Tracking the seasonal calcification of *Cyprideis torosa* (Crustacea, Ostracoda) using Mg/Ca-inferred temperatures, and its implications for palaeotemperature reconstruction

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Abstract

Ostracod shell chemistry data are widely used for palaeoenvironmental reconstruction. Despite this, there has been little systematic research into the implications of the timing of calcification or the duration of each moult stage. Consequently, it is unclear whether palaeoenvironmental reconstructions are recording restricted (inter-seasonal) time periods or reflect the mean annual conditions. The seasonality of shell formation can therefore have implications for palaeoenvironmental reconstructions based on geochemical signatures, especially palaeotemperature, particularly in environments that show large inter-annual variations in water conditions. *Cyprideis torosa* is a geographically widespread and eurytopic species that has great potential for a range of palaeoenvironmental reconstructions, but inhabits environments with large seasonal and inter-annual variation. Using hourly water and air temperature data, ostracod shell and surface water chemistry from a shallow coastal pond in SE UK, we improve knowledge of the timing of *Cyprideis torosa* calcification, and thus our understanding of the potential seasonality of signals in palaeotemperature datasets. We suggest seasonal calcification in spring and autumn, with persistence at the adult life stage for up to 12-18 months. Sr/Ca values of *C. torosa* appear to reflect a Sr/Ca$_{water}$ control on calcification timing and have no temperature dependence. For Mg/Ca, we show a minimum temperature control on calcification of 7 ˚C, with *C. torosa* Mg/Ca-inferred temperatures broadly tracking spring and autumn temperatures.

Keywords: Mg/Ca, calcification, ostracods, life cycle, *Cyprideis torosa*, palaeotemperature

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1. Introduction

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1. Introduction

Ostracods are microscopic aquatic crustaceans, whose bivalved calcitic shells are readily preserved in sediments, and are thus commonly used for palaeoenvironmental reconstruction. However, despite their widespread use, relatively little is understood about the life cycle and calcification of many routinely analysed ostracod species. Seasonality of shell formation, which differs between species, can have important implications for palaeoenvironmental reconstructions. This is especially the case for palaeotemperature. In this study, we analyse variations in the trace-element shell chemistry of *Cyprideis torosa* a ubiquitous brackish water species, commonly used in palaeoenvironmental reconstructions*,* to infer seasonal cycles of calcification.

Ostracods in the Subclass Podocopa, such as *C. torosa*, grow through nine instars (eight juvenile and one adult stage) (Horne, 2005), with juvenile stages designated as (in descending order of size) A-1, A-2, A-3, A-4 etc., but the persistence at each life stage is largely unknown. Temperate environments with stable salinity produce a single generation of *C. torosa* per year, but with two peaks of adults. A-1 to adult calcification occurs biannually in spring and late summer to early autumn (Heip, 1976). Adults overwinter alongside populations of A-1 juveniles that produce new adult populations in the spring and autumn; the generations overlap so that the population comprises both overwintering adults and newly-matured adults arising from overwintering juveniles, producing peak abundances of *C. torosa* during calcification months in the spring. The overwintering female adults release early instar juveniles from the brood chamber in the spring that moult to adulthood by the autumn (Heip, 1976; Horne, 1983). It is sometimes possible to distinguish between adults that overwintered and those that are newly calcified because the former may have material (e.g. diatoms) attached to their shells whereas the latter have relatively ‘clean’ looking shells. In warmer regions, for example the Mediterranean, *C. torosa* produces two or more generations per year although the increased temperatures reduce the mean duration of early-juvenile instar stages (Mezquita *et al.,* 2000). Previous ecological monitoring of *C. torosa* has shown that environmental conditions, such as temperature, are an important control on the life cycle of the species, but relatively little is understood about these controls.

Previous studies of the seasonal life cycle of *C. torosa* (e.g. Heip, 1976; Horne, 1983) have not considered the implications for palaeoenvironmental reconstruction using this species. Consequently, relatively little is understood about whether palaeoenvironmental reconstructions are recording restricted periods (i.e. only spring or late summer temperatures), which may be linked to a temperature preference for calcification. Some authors have argued that shell calcification can occur at different times of the year, so that the resulting intra-annual noise in inferred temperatures may interfere with long-term palaeoenvironmental signals (Börner *et al*., 2013). Because *C. torosa* inhabits highly dynamic waterbodies, such as estuaries and coastal lakes, it is likely to experience wide variations in environmental conditions on short timescales. Understanding these variations in the hydrological system coupled with the population dynamics of the species will allow more detailed understanding of ostracod-shell trace-element datasets.

The Mg content of ostracod shells is commonly used as a palaeotemperature proxy in Quaternary sediments. Typically, the Mg content of ostracod valves correlates with the Mg content of water and with water temperature (Chivas *et al*., 1986; De Deckker and Forester, 1988; Holmes and Chivas, 2002). In situations where the composition of the water shows minimal variation, temperature is the major control and the Mg content of ostracod shells can be used as a palaeothermometer. *Cyprideis torosa* provides an ideal target species to track calcification timings since there are existing Mg/Ca temperature calibrations (e.g. Wansard 1996; De Deckker *et al.* 1999). When the Mg/Ca ratio of the host water is known, the calcification temperature for *C. australiensis* is given by the equation of De Deckker *et al*. (1999) of $T(^\circ C) = 2.69 + 5230([\text{Mg/Ca}]_{\text{shell}} / [\text{Mg/Ca}]_{\text{water}})$. Whilst *C. australiensis* is now considered a separate species to *C. torosa* (Schön *et al.,* 2017), this equation has been successfully applied to living and fossil specimens of *C. torosa* in water of marine-type ionic composition (Holmes and De Deckker, 2017). Where the waters are more dilute and/or of contrasting ionic composition, it is possible to use the palaeotemperature equation of Wansard (1996), developed for *C. torosa*, of $T(^\circ C) = 3.3 + (1971.0 * [\text{Mg/Ca}]_{\text{ostracod}})$. Similarly, for *C. australiensis,* the Sr/Ca of the shell is often positively correlated with the Sr/Ca of the host water, which in some circumstances covaries with salinity (Chivas *et al*., 1985). De Deckker *et al.* (1999) have also suggested a small temperature dependence on the incorporation of Sr into shells.

Currently, there are three large unknowns when calculating Mg/Ca-inferred temperatures: 1) the exact timing of *C. torosa* calcification, 2) the implications of seasonal water variations on the range of calculated temperatures, and 3) the $\text{Mg/Ca}_{\text{water}}$ scenarios that the De Deckker *et al*. (1999) and Wansard (1996) equations apply. It is common practice to use 'spot samples' or annual mean $\text{Mg/Ca}_{\text{water}}$ conditions to calculate partition coefficients (K_D values) and/or in temperature calculations (e.g. Holmes *et al.*, 1995; Wansard *et al.*, 1998; Keatings *et al*., 2007). However, by doing so, it is possible that the seasonal signal in temperature and water composition is masked. *Cyprideis torosa* has strong seasonal calcification patterns (Heip, 1976), yet we currently have little understanding of whether seasonal temperatures are reflected in $\text{Mg/Ca}_{\text{shell}}$ values, thus there are large uncertainties in reconstructed temperatures.

Understanding calcification, the seasonal water conditions and constraining the use of Mg/Ca$_{water}$ values for temperature calculations will have implications for the interpretation of palaeotemperature records. Here, we aim to improve understanding of the timing of *C. torosa* calcification using Mg/Ca-inferred temperatures coupled with hourly water temperatures and seasonal water chemistry. In addition to contributing to the understanding of the *C. torosa* life cycle, we demonstrate how the improvement in estimation of the timing of *C. torosa* calcification could enable the identification of seasonal signals in Mg/Ca-inferred temperatures, and thus decrease uncertainties in the interpretation of palaeotemperatures derived from fossil shells.

2. Materials and methods

2.1 Field collections of ostracods and waters

Water samples and living specimens of *C. torosa* were collected in August and December 2016 and April, June and September 2017 from a shallow coastal pond in Pegwell Bay Nature Reserve, Kent, SE UK (Fig. 1) where the species is particularly abundant. The ~ 0.32 by 0.32 km, shallow (< 1 m) saltmarsh pond has a muddy substrate and low macrophyte abundance. The pond is situated above Mean High Water level, but is still within the intertidal zone. Ostracods were collected in a 250 µm zooplankton net from the top 1 cm of sediment at location 'X' (Fig. 1). Sediment was then washed through a 250 µm sieve (the adult shells being ~1000 µm) to remove any remaining fine sediment and dried in an oven at 50 °C. Adult carapaces with soft tissue and appendages (indicating that the individuals were alive at the time of collection) were selected for geochemical analyses. The right valves were reserved for trace metal analysis and the left for stable isotope analyses (results will be described elsewhere). To constrain the variation in water chemistry over the expected period of calcification, water samples were collected as one-off spot samples in April and September and hourly from low to high tide in June 2017 in sterile 50 mL centrifuge tubes and filtered prior to analysis. In situ measurements of conductivity and temperature were taken using a YSI 30 handheld probe calibrated and recorded at 25 °C. For the April and June 2017 sampling, in situ alkalinity as $CaCO_3$ equivalent was determined using a Hach Digital Titrator, 1.6N Sulphuric acid (H_2SO_4) cartridge and Phenolphthalein and Bromcresol Green-Methyl Red indicators.

2.2 Population dynamics

For each sample collection, the length-height relationship of a sub-sample of 100 individuals was established under a low-power stereo microscope using a calibrated eyepiece reticule.

This allowed the population age structure at intervals across the year to be established. The large mesh size used may restrict the collection of all instars, but since the primary purpose of sampling was to determine peak abundances of adult populations, the collection of small juveniles was not considered important. Additional information, including adult sex and the presence of adhering material, was also noted.

2.3 Annual temperature datasets

A Tinytag Aquatic 2 temperature logger with temperature range –40 to +70°C was deployed at a depth of ~10 cm to record subsurface water temperature from August 2016 to September 2017. Complementary hourly air temperature data were downloaded from the Met Office weather station dataset on the Centre for Environmental Data Analysis database. Temperature data were extracted for Kent International Airport, Manston weather station (WMO ID 3797) located 2 km inland from the study pond (Fig. 1).

2.3 Geochemical analyses

2.3.1 Ostracods

Prior to geochemical analyses, ostracod valves were cleaned according to Roberts *et al*. (2018). Soft tissue and any adhering dried sediment were removed from valves using needles, a fine paint brush wetted with methanol and ultra-pure 18.2Ω Milli Q deionised water under a binocular microscope. Valves were then sonicated in methanol and 18.2Ω Milli Q deionised water and dried at 50 °C prior to analysis.

For trace metal analysis, single ostracod valves were dissolved in 500 µL of 1.07 M HNO_3 (trace metal grade) in an acid–leached (48h in 80 °C 10 % HNO_3) 600 µL micro-centrifuge tube. The Mg/Ca, Sr/Ca, Fe/Ca, Mn/Ca and Al/Ca ratios of valves were determined using the intensity ratio calibration of de Villiers *et al*. (2002) using a Varian 720 ES ICP-OES at University College London (UCL). The results were corrected for blank intensity. Analysis of the carbonate standard BCS-CRM 393 gave an Mg/Ca of 3.9 ±0.01 mmol/mol and Sr/Ca of 0.19 ±0.004 mmol/mol for 12 determinations across three runs. The values are in good agreement with the mean values of 3.9 mmol/mol and 0.19 mmol/mol quoted in Greaves *et al.* (2008). The Fe/Ca, Mn/Ca and Al/Ca ratios were monitored as contamination indicators to check for elevated Mg/Ca ratios due to high–Mg marine clays and Fe-Mn oxyhydroxide coatings, which form on biogenic calcite in marine-type waters. The inefficient removal of these coatings can cause bias in Mg/Ca ratios and thus Mg/Ca-inferred temperatures.

