** (1996) **and Dwyer et al.,** (2002)**.**

The lower Mg/Ca in *S. impressa* and *N. delicata* ostracods in comparison to other species from the same superfamily such as *Krithe* or *Loxoconcha* may be the result of other variables such as pH, DIC, CO_3 concentration or the calcite saturation index (Ω). These variables have been suggested to partially control the incorporation of ostracod Mg/Ca ratios (Elmore et al., 2012; Farmer et al., 2012; Holmes and De Deckker, 2012). *Loxoconcha* from Chesapeake Bay (Cronin et al., 2003, 2005a) most likely dwells in the most similar environment as *Sinocytheridea* and *Neomonoceratina* due to its presence in a large estuary in a polyhaline system at similar depths. Measurements of pH and dissolved inorganic carbon (DIC) in HK and Chesapeake Bay waters allow us to compare the saturation index in both basins. A bottom water pH ranging from 7.8 to 8.2 (EPD, 2018) and DIC over 1850 µM (Guo et al., 2008; Yuan et al., 2011) indicate that the seawater is oversaturated with calcite (Ω ~3) in our sampling sites for most of the year. On the other hand, pH and DIC in the bottom waters of the Chesapeake lower bay in 2006 ranged from 7.92 to 7.96 and 1717.4 µM to 1865.4 µM respectively, with a calcite saturation index over 2.8 (Brodeur et al., 2019), suggesting that the lower ostracod Mg/Ca ratios in *S. impressa* and *N. delicata* ostracods are not a direct result of different conditions of pH, DIC or calcite saturation index. Species-specific biomineralization types (i.e. ostracod calcification) may also play an important role in the temperature response to ostracod Mg/Ca ratios of different species, but our measurements do not allow us to explore this factor.

5 Conclusion

The Mg/Ca ratios of *S. impressa* and *N. delicata* ostracods can be used as proxies of water temperature in shallow marine environments. The temperature dependence of *S. impressa* is higher and therefore it is more suitable for temperature reconstructions. This study shows that: 1) the number of shells per sampling site has an important impact on the calibration robustness due to the strong seasonal variability of temperature in estuaries and coastal areas, and therefore we recommend the use of four or more shells per sampling site, 2) ostracods can give information on monthly water temperatures 3) the temperature reconstruction based on Mg/Ca ratios of *S. impressa* and *N. delicata* specimens has the potential to give insight to past ocean circulation in coastal areas of the South China Seas and 4) ostracods from the same superfamily show different calibration curves, which does not seem to be controlled by the Mg/Ca ratios of marine waters, temperature or the carbonate system. Better understanding of ostracod molting time will likely improve calibrations and the identification of the calcification temperature for *S. impressa* and *N. delicata* ostracods.

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