2.3.2 Waters

Major and minor cations (Na^+, K^+, Ca^{2+}, Mg^{2+} and Sr^{2+}) were analysed using a Varian 720 ES ICP-OES at UCL. Standards were prepared volumetrically using single element standard solutions of known concentrations. Analysis of the standard river water SLRS-4 gave concentrations of 24.6 ±0.3 mg L^{-1} for Na, 0.07 ±0.005 for K, 6.1 ±0.2 mg L^{-1} for Ca, 1.6 ±0.01 for Mg and 0.029 ±0.003 for Sr, in good agreement with the published values of 24.0 for Na, 0.07 mg L^{1} for K, 6.2 mg L^{-1} for Ca, 1.6 mg L^{-1} for Mg and 0.026 mg L^{-1} for Sr (Yeghicheyan *et al.*, 2001). Major anions (Cl^- and SO_4^{2-}) were analysed using a Dionex Ion Chromatograph at UCL with KOH eluent at concentration of 12.5 mM, SRS current of 20 mA and flow rate of 0.47 mL/min. Analysis of the multianion standard solution PRIMUS gave values of 9.9 ±2.7 mg L^{-1} for Cl^- and 10.42 ±0.16 mg L^{-1} for SO_4^{2-}, in good agreement with the certified values of 10 mg L^{-1} for all anions.

3. Results

3.2 Water chemistry

The electrical conductivity of the pond was highest in June reaching an average of 75.2 mS cm^{-1} with the lowest values of 40.2 mS cm^{-1} recorded in December (Table 1; Fig. 2). Conductivity shows a strong seasonal pattern with similar values recorded between September and April (Fig. 2).

Between low and high tide, there was little variation in water Sr^{2+} and Sr/Ca_{water} with variations of ±1.17 mg L^{-1} and ±0.17 mmol/mol respectively (Table 2). The lowest Sr/Ca_{water} of 9.87 mmol/mol was recorded at 09:00. The largest variation across the day was in Na^+ concentrations (±14784 mg L^{-1}) with peak concentrations at 06:00 of 91132 mg L^{-1}; lowest values were observed in the centre of the pond at locations 3 and 6 (Table 2). Otherwise, there was low variability in all cation concentrations. Cl^- concentrations were consistent with diluted seawater and were fairly constant throughout the day. Alkalinity varied little throughout the day (± 9.8 mg L^{-1}) with an average of 259.6 mg L^{-1} $CaCO_3$ total alkalinity (Table 2). The alkalinity in April was lower, but still high at 210 mg L^{-1}. Whilst there is little diurnal variation in trace-element/Ca_{water}, there is an indication of seasonal control on Sr/Ca_{water} with values in

April lower than in June at 7.45 mmol/mol (compared with an average of 10.25 mmol/mol). Mg/Ca_{water} values were similar during April and June at 4427.89 mmol/mol (compared with an average of 4144.77). Spatially, there was little variation in water cation and trace-element/Ca concentrations (Fig. 3). There was slightly greater variation in anion concentrations with much lower Cl^- 11292.15 mg L^{-1} recorded at Site 3 on the eastern edge of the pond. The northern end of the pond (Sites 5 and 6) had lower conductivity (71.9 mS cm^{-1} compared to 77.8 mS cm^{-1} at the southern end of the pond at location 1).

3.3 Ostracod population dynamics

Females accounted for the highest proportion of the adult population in all sampled months except August, when the percentage of females dropped to 20 % from 56 % in February and September (Table 3). In August, the population was dominated by large A-1 individuals or small adults, which may account for the lower number of female adults during this collection (Fig. 4). Large numbers of A-1 individuals were also present in June. The large number of A-1 juveniles during the summer months could be individuals preparing to moult to adulthood in the autumn if the population dynamics of *C. torosa* at Pegwell Bay adheres to the model of Heip (1976). The highest number of juveniles across instars was recorded in August (39) and December (32) (Fig. 4). Conversely, a lower number of juveniles was recorded in April and September with a lack of juveniles below instar A-2. The smaller number of juveniles in April and September is likely due to an increase in adults from spring and autumn calcification (Heip, 1976). A lack of juvenile instars below A-3 (< 250 µm) was however likely due to them passing through the net and sieve mesh size used during sample collection and processing. In general, the percentage of individuals noted to have adhering material is minimal in all samples with a maximum of 13 % in December and a minimum of 7 % in April and February respectively (Table 3).

3.1 Temperature

There was a large variation in water temperatures with a range from –1.6 to 34.2 ˚C, displaying a seasonal pattern over the sampling period with minimum temperatures recorded in January and maximum temperatures in June (Table 5). Diurnal variation in average water temperature is low at an average of ± 1 ˚C difference between day and night temperatures. Despite the inland and higher altitude location of the weather station (~49 m a.s.l), and therefore expected cooler temperatures, the water temperature broadly matched the air temperature in all months. Precipitation was highest in November at 103.4 mm and lowest in December at 9.2 mm, but with no obvious seasonal pattern.

3.4 Ostracod Shell chemistry

The shell chemistry displays a seasonal trend (Fig. 5). Valves collected on each sampling day had high Sr/Ca$_{shell}$ (up to 4.23 mmol/mol in June), typical of a marine-influenced system (Table 4). The Sr/Ca$_{shell}$ is similar throughout the year (± 2.19 mmol/mol), but the lowest values are recorded in December and February, suggesting a slight seasonal control (Fig. 5). Variability in Mg/Ca$_{shell}$ is more marked than that in Sr/Ca$_{shell}$; Mg/Ca$_{shell}$ is strongly seasonal with gradually decreasing values recorded April to September (Fig. 5) and the lowest average values of 7.88 and 8.24 mmol/mol recorded in December and February respectively (Table 4). There is no systematic relationship between Mg/Ca$_{shell}$ and Sr/Ca$_{shell}$. Using the valves and water collected in June 2017, M/Ca$_{ostracod}$ and M/Ca$_{water}$ show a positive, but statistically insignificant linear relationships ($R^2 = 0.12$, $p = 0.4$ for Sr/Ca and $R^2 = 0.30$, $p = 0.2$ for Mg/Ca) (Fig. 6).

4. Discussion

The Mg/Ca$_{water}$ over the calcification period (spring to autumn) varied between 3.9 and 4.4 mol/mol (averaging at 4.2 mol/mol). With the Mg/Ca$_{water}$ variation over the calcification period constrained, it is possible to compare the monitored water temperatures with Mg/Ca-inferred temperatures and predict the possibility of calcification over the monitoring period. We can calculate the Mg/Ca-inferred temperature using the temperature calibration of De Deckker *et al*. (1999), since the waters are of marine-like ionic composition (Table 2):

$$T\ (^{\circ}\mathrm{C}) = 2.69 + (5230 * [\mathrm{Mg/Ca}]_{ostracod}/[\mathrm{Mg/Ca}]_{water}) \quad (1)$$

4.1 Tracking the calcification of *C. torosa*

Temperatures for each collected individual have been calculated using Mg/Ca$_{water}$ values of 3.9, 4.2, 4.4 and 5.1 mol/mol, and equation (1). For the August 2016 collections, there is a bimodal distribution of inferred temperature, which provides some support for the model of Heip (1976) (Fig. 7). Under all Mg/Ca$_{water}$ scenarios, there appears to be two generations of *C. torosa*, calcifying in the spring (valves with Mg/Ca inferred temperatures of 20.6 to 34.5 ˚C at Mg/Ca$_{water}$ of 4.2 mol/mol for 04-Aug-2016; Table 4), and autumn (inferred temperatures of 7.0 to 9.2 ˚C). In April and early May 2017, water temperatures reach 21.6 and 31.6 ˚C, suggesting that, with similar temperatures in the spring of 2016 (16.7 to 22.8 compared with 17.9 to 22.8

in 2017; Met Office, 2012), adult valves collected in August 2016 could be recording spring temperatures.

There is also a bimodal distribution of inferred temperatures in April 2017 using the Mg/Ca$_{water}$ scenario of 3.9 mol/mol and in June 2017 under all Mg/Ca$_{water}$ scenarios. However, the upper-limits of calculated temperatures are untenable with values between 36.8 and 47.4 ˚C at Mg/Ca$_{water}$ of 3.9 to 4.4 mol/mol (Table 4), suggesting that these individuals calcified under higher Mg/Ca$_{water}$ concentrations, which were not recorded over the monitored period. It is therefore likely that these individuals calcified in the previous year when Mg/Ca$_{water}$ values could have reached 5.1 mol/mol (average seawater concentrations due to a direct marine connection at high tides – tidal connection is unpredictable, but has been recorded by camera traps), and thus lowering inferred temperatures to 32.1 to 36.9 ˚C (within the expected summer to spring temperatures for the region). Individuals from the previous calcification cycle becoming a larger proportion of the sample size may relate to A-1 valves calcifying after the collection date, and therefore there is a higher proportion of the previous year's adult population. This is confirmed by the Mg/Ca-inferred temperature of the majority of individuals collected in June 2017 closely mirroring monitored temperatures (Fig. 7). Furthermore, by June there is a lack of individuals with an Mg/Ca-inferred temperature below 16 ˚C (with Mg/Ca$_{water}$ of 4.2 mol/mol), suggesting that adults that calcified in the previous autumn and spring have died and are no longer in the living assemblage (i.e. there is a turnover in population to individuals that have calcified within the current year).

There is further evidence of the loss of adults from the population in the winter months. Water temperatures of 7 ˚C were measured in November 2016, May 2017, and April 2017 with Mg/Ca-inferred temperatures of 7 ˚C from individuals collected in August 2016, December 2016, February 2017 and September 2017. The lack of Mg/Ca-inferred temperatures below 7 ˚C from the December 2016 and February 2017 datasets could be related to the fact that 1) adult individuals calcifying in the spring of the previous year were no longer present in the population or 2) adults calcifying in September 2016 were a larger percentage of the population and thus a larger percentage of the sample. However, there is no consensus over how long *C. torosa* individuals live as adults. The culture experiments of P. Frenzel suggest an adult life-span of 6-12 months; the adults from cultures were harvested at 6 months after moulting with the majority of the population alive (P. Frenzel, personal communication, 2018). Furthermore, it is possible that female life-span is longer than that of males due to the brood care of eggs and small juveniles. It is unlikely that it is necessary for males to overwinter in order to mate with newly-moulted adult females in spring (confirmed by the larger percentage of adult females from December 2016 onwards once overwintering commences; Table 4).

Adults collected in February 2017 may therefore have calcified in the previous autumn, or earlier. Based on an adult life span of 6-12 months, it is possible that the lack of valves yielding an Mg/Ca-inferred temperature below 7 ˚C, when spring temperatures were on average lower, is based on a combination of the loss of the previous adult generation and the increase in percentage of adults calcifying at a later date. Population data and our interpretation of Mg/Ca-inferred temperatures presented here suggest that individuals may be present in the population for 12 to 18 months, but that there is a replacement of adults from June to September; the percentage of juvenile individuals with adhering material drops to 0 % from June (Table 3) and the number of A-1 individuals in the sample reduces between June and September (Fig. 4), suggesting moulting to adulthood commences in May to June.

It is reasonable to assume that there was no life-cycle development (release of broods or moulting) from December 2016 to April 2017, due to the overwintering of valves (Heip, 1976; Horne, 1983). However, based on the assumption that valves calcify in early to mid-autumn, we would expect the samples collected during December to April to have more individuals that reflect the temperature of autumn calcification (e.g. 2.1 to 17.1 ˚C for October to November) than spring calcification (e.g. 4.7 to 31.0 ˚C for March to May). Using the average Mg/Ca_{water} value of 4.2 mol/mol, the majority of valves collected in December 2016 and February 2017 appear to reflect the mean temperatures from October 2016 with average Mg/Ca-inferred ostracod values of 12.7 ˚C and an average measured water temperature of 12.8 ˚C (Fig. 7). Furthermore, for June an increase in valves recording temperatures between 10 and 20 ˚C may reflect the increase in water temperatures from May when A-1 valves would have been calcifying.

Despite the fact that valve Mg/Ca appears to reflect the mean temperatures from the preceding months, no significant relationship was established between Mg/Ca-inferred temperatures and water temperatures averaged for up to six months prior to collection (Table 6). This may be due to adults having calcified more than six months prior to collection or that the samples are composed of a mixed population containing individuals that calcified under different conditions. The exact timing of calcification is an important variable in determining a significant relationship between Mg/Ca-inferred temperatures and water temperatures. Although relatively little difference is observed between day and night temperatures in the pond, it is unknown if ostracods calcify at night, during the day or both. It has been shown for foraminifera that calcification occurs in light-limited environments and that diurnal patterns in Mg/Ca observed as bands of high and low Mg/Ca through the test have important implications for palaeothermometry (Fehrenbacher *et al*., 2017). Notwithstanding differences in and problems with vital effects (e.g. Weiner and Dove, 2003), the mechanisms of biomineralisation for ostracods and foraminifera are broadly similar based on the assumptions of mineral precipitation (e.g. Mucci and Morse, 1983) and, therefore, it is possible that ostracod Mg/Ca is, at times, recording night temperatures. More information on the controls on biomineralisation would, however, be needed to fully understand these patterns.

Despite the Mg/Ca$_{water}$ scenario used in equation (1), no valves have an Mg/Ca-inferred temperature below 7 ˚C during the period that temperatures were monitored, perhaps suggesting a minimum temperature control on calcification. Temperature controls on calcification have been suggested by several authors (Heip, 1976; Wansard *et al*., 2017). Originally the temperature control on calcification was proposed to be purely biological; the initial findings of Heip (1976) suggested that the first three instar stages tolerate lower temperatures to moult since they develop in brood care in the female valves, but a minimum ambient water temperature of 7.3 ˚C is required for individuals to be released from brood care, at least 16.3 ˚C for instars 4 and 5 to moult, and a minimum temperature of 9.3 ˚C to reach adulthood. The findings presented here suggest that there may be a combined biological and environmental control on calcification; the lack of Mg-inferred temperatures below 7 ˚C is likely due to a biologically linked minimum temperature control on calcification of A-1 to adult moult stage, which coincides with the environmental conditions at the time of overwintering of populations.

4.2 Implications for palaeotemperature reconstruction

The trace-element composition of *C. torosa* has been used extensively for palaeoenvironmental reconstruction for sites with marine-type waters (e.g. Anadón *et al.* 1987; Gasse *et al.* 1987; De Deckker *et al.* 1988a, b; Gibert *et al.* 1990; De Deckker & Williams 1993; Wansard 1996; Ingram *et al.* 1998; Holmes *et al.* 2010; Marco-Barba *et al.* 2013; Grossi *et al.* 2015). However, quantifying trace-element partitioning for *C. torosa* is problematic due its eurytopic nature and, therefore, it is often impossible to determine the composition of host water and temperature at the time of calcification.

With detailed temperature monitoring, this study at the coastal pond in Pegwell Bay extends the understanding of the Mg/Ca signal that may be represented in a fossil *C. torosa* record from marine-type waters. A minimum Mg-inferred temperature of 7 ˚C that coincided with the overwintering of valves suggests that in some environments winter temperatures (i.e. minimum temperature values) will not be recorded. Reconstructions will therefore have a strong bias towards warmer temperatures. The use of Mg/Ca-inferred temperatures to reconstruct minimum temperatures should therefore be avoided. Furthermore, based on three years of 5-day averaged temperatures Heip (1976) suggests a minimum temperature to moult to adulthood of 9.3 ˚C. In environments where spring and autumn temperatures are not substantially lower than 9 ˚C, any Mg/Ca-reconstructed temperature significantly below this may be an artefact of the calibration or a result of a lowered Mg/Ca_{water} value rather than a true temperature signal. Maximum temperatures (taking 34.2 ˚C in June 2017 as an analogue for expected maximum temperatures) are, however, recorded by individuals (e.g. those collected in April with an Mg/Ca temperature of 38.4˚C), suggesting that, there is no upper temperature limit on calcification in the pond at Pegwell Bay. However, in sites with substantial evaporation, the increase in Mg/Ca_{water} may mask the temperature dependence of calcification at higher temperatures. In marginal-marine waters, however, where multiple individual valves from the same stratigraphic interval are analysed for a palaeotemperature dataset, maximum temperatures are likely to be captured. With bulk multiple shell analyses (i.e. many shells combined together and analysed as one), the presence of multiple generations in a fossil assemblage is likely to limit the accurate reconstruction of minimum or maximum temperatures. Instead the analysis of a large number of single valves in likely to be more informative. The results presented here show that under conditions of constant Mg/Ca_{water}, the Mg/Ca of *Cyprideis* can be used to reconstruct water temperatures exceeding ~9.3 ˚C, the minimum temperature at which the species moults to adulthood (Heip, 1976). Good understanding of modern site systematics and hydrochemistry are, however, necessary to confirm this relationship for palaeo-datasets.

Sr/Ca_{shell} values show a small increase in June 2017 and September 2017 (Fig. 5), which could be reflecting high EC values recorded in June 2017, and therefore spring calcification (Fig. 8). However, there is little change in Sr/Ca_{shell} throughout the sampling period, whereas there is a large spread of Mg/Ca-inferred temperatures for each dataset; Sr/Ca_{shell} values vary by 2.19 mmol/mol compared to 29.84 mmol/mol for Mg/Ca_{shell}, implying that calcification temperature has large variations and that the Sr/$Ca_{ostracod}$ uptake is not thermodependent (c.f. De Deckker *et al*., 1999). Whilst there is no significant relationship between Sr/$Ca_{ostracod}$ and Sr/Ca_{water} in June 2017 (R^2 = 0.12, p = 0.4; Fig. 6), it is likely that Sr/Ca_{water} varies little despite large changes in EC (Table 2) or that there is a Sr/Ca_{water} control on calcification that is more limited than the temperature control. Whilst we have shown that the Mg/Ca_{shell} is controlled primarily by temperature in this environment, in a highly variable environment with higher diurnal variation than the pond at Pegwell Bay, such as estuaries, where *C. torosa* is often abundant, there may be small windows of time that water conditions are optimal for calcification and there is therefore potential for this to mask the temperature dependence of Mg/Ca_{shell}.

5. Conclusions

Annual monitoring can be used to broadly track the calcification of *C. torosa.* However, given the longevity of *C. torosa* adults and hydrological variability, it may be difficult to confirm that calcification took place during the period when temperatures were measured. Currently, studies are limited by a lack of knowledge on *C. torosa* adult longevity. Based on the Mg/Ca-inferred temperatures from adult valves, the results presented here support the suggestion that *C. torosa* calcification occurs in spring and autumn and support the model of two overlapping populations proposed by Heip (1976). Due to the range of temperatures recorded, we suggest that calcification can occur as late as November and occurs throughout the summer months, and there is potential for adult longevity of 12-18 months. Due to autumnal temperatures in the UK, we propose a minimum temperature control on calcification to adulthood of 7, lower than the 9.3 ˚C proposed by Heip (1976).

The exact timing of calcification for these samples remains unclear and conditions from the previous year may be recorded. Börner *et al*. (2013) raised concerns that individuals calcifying under different seasonal conditions may result in intra-sample noise, which may interfere with long-term patterns in palaeolimnological studies. Due to the often low resolution (at least five to ten years) and relative rarity of annually-laminated sediments (which often do not contain ostracods), it is likely that these seasonal signals would be lost in a fossil assemblage unless a particularly strong seasonal pattern were present; for the pond at Pegwell Bay, spring and autumn calcification is likely to produce similar Mg/Ca-inferred temperatures. When paired with modern hydrological systematics of the site, this intra-annual noise should not be an issue for the majority of paleolimnological studies especially where multiple valves are analysed to give an indication of the variability within the sample. To determine the number of individual valves required to capture the expected variability, a simple sample size calculator can be used that incorporates the desired error at a given level of significance and estimated variability using the t distribution (see Holmes, 2008 for further details). However, where palaeotemperatures are required to inform minimum temperatures, it is likely that the lowest Mg/Ca-inferred temperatures are reflecting autumn, and therefore unlikely absolute minimum temperatures. Where multiple individual valves are analysed there should be good indication of maximum and mean inferred temperatures, reflecting spring to autumn conditions.

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Tables

Table 1. Electrical conductivity, salinity, and average water temperature for each of the sampling days. Temperature is the average recorded over a 24-hour period, except for 4-Aug-2016 which is averaged from data logger deployment at 14:40.

Table 2. Water chemistry variables recorded on 18-Apr-2017, from high to low tide on 27-June-2017, and 28-Sep-2017 . Numbers appearing after the 12:00 sampling times (1,2 etc.) relate to the locations in Figure 4..

Table 3. Percentage male (M), female (F) and juvenile (juv.) individuals for each sampling month. The number of individuals with adhering detritus is also noted and broken down by M, F and juv.

Table 4. Ostracod Mg/Ca and Mg/Ca inferred temperature alongside measured water temperature. Water temperature is the average recorded over a 24-hour period, except for 4-Aug-2016 which is averaged from data logger deployment at 14:40.

Table 5. Minimum, maximum and average monthly air and water temperature, and monthly rainfall for the monitoring period August 2016 to September 2017. Air temperature and precipitation data were downloaded from Met Office (2012)

Table 6. Pearson correlation coefficient for Mg/Ca vs average temperature of months preceding collection

Figures

Figure 1. Location of the coastal pond at Pegwell Bay. The inset map shows the location of samples taken on 27/6/2017. Samples were collected at ‘X’ for all sample dates.

Figure 2. Electrical conductivity and average water temperature for each sampling day. Note that there is no Mg/Ca_{water} or Sr/Ca_{water} data for the seasonal sampling.

Figure 3. Variations in trace-element/Ca, Cl^-, electrical conductivity and temperature across the pond at locations 1, 2, 3, 4, 5, 6 between 12:00 and 13:00 on 27/06/2017. Note the logarithmic scale. Ostracod samples were collected at ‘X’ for all sample dates

Figure 4. Length-height relationship for a subsample of 100 individuals of *Cyprideis torosa* for each collection in a) August, b) December, c) February, d) April, e) June and f) September. Black circles denote juvenile individuals, blue adult males and purple adult females

Figure 5. Ostracod Sr/Ca$_{\text{shell}}$ (a) and Mg/Ca$_{\text{shell}}$ (b) for samples collected on each sampling day. Data from individual valves are represented by the grey circles and the mean is denoted by the black line

Figure 6. Relationship between a) Mg/Ca$_{\text{shell}}$ and Mg/Ca$_{\text{water}}$ and b) Sr/Ca$_{\text{shell}}$ and Sr/Ca$_{\text{water}}$ using the values for water and ostracods collected in June 2017.

Figure 7. Water, air and ostracod Mg/Ca-inferred temperatures calculated under Mg/Ca$_{\text{water}}$ scenarios of a) 3.9 mol/mol, b) 4.2 mol/mol, c) 4.4 mol/mol, and d) 5.1 mol/mol. Air and water temperature are displayed by the red and blue lines respectively. The frequency distributions of ostracod Mg-inferred temperature for each collection (the red bars) are placed along the x-axis date of collection. Air temperature was obtained from Met Office (2012)

Figure 8. Average Sr/Ca$_{\text{shell}}$, Mg/Ca$_{\text{shell}}$, electrical conductivity and average water temperature for each sampling day. The grey line relates to the right y-axis in each graph.

Table 1. Electrical conductivity, salinity, and average water temperature for each of the sampling days. Temperature is the average recorded over a 24-hour period, except for 4-Aug-2016 which is averaged from data logger deployment at 14:40.

Date	Electrical conductivity (mS cm^{-1})	Salinity PSU	Average water temperature (°C)
04-Aug-2016	55.2	36.6	20.9
01-Dec-2016	40.2	25.7	3.0
02-Feb-2017	45.1	29.2	8.3
18-Apr-2017	44.6	28.8	10.2
27-Jun-2017	75.2	~53*	17.2
28-Sept-2017	33.3	20.8	18.3

*above scale for accurate conversion

Table 2. Water chemistry variables recorded on 18-Apr-2017, from high to low tide on 27-June-2017, and 28-Sep-2017 . Numbers appearing after the 12:00 sampling times (1,2 etc.) relate to the locations in Figure 4.

Date	**Time / Location**	**Cation concentration (mg L^{-1})**					**Trace-element/Ca (mmol/mol)**		**Anion concentration (mg L^{-1})**	
		Na^+	**K^+**	**Ca^{2+}**	**Sr^{2+}**	**Mg^{2+}**	**Sr/Ca**	**Mg/Ca**	**Cl^-**	**SO_4^{2-}**
18-Apr-2017		11934.60	445.35	496.16	8.08	1332.25	7.45	4427.89	14568.01	5132.92
27-June-2017	**06:00**	91132.00	243.42	804.46	18.05	2060.38	10.26	4223.52	14662.74	2117.87
	07:00	47536.80	139.65	795.55	17.72	2024.14	10.19	4195.70	14584.57	2164.07
	08:00	44429.20	132.66	693.89	15.59	1760.88	10.28	4184.80	13239.73	1942.17
	08:30	65715.80	188.87	661.52	14.59	1646.83	10.09	4105.26	13136.87	1736.55
	09:00	48956.00	143.25	796.15	17.19	2032.71	9.87	4210.29	14293.15	2121.86
	10:00	43324.70	128.07	721.28	16.09	1811.96	10.21	4105.26	13326.92	1967.73
	12:00-1	72766.50	201.52	771.77	17.42	1925.81	10.32	4114.91	14727.81	2059.38
	12:00-2	50562.30	145.69	715.88	15.96	1780.74	10.20	4101.97	12972.51	1840.22
	12:00-3	33012.40	105.05	630.11	14.13	1555.84	10.25	4071.78	11292.15	1803.05
	12:00-4	42708.70	128.89	699.98	15.51	1718.52	10.14	4048.56	13046.82	1806.51
	12:00-5	45115.70	135.69	742.60	16.60	1843.63	10.23	4094.05	13407.76	1928.17
	12:00-6	39816.40	123.77	683.65	15.39	1695.61	10.29	4090.03	12671.54	1871.37
	14:00	53578.00	153.83	779.12	17.68	1988.87	10.38	4209.54	14405.43	2080.02
	15:00	51046.10	146.94	705.34	16.01	1802.53	10.38	4211.21	13870.14	2003.66
	17:00	41804.80	123.11	716.16	16.70	1808.51	10.67	4164.34	14249.60	2349.22
	Average	**51433.69**	**149.36**	**727.83**	**16.31**	**1830.46**	**10.25**	**4144.77**	**13592.52**	**1986.12**
	Std Dev.	**±14783.53**	**±35.80**	**±52.48**	**±1.17**	**±149.86**	**±0.17**	**±58.88**	**±938.76**	**±165.17**
28-Sept-2017		7987.84	330.99	379.55	6.00	896.62	7.23	3895.53		

Table 2. Continued

Date	Time / Location	Electrical conductivity (mS cm^{-1})	Water Temp. (°C)	Alkalinity as $CaCO_3$ equivalence (mg L^{-1})	
				CO_3^{2-}	HCO_3^-
18-Apr-2017		44.6	10.2	0	210
27-June-2017	**06:00**	70.5	15.8	0	266
	07:00	75.0	16.4	0	266
	08:00	75.9	17.6	0	244
	09:00	77.8	19.0	0	256
	10:00	76.9	19.4	0	272
	12:00-1	77.8	22.5	0	270
	12:00-2	76.7	21.6		
	12:00-3	72.3	21.5		
	12:00-4	70.2	23.2		
	12:00-5	71.7	21.9		
	12:00-6	71.9	22.2		
	14:00	77.9	23.3	0	260
	15:00	78.2	24.7	14	254
	17:00	78.8	22.6	0	248
	Average	**75.2**	**20.8**		**259.6**
	Std Dev.	**±3.0**	**±2.7**		**±9.8**
28-Sept-2017		**33.3**	**18.3**		

Table 3. Percentage male (M), female (F) and juvenile (Juv) individuals for each sampling month. The number of individuals with adhering detritus is also noted and broken down by M, F and juv.

	% individuals					
	April	**Aug.**	**Dec.**	**Feb.**	**June**	**Sept.**
F	51	20	40	56	47	56
M	42	41	28	32	24	36
Juv.	7	39	32	12	29	8
Detritus	7	8	13	7	9	11
	F 4	**F** 4	**F** 6	**F** 2	**F** 8	**F** 10
	M 3	**M** 1	**M** 6	**M** 1	**M** 1	**M** 1
	Juv. 0	**Juv.** 3	**Juv.** 1	**Juv.** 4	**Juv.** 0	**Juv.** 0

Table 4. Ostracod Mg/Ca and Mg/Ca inferred temperature alongside measured water temperature. Water temperature is the average recorded over a 24-hour period, except for 4-Aug-2016 which is averaged from data logger deployment at 14:40.

Collected	**Water temp. (°C)**	**Mg/Ca$_{\text{ostracod}}$ (mmol/mol)**	**Average Mg/Ca$_{\text{ostracod}}$ (mmol/mol)**	**Mg/Ca temp. (°C) Mg/Ca$_{\text{water}}$ 3.9 mol/mol**	**Mg/Ca temp. (°C) Mg/Ca$_{\text{water}}$ 4.2 mol/mol**	**Mg/Ca temp. (°C) Mg/Ca$_{\text{water}}$ 4.4 mol/mol**	**Mg/Ca temp. (°C) Mg/Ca$_{\text{water}}$ 5.1 mol/mol**	**Average Mg/Ca temp. (°C) Mg/Ca$_{\text{water}}$ 4.2 mol/mol**
04-Aug-2016	20.9	18.57	12.74	27.6	25.8	24.8	21.7	18.55
		4.68		9.0	8.5	8.3	7.5	
		3.58		7.5	7.1	6.9	6.4	
		5.24		9.7	9.2	8.9	8.1	
		17.78		26.5	24.8	23.8	20.9	
		14.39		22.0	20.6	19.8	17.4	
		3.48		7.4	7.0	6.8	6.3	
		25.57		37.0	34.5	33.1	28.9	
		21.34		31.3	29.3	28.1	24.6	
01-Dec-2016	9.2	12.75	7.88	19.8	18.6	17.8	15.8	12.50
		4.93		9.3	8.8	8.5	7.7	
		7.64		12.9	12.2	11.8	10.5	
		4.35		8.5	8.1	7.9	7.2	
		4.42		8.6	8.2	7.9	7.2	
		7.33		12.5	11.8	11.4	10.2	
		4.27		8.4	8.0	7.8	7.1	
		20.05		29.6	27.7	26.5	23.2	
		5.19		9.7	9.2	8.9	8.0	
02-Feb-2017	6.4	4.57	8.24	8.8	8.4	8.1	7.4	12.95
		4.24		8.4	8.0	7.7	7.0	
		5.48		10.0	9.5	9.2	8.3	
		5.92		10.6	10.1	9.7	8.8	
		15.37		23.3	21.8	21.0	18.4	
		10.03		16.1	15.2	14.6	13.0	
		12.46		19.4	18.2	17.5	15.5	
		9.72		15.7	14.8	14.2	12.7	
		5.94		10.7	10.1	9.8	8.8	
		8.66		14.3	13.5	13.0	11.6	

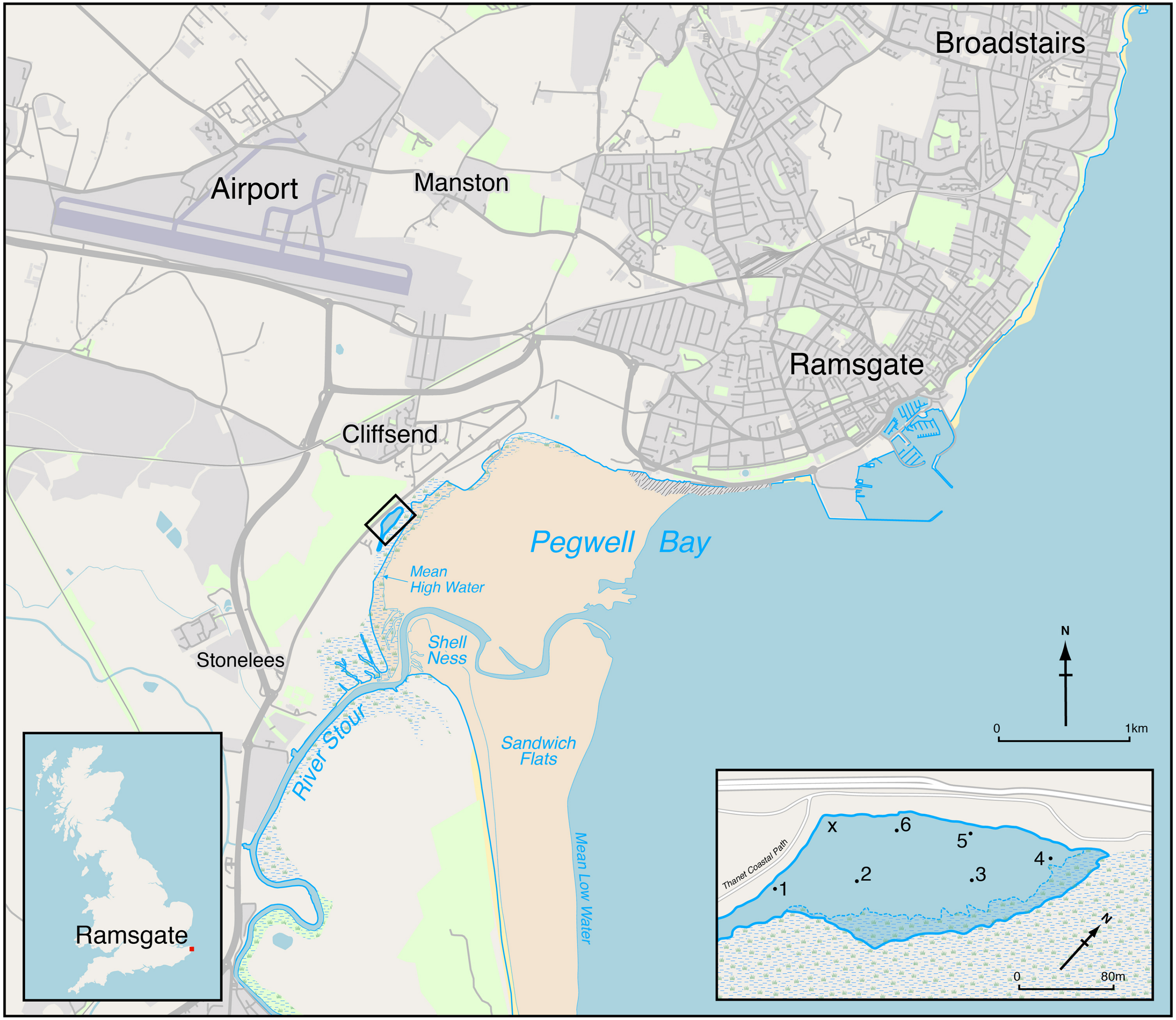
18-April-2017	17.6	17.56	23.26	26.2	24.6	23.6	20.7	31.65
		18.02		26.9	25.1	24.1	21.2	
		28.67		41.1	38.4	36.8	32.1	
		15.87		24.0	22.5	21.6	19.0	
		17.10		25.6	24.0	23.0	20.2	
		24.24		35.2	32.9	31.5	27.6	
		19.61		29.0	27.1	26.0	22.8	
		33.32		47.4	44.2	42.3	36.9	
		31.91		45.5	42.4	40.6	35.4	
		26.26		37.9	35.4	33.9	29.6	
27-Jun-2017	21.4	13.06	16.73	20.2	19.0	18.2	16.1	23.53
		33.12		47.1	43.9	42.1	36.7	
		21.34		31.3	29.3	28.1	24.6	
		11.80		18.5	17.4	16.7	14.8	
		23.50		34.2	32.0	30.6	26.8	
		15.11		22.9	21.5	20.6	18.2	
		19.63		29.0	27.1	26.0	22.8	
		11.01		17.5	16.4	15.8	14.0	
		14.18		21.7	20.3	19.5	17.2	
		10.67		17.0	16.0	15.4	13.6	
		10.68		17.0	16.0	15.4	13.6	
28-Sept-2017	16.7	9.89	9.77	16.0	15.0	14.5	12.8	14.87
		8.07		13.5	12.7	12.3	11.0	
		5.08		9.5	9.0	8.7	7.9	
		8.63		14.3	13.4	13.0	11.5	
		11.22		17.7	16.7	16.0	14.2	
		15.77		23.8	22.3	21.4	18.9	

Table 5. Minimum, maximum and average monthly air and water temperature, and monthly rainfall for the monitoring period August 2016 to September 2017. Air temperature and precipitation data were downloaded from Met Office (2012)

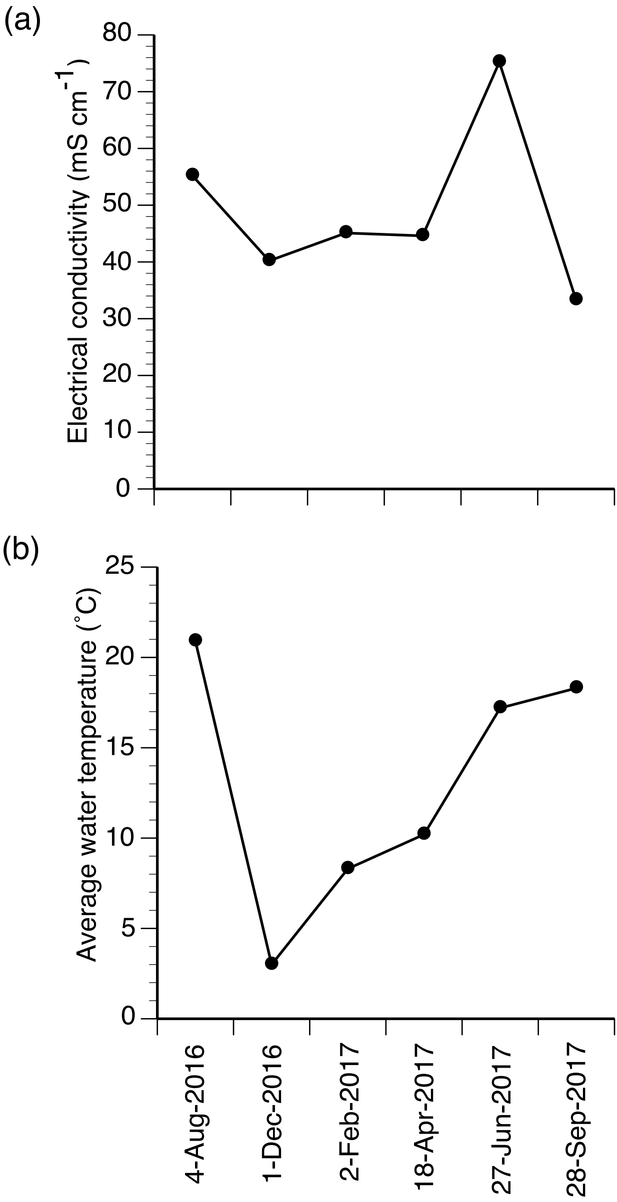
	Air temp. (˚C)			**Water temp. (˚C)**			**Precipitation (mm)**
Month/Year	**Max.**	**Min.**	**Average**	**Max.**	**Min.**	**Average**	
08/2016	23.3	14.4	18.5	27.4	13.0	19.4	18.0
09/2016	22.5	14.4	17.7	26.6	14.7	19.6	76.2
10/2016	15.0	9.4	11.9	17.1	9.8	12.8	34.8
11/2016	10.1	4.4	7.4	12.6	2.1	7.8	103.4
12/2016	9.3	3.8	6.8	10.1	0.4	5.7	9.2
01/2017	6.3	0.7	3.5	7.7	-1.6	3.3	48.2
02/2017	9.2	4.5	6.6	11.3	1.2	6.4	26.4
03/2017	13.0	6.0	9.2	16.4	4.7	10.2	17.2
04/2017	13.7	5.9	9.4	21.6	5.5	13.9	10.8
05/2017	17.9	9.9	13.5	31.0	8.1	17.6	57.8
06/2017	22.4	13.1	17.4	34.2	11.8	21.4	37.8
07/2017	22.8	14.5	18.0	30.9	13.9	20.6	74.2
08/2017	21.4	13.3	16.9	25.9	15.6	20.2	85.6
09/2017	18.3	11.1	14.3	22.9	12.3	16.8	37.0

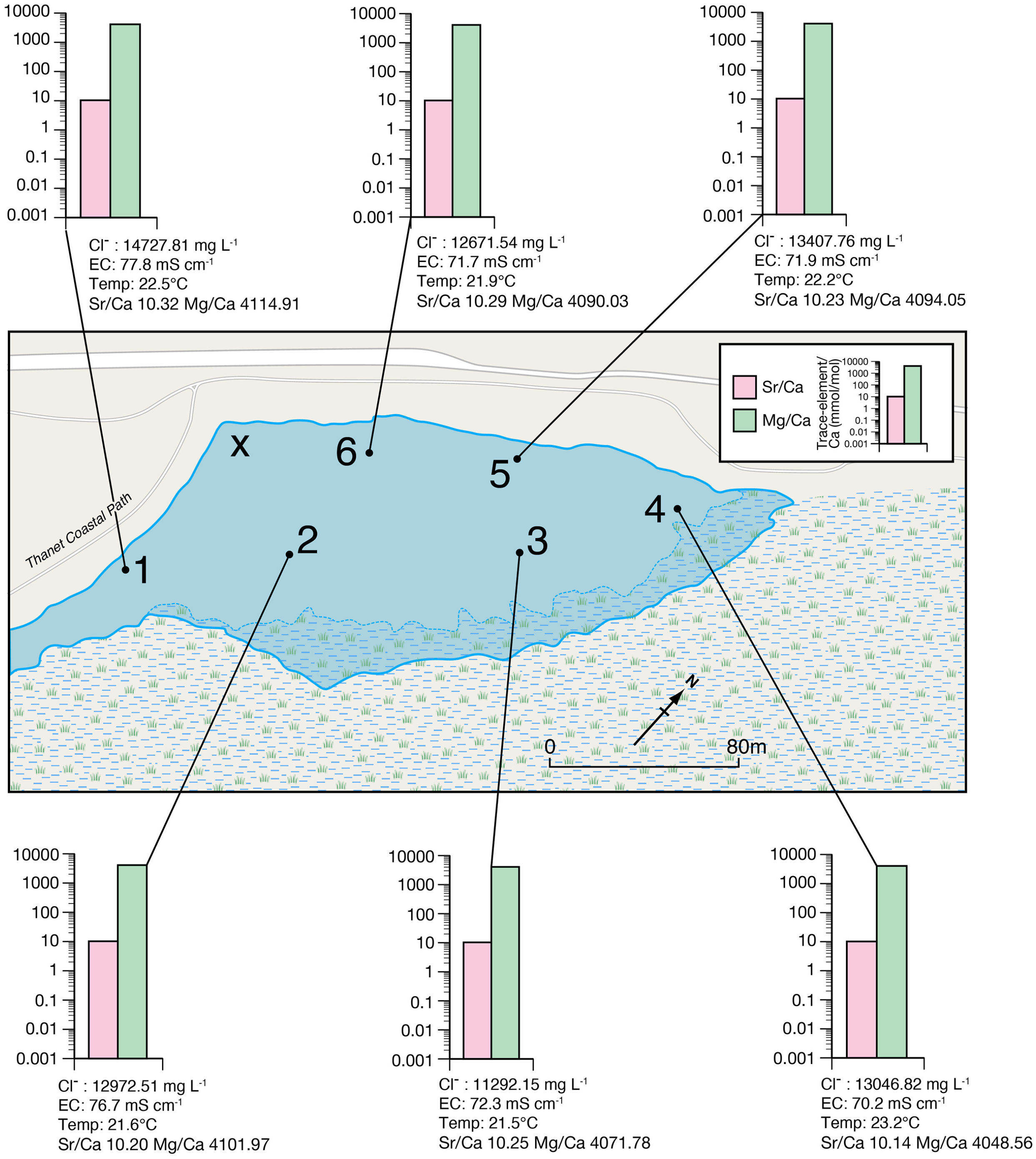
Table 6. T-test for Mg/Ca-inferred temperatures vs average temperature of months preceding collection

	T-test	p-value
Collection month	1.03	>0.3
1 month before	0.56	>0.6
2 months before	0.22	>0.8
3 months before	-0.20	>0.8
6 months before	-1.12	>0.3



Map data © OpenStreetMap contributors, CC-BY-SA, opendatacommons.org


(a)
Electrical conductivity (mS cm^{-1})
0
10
20
30
40
50
60
70
80
(b)
Average water temperature (°C)
0
5
10
15
20
25
4-Aug-2016
1-Dec-2016
2-Feb-2017
18-Apr-2017
27-Jun-2017
28-Sep-2017



1: Cl⁻ : 14727.81 mg L^{-1}
EC: 77.8 mS cm^{-1}
Temp: 22.5°C
Sr/Ca 10.32 Mg/Ca 4114.91

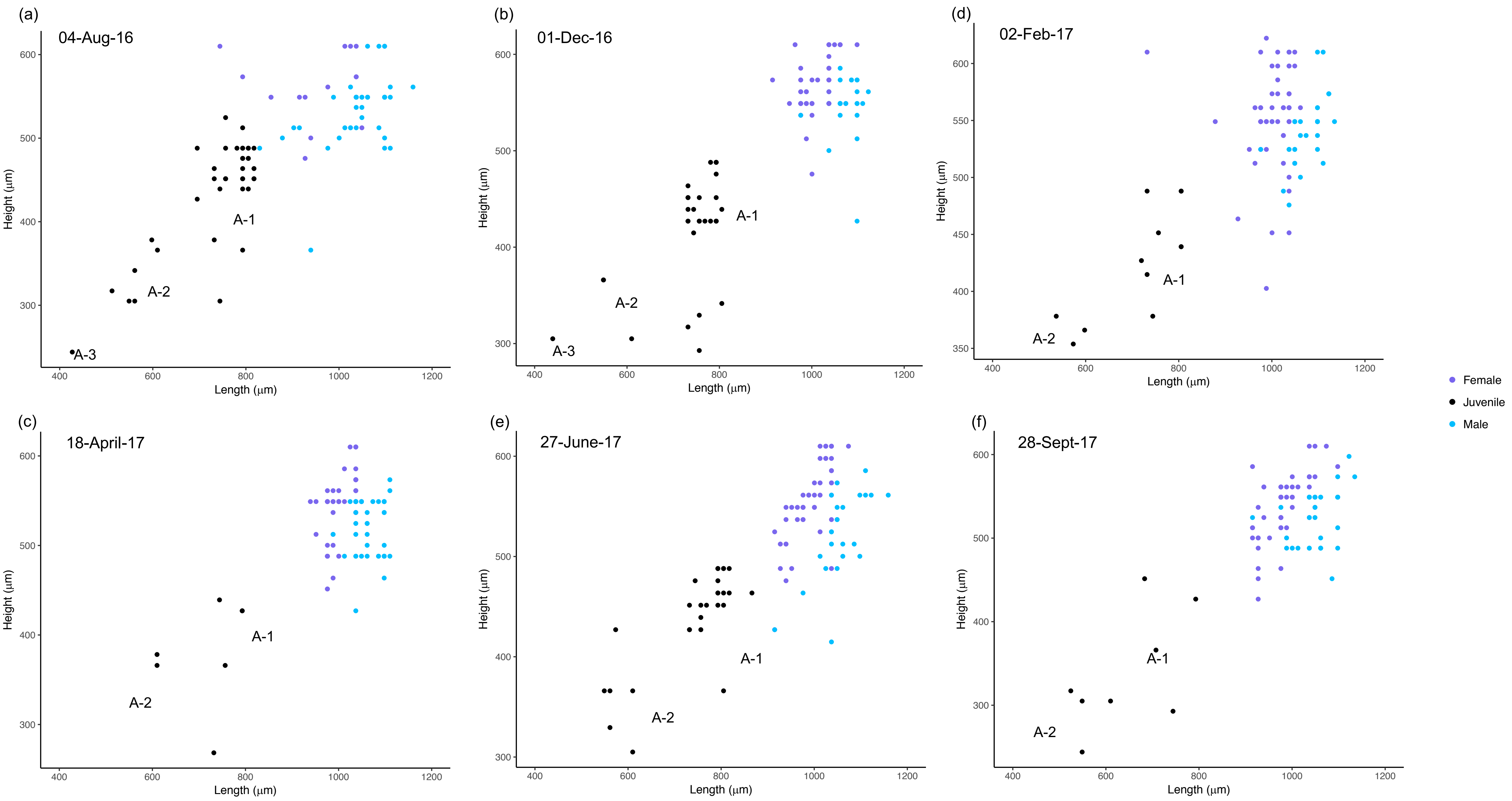
6: Cl⁻ : 12671.54 mg L^{-1}
EC: 71.7 mS cm^{-1}
Temp: 21.9°C
Sr/Ca 10.29 Mg/Ca 4090.03

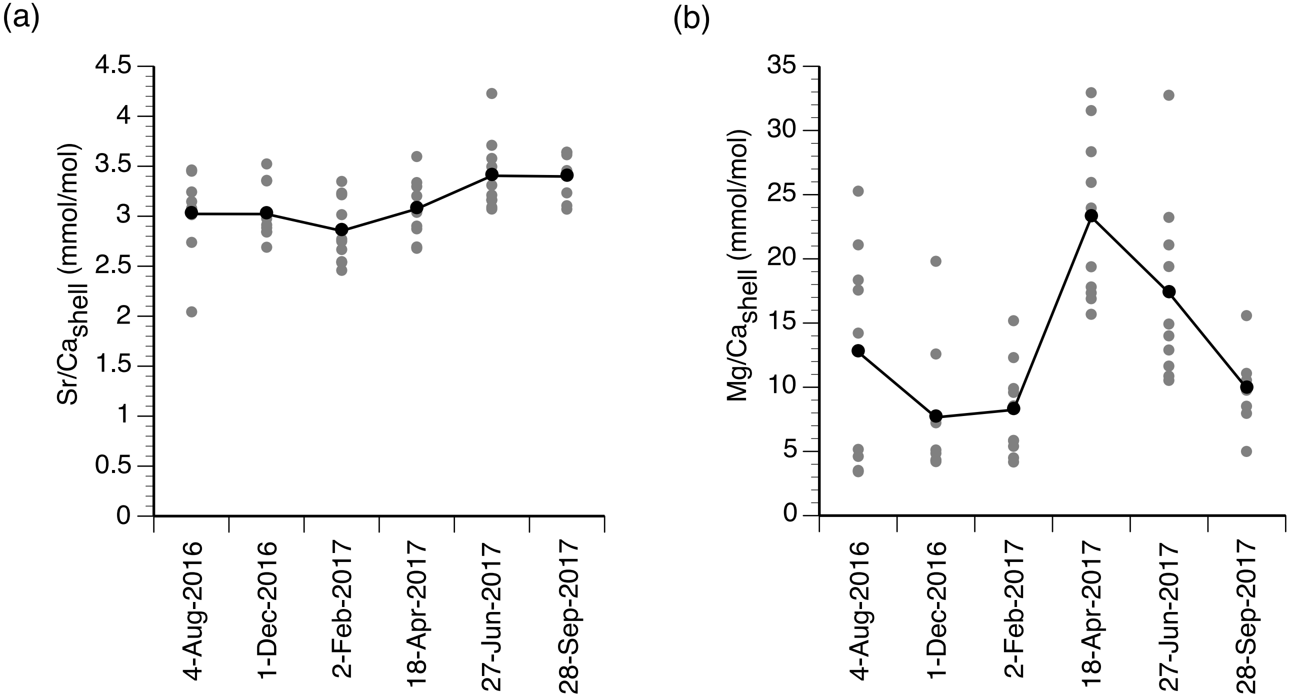
5: Cl⁻ : 13407.76 mg L^{-1}
EC: 71.9 mS cm^{-1}
Temp: 22.2°C
Sr/Ca 10.23 Mg/Ca 4094.05

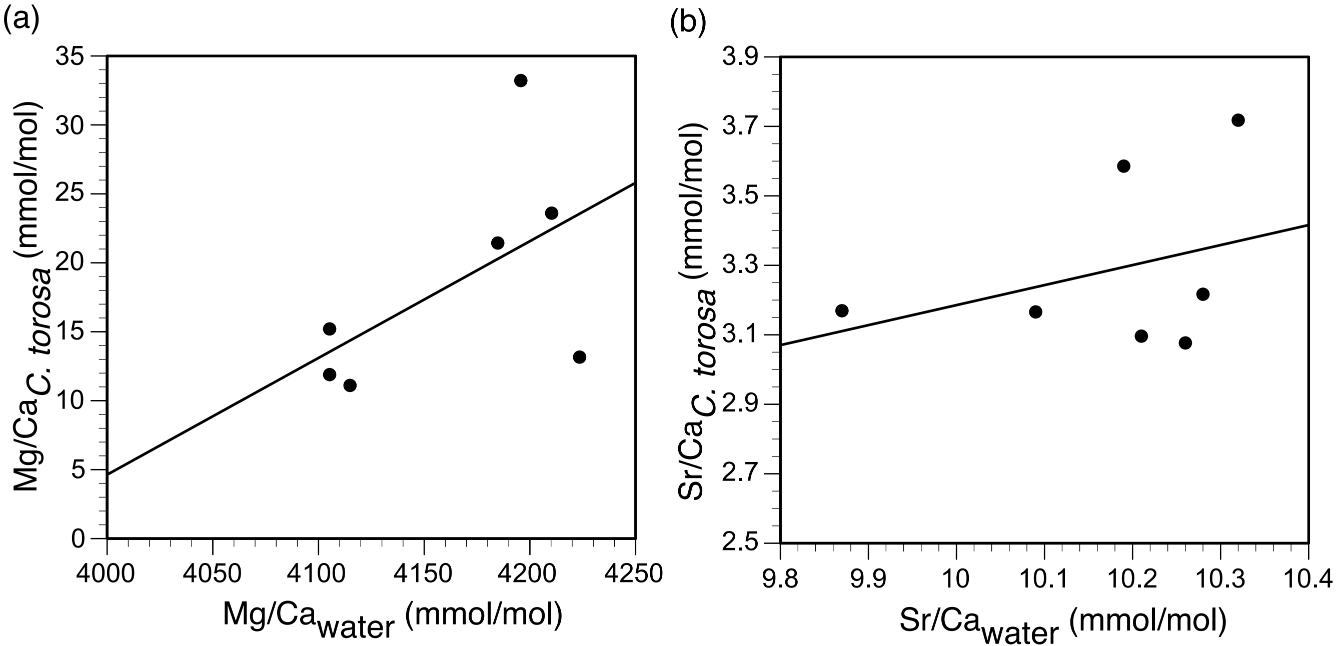
2: Cl⁻ : 12972.51 mg L^{-1}
EC: 76.7 mS cm^{-1}
Temp: 21.6°C
Sr/Ca 10.20 Mg/Ca 4101.97

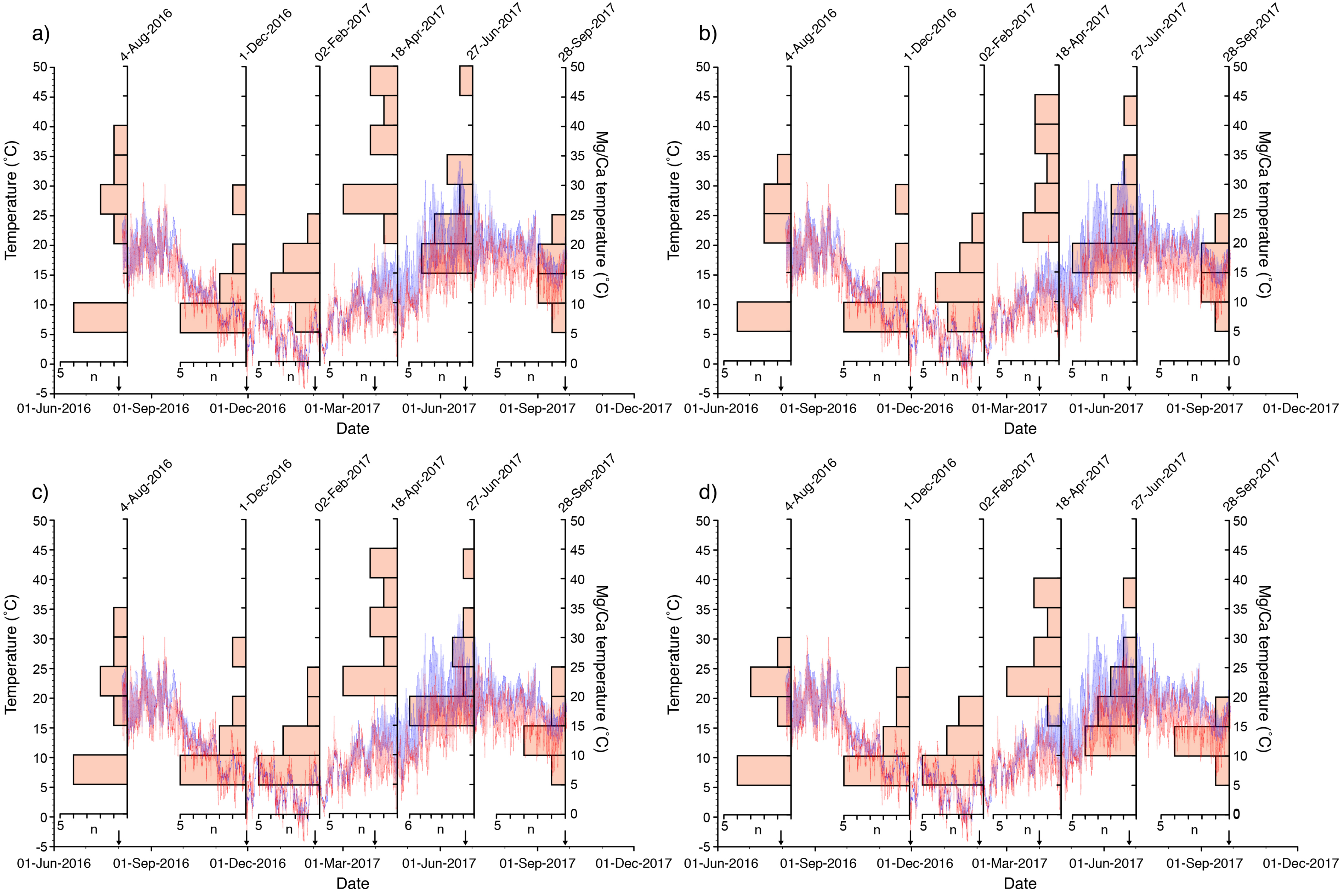
3: Cl⁻ : 11292.15 mg L^{-1}
EC: 72.3 mS cm^{-1}
Temp: 21.5°C
Sr/Ca 10.25 Mg/Ca 4071.78

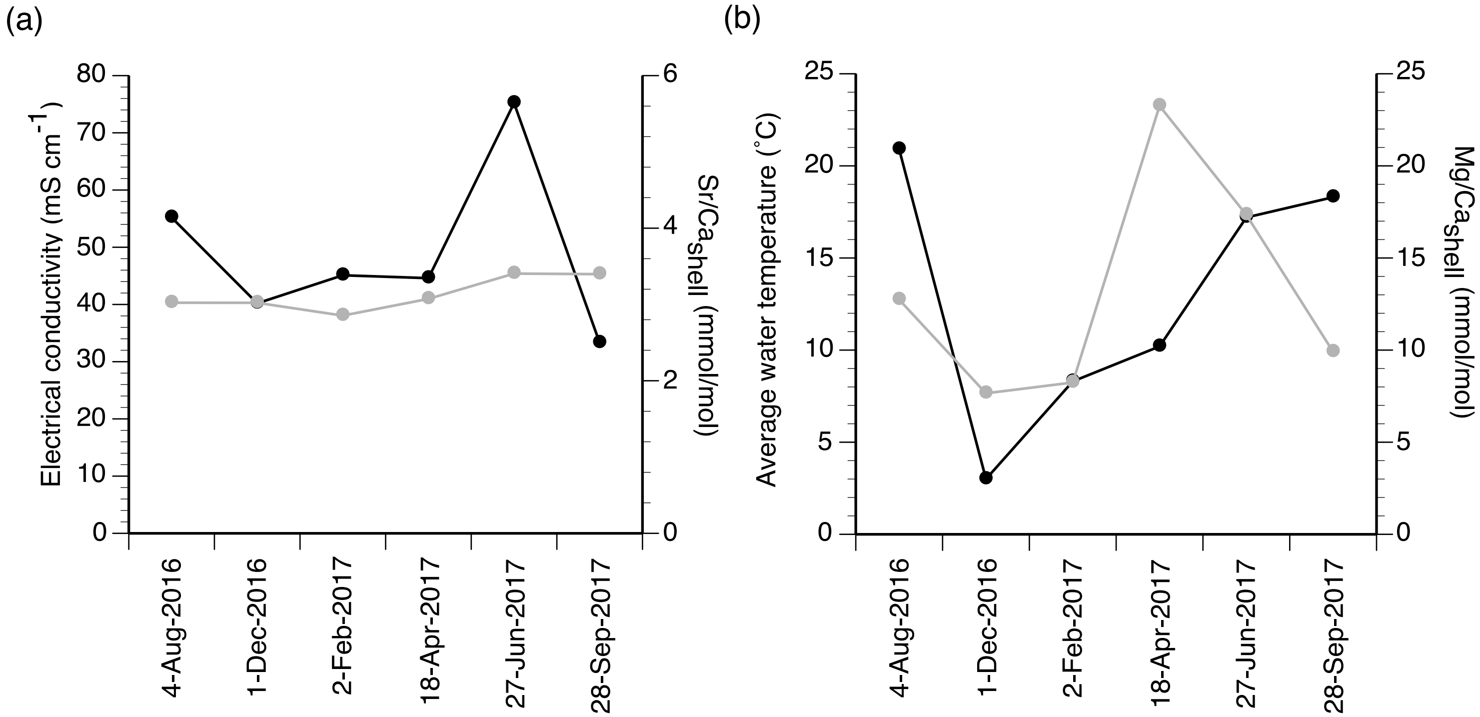
4: Cl⁻ : 13046.82 mg L^{-1}
EC: 70.2 mS cm^{-1}
Temp: 23.2°C
Sr/Ca 10.14 Mg/Ca 4048.56


(a)
04-Aug-16
A-1
A-2
A-3
(b)
01-Dec-16
A-1
A-2
A-3
(d)
02-Feb-17
A-1
A-2
(c)
18-April-17
A-1
A-2
(e)
27-June-17
A-1
A-2
(f)
28-Sept-17
A-1
A-2
Height (µm)
Length (µm)
400
600
800
1000
1200
300
350
400
450
500
550
600
Female
Juvenile
Male


(a)
Sr/Ca$_{shell}$ (mmol/mol)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
4-Aug-2016
1-Dec-2016
2-Feb-2017
18-Apr-2017
27-Jun-2017
28-Sep-2017
(b)
Mg/Ca$_{shell}$ (mmol/mol)
35
30
25
20
15
10
5
0
4-Aug-2016
1-Dec-2016
2-Feb-2017
18-Apr-2017
27-Jun-2017
28-Sep-2017


(a)
35
30
25
20
15
10
5
0
4000
4050
4100
4150
4200
4250
Mg/Ca$_{C.\ torosa}$ (mmol/mol)
Mg/Ca$_{water}$ (mmol/mol)
(b)
3.9
3.7
3.5
3.3
3.1
2.9
2.7
2.5
9.8
9.9
10
10.1
10.2
10.3
10.4
Sr/Ca$_{C.\ torosa}$ (mmol/mol)
Sr/Ca$_{water}$ (mmol/mol)


a)
b)
c)
d)
4-Aug-2016
1-Dec-2016
02-Feb-2017
18-Apr-2017
27-Jun-2017
28-Sep-2017
Temperature (˚C)
Mg/Ca temperature (˚C)
n
Date
01-Jun-2016
01-Sep-2016
01-Dec-2016
01-Mar-2017
01-Jun-2017
01-Sep-2017
01-Dec-2017


(a)
Electrical conductivity (mS cm^{-1})
80
70
60
50
40
30
20
10
0
Sr/Ca$_{shell}$ (mmol/mol)
6
4
2
0
4-Aug-2016
1-Dec-2016
2-Feb-2017
18-Apr-2017
27-Jun-2017
28-Sep-2017
(b)
Average water temperature (°C)
25
20
15
10
5
0
Mg/Ca$_{shell}$ (mmol/mol)
25
20
15
10
5
0
4-Aug-2016
1-Dec-2016
2-Feb-2017
18-Apr-2017
27-Jun-2017
28-Sep-2017

Ostracods are microscopic aquatic crustaceans, whose bivalved calcitic shells are readily preserved in sediments, and are thus commonly used for palaeoenvironmental reconstruction. However, despite their widespread use, relatively little is understood about the life cycle and calcification of many routinely analysed ostracod species. Seasonality of shell formation, which differs between species, can have important implications for palaeoenvironmental reconstructions. This is especially the case for palaeotemperature. In this study, we analyse variations in the trace-element shell chemistry of *Cyprideis torosa* a ubiquitous brackish water species, commonly used in palaeoenvironmental reconstructions, to infer seasonal cycles of calcification.

Ostracods in the Subclass Podocopa, such as *C. torosa*, grow through nine instars (eight juvenile and one adult stage) (Horne, 2005), with juvenile stages designated as (in descending order of size) A-1, A-2, A-3, A-4 etc., but the persistence at each life stage is largely unknown. Temperate environments with stable salinity produce a single generation of *C. torosa* per year, but with two peaks of adults. A-1 to adult calcification occurs biannually in spring and late summer to early autumn (Heip, 1976). Adults overwinter alongside populations of A-1 juveniles that produce new adult populations in the spring and autumn; the generations overlap so that the population comprises both overwintering adults and newly-matured adults arising from overwintering juveniles, producing peak abundances of *C. torosa* during calcification months in the spring. The overwintering female adults release early instar juveniles from the brood chamber in the spring that moult to adulthood by the autumn (Heip, 1976; Horne, 1983). It is sometimes possible to distinguish between adults that overwintered and those that are newly calcified because the former may have material (e.g. diatoms) attached to their shells whereas the latter have relatively ‘clean’ looking shells. In warmer regions, for example the Mediterranean, *C. torosa* produces two or more generations per year although the increased temperatures reduce the mean duration of early-juvenile instar stages (Mezquita *et al.,* 2000). Previous ecological monitoring of *C. torosa* has shown that environmental conditions, such as temperature, are an important control on the life cycle of the species, but relatively little is understood about these controls.

Previous studies of the seasonal life cycle of *C. torosa* (e.g. Heip, 1976; Horne, 1983) have not considered the implications for palaeoenvironmental reconstruction using this species. Consequently, relatively little is understood about whether palaeoenvironmental reconstructions are recording restricted periods (i.e. only spring or late summer temperatures), which may be linked to a temperature preference for calcification. Some authors have argued that shell calcification can occur at different times of the year, so that the resulting intra-annual noise in inferred temperatures may interfere with long-term palaeoenvironmental signals (Börner *et al*., 2013). Because *C. torosa* inhabits highly dynamic waterbodies, such as estuaries and coastal lakes, it is likely to experience wide variations in environmental conditions on short timescales. Understanding these variations in the hydrological system coupled with the population dynamics of the species will allow more detailed understanding of ostracod-shell trace-element datasets.

The Mg content of ostracod shells is commonly used as a palaeotemperature proxy in Quaternary sediments. Typically, the Mg content of ostracod valves correlates with the Mg content of water and with water temperature (Chivas *et al*., 1986; De Deckker and Forester, 1988; Holmes and Chivas, 2002). In situations where the composition of the water shows minimal variation, temperature is the major control and the Mg content of ostracod shells can be used as a palaeothermometer. *Cyprideis torosa* provides an ideal target species to track calcification timings since there are existing Mg/Ca temperature calibrations (e.g. Wansard 1996; De Deckker *et al.* 1999). When the Mg/Ca ratio of the host water is known, the calcification temperature for *C. australiensis* is given by the equation of De Deckker *et al*. (1999) of $T(^{\circ}C) = 2.69 + 5230([Mg/Ca]_{shell} /[Mg/Ca]_{water})$. Whilst *C. australiensis* is now considered a separate species to *C. torosa* (Schön *et al.,* 2017), this equation has been successfully applied to living and fossil specimens of *C. torosa* in water of marine-type ionic composition (Holmes and De Deckker, 2017). Where the waters are more dilute and/or of contrasting ionic composition, it is possible to use the palaeotemperature equation of Wansard (1996), developed for *C. torosa*, of $T(^{\circ}C) = 3.3 + (1971.0 * [Mg/Ca]_{ostracod})$. Similarly, for *C. australiensis,* the Sr/Ca of the shell is often positively correlated with the Sr/Ca of the host water, which in some circumstances covaries with salinity (Chivas *et al*., 1985). De Deckker *et al.* (1999) have also suggested a small temperature dependence on the incorporation of Sr into shells.

Currently, there are three large unknowns when calculating Mg/Ca-inferred temperatures: 1) the exact timing of *C. torosa* calcification, 2) the implications of seasonal water variations on the range of calculated temperatures, and 3) the Mg/Ca_{water} scenarios that the De Deckker *et al*. (1999) and Wansard (1996) equations apply. It is common practice to use 'spot samples' or annual mean Mg/Ca_{water} conditions to calculate partition coefficients (K_D values) and/or in temperature calculations (e.g. Holmes *et al.*, 1995; Wansard *et al.*, 1998; Keatings *et al*., 2007). However, by doing so, it is possible that the seasonal signal in temperature and water composition is masked. *Cyprideis torosa* has strong seasonal calcification patterns (Heip, 1976), yet we currently have little understanding of whether seasonal temperatures are reflected in Mg/Ca_{shell} values, thus there are large uncertainties in reconstructed temperatures.

Understanding calcification, the seasonal water conditions and constraining the use of Mg/Ca$_{water}$ values for temperature calculations will have implications for the interpretation of palaeotemperature records. Here, we aim to improve understanding of the timing of *C. torosa* calcification using Mg/Ca-inferred temperatures coupled with hourly water temperatures and seasonal water chemistry. In addition to contributing to the understanding of the *C. torosa* life cycle, we demonstrate how the improvement in estimation of the timing of *C. torosa* calcification could enable the identification of seasonal signals in Mg/Ca-inferred temperatures, and thus decrease uncertainties in the interpretation of palaeotemperatures derived from fossil shells.

2. Materials and methods

2.1 Field collections of ostracods and waters

Water samples and living specimens of *C. torosa* were collected in August and December 2016 and April, June and September 2017 from a shallow coastal pond in Pegwell Bay Nature Reserve, Kent, SE UK (Fig. 1) where the species is particularly abundant. The ~ 0.32 by 0.32 km, shallow (< 1 m) saltmarsh pond has a muddy substrate and low macrophyte abundance. The pond is situated above Mean High Water level, but is still within the intertidal zone. Ostracods were collected in a 250 μm zooplankton net from the top 1 cm of sediment at location ‘X’ (Fig. 1). Sediment was then washed through a 250 μm sieve (the adult shells being ~1000 μm) to remove any remaining fine sediment and dried in an oven at 50 °C. Adult carapaces with soft tissue and appendages (indicating that the individuals were alive at the time of collection) were selected for geochemical analyses. The right valves were reserved for trace metal analysis and the left for stable isotope analyses (results will be described elsewhere). To constrain the variation in water chemistry over the expected period of calcification, water samples were collected as one-off spot samples in April and September and hourly from low to high tide in June 2017 in sterile 50 mL centrifuge tubes and filtered prior to analysis. In situ measurements of conductivity and temperature were taken using a YSI 30 handheld probe calibrated and recorded at 25 °C. For the April and June 2017 sampling, in situ alkalinity as $CaCO_3$ equivalent was determined using a Hach Digital Titrator, 1.6N Sulphuric acid (H_2SO_4) cartridge and Phenolphthalein and Bromcresol Green-Methyl Red indicators.

2.2 Population dynamics

For each sample collection, the length-height relationship of a sub-sample of 100 individuals was established under a low-power stereo microscope using a calibrated eyepiece reticule.

This allowed the population age structure at intervals across the year to be established. The large mesh size used may restrict the collection of all instars, but since the primary purpose of sampling was to determine peak abundances of adult populations, the collection of small juveniles was not considered important. Additional information, including adult sex and the presence of adhering material, was also noted.

2.3 Annual temperature datasets

A Tinytag Aquatic 2 temperature logger with temperature range –40 to +70°C was deployed at a depth of ~10 cm to record subsurface water temperature from August 2016 to September 2017. Complementary hourly air temperature data were downloaded from the Met Office weather station dataset on the Centre for Environmental Data Analysis database. Temperature data were extracted for Kent International Airport, Manston weather station (WMO ID 3797) located 2 km inland from the study pond (Fig. 1).

2.3 Geochemical analyses

2.3.1 Ostracods

Prior to geochemical analyses, ostracod valves were cleaned according to Roberts *et al*. (2018). Soft tissue and any adhering dried sediment were removed from valves using needles, a fine paint brush wetted with methanol and ultra-pure 18.2Ω Milli Q deionised water under a binocular microscope. Valves were then sonicated in methanol and 18.2Ω Milli Q deionised water and dried at 50 °C prior to analysis.

For trace metal analysis, single ostracod valves were dissolved in 500 µL of 1.07 M HNO_3 (trace metal grade) in an acid–leached (48h in 80 °C 10 % HNO_3) 600 µL micro-centrifuge tube. The Mg/Ca, Sr/Ca, Fe/Ca, Mn/Ca and Al/Ca ratios of valves were determined using the intensity ratio calibration of de Villiers *et al*. (2002) using a Varian 720 ES ICP-OES at University College London (UCL). The results were corrected for blank intensity. Analysis of the carbonate standard BCS-CRM 393 gave an Mg/Ca of 3.9 ±0.01 mmol/mol and Sr/Ca of 0.19 ±0.004 mmol/mol for 12 determinations across three runs. The values are in good agreement with the mean values of 3.9 mmol/mol and 0.19 mmol/mol quoted in Greaves *et al.* (2008). The Fe/Ca, Mn/Ca and Al/Ca ratios were monitored as contamination indicators to check for elevated Mg/Ca ratios due to high–Mg marine clays and Fe-Mn oxyhydroxide coatings, which form on biogenic calcite in marine-type waters. The inefficient removal of these coatings can cause bias in Mg/Ca ratios and thus Mg/Ca-inferred temperatures.

2.3.2 Waters

Major and minor cations (Na^+, K^+, Ca^{2+}, Mg^{2+} and Sr^{2+}) were analysed using a Varian 720 ES ICP-OES at UCL. Standards were prepared volumetrically using single element standard solutions of known concentrations. Analysis of the standard river water SLRS-4 gave concentrations of 24.6 ±0.3 mg L^{-1} for Na, 0.07 ±0.005 for K, 6.1 ±0.2 mg L^{-1} for Ca, 1.6 ±0.01 for Mg and 0.029 ±0.003 for Sr, in good agreement with the published values of 24.0 for Na, 0.07 mg L^{1} for K, 6.2 mg L^{-1} for Ca, 1.6 mg L^{-1} for Mg and 0.026 mg L^{-1} for Sr (Yeghicheyan *et al.*, 2001). Major anions (Cl^- and SO_4^{2-}) were analysed using a Dionex Ion Chromatograph at UCL with KOH eluent at concentration of 12.5 mM, SRS current of 20 mA and flow rate of 0.47 mL/min. Analysis of the multianion standard solution PRIMUS gave values of 9.9 ±2.7 mg L^{-1} for Cl^- and 10.42 ±0.16 mg L^{-1} for SO_4^{2-}, in good agreement with the certified values of 10 mg L^{-1} for all anions.

3. Results

3.2 Water chemistry

The electrical conductivity of the pond was highest in June reaching an average of 75.2 mS cm^{-1} with the lowest values of 40.2 mS cm^{-1} recorded in December (Table 1; Fig. 2). Conductivity shows a strong seasonal pattern with similar values recorded between September and April (Fig. 2).

Between low and high tide, there was little variation in water Sr^{2+} and Sr/Ca_{water} with variations of ±1.17 mg L^{-1} and ±0.17 mmol/mol respectively (Table 2). The lowest Sr/Ca_{water} of 9.87 mmol/mol was recorded at 09:00. The largest variation across the day was in Na^+ concentrations (±14784 mg L^{-1}) with peak concentrations at 06:00 of 91132 mg L^{-1}; lowest values were observed in the centre of the pond at locations 3 and 6 (Table 2). Otherwise, there was low variability in all cation concentrations. Cl^- concentrations were consistent with diluted seawater and were fairly constant throughout the day. Alkalinity varied little throughout the day (± 9.8 mg L^{-1}) with an average of 259.6 mg L^{-1} $CaCO_3$ total alkalinity (Table 2). The alkalinity in April was lower, but still high at 210 mg L^{-1}. Whilst there is little diurnal variation in trace-element/Ca_{water}, there is an indication of seasonal control on Sr/Ca_{water} with values in

April lower than in June at 7.45 mmol/mol (compared with an average of 10.25 mmol/mol). Mg/Ca_{water} values were similar during April and June at 4427.89 mmol/mol (compared with an average of 4144.77). Spatially, there was little variation in water cation and trace-element/Ca concentrations (Fig. 3). There was slightly greater variation in anion concentrations with much lower Cl^- 11292.15 mg L^{-1} recorded at Site 3 on the eastern edge of the pond. The northern end of the pond (Sites 5 and 6) had lower conductivity (71.9 mS cm^{-1} compared to 77.8 mS cm^{-1} at the southern end of the pond at location 1).

3.3 Ostracod population dynamics

Females accounted for the highest proportion of the adult population in all sampled months except August, when the percentage of females dropped to 20 % from 56 % in February and September (Table 3). In August, the population was dominated by large A-1 individuals or small adults, which may account for the lower number of female adults during this collection (Fig. 4). Large numbers of A-1 individuals were also present in June. The large number of A-1 juveniles during the summer months could be individuals preparing to moult to adulthood in the autumn if the population dynamics of *C. torosa* at Pegwell Bay adheres to the model of Heip (1976). The highest number of juveniles across instars was recorded in August (39) and December (32) (Fig. 4). Conversely, a lower number of juveniles was recorded in April and September with a lack of juveniles below instar A-2. The smaller number of juveniles in April and September is likely due to an increase in adults from spring and autumn calcification (Heip, 1976). A lack of juvenile instars below A-3 (< 250 μm) was however likely due to them passing through the net and sieve mesh size used during sample collection and processing. In general, the percentage of individuals noted to have adhering material is minimal in all samples with a maximum of 13 % in December and a minimum of 7 % in April and February respectively (Table 3).

3.1 Temperature

There was a large variation in water temperatures with a range from –1.6 to 34.2 ˚C, displaying a seasonal pattern over the sampling period with minimum temperatures recorded in January and maximum temperatures in June (Table 5). Diurnal variation in average water temperature is low at an average of ± 1 ˚C difference between day and night temperatures. Despite the inland and higher altitude location of the weather station (~49 m a.s.l), and therefore expected cooler temperatures, the water temperature broadly matched the air temperature in all months. Precipitation was highest in November at 103.4 mm and lowest in December at 9.2 mm, but with no obvious seasonal pattern.

3.4 Ostracod Shell chemistry

The shell chemistry displays a seasonal trend (Fig. 5). Valves collected on each sampling day had high Sr/Ca$_{shell}$ (up to 4.23 mmol/mol in June), typical of a marine-influenced system (Table 4). The Sr/Ca$_{shell}$ is similar throughout the year (± 2.19 mmol/mol), but the lowest values are recorded in December and February, suggesting a slight seasonal control (Fig. 5). Variability in Mg/Ca$_{shell}$ is more marked than that in Sr/Ca$_{shell}$; Mg/Ca$_{shell}$ is strongly seasonal with gradually decreasing values recorded April to September (Fig. 5) and the lowest average values of 7.88 and 8.24 mmol/mol recorded in December and February respectively (Table 4). There is no systematic relationship between Mg/Ca$_{shell}$ and Sr/Ca$_{shell}$. Using the valves and water collected in June 2017, M/Ca$_{ostracod}$ and M/Ca$_{water}$ show a positive, but statistically insignificant linear relationships ($R^2 = 0.12$, $p = 0.4$ for Sr/Ca and $R^2 = 0.30$, $p = 0.2$ for Mg/Ca) (Fig. 6).

4. Discussion

The Mg/Ca$_{water}$ over the calcification period (spring to autumn) varied between 3.9 and 4.4 mol/mol (averaging at 4.2 mol/mol). With the Mg/Ca$_{water}$ variation over the calcification period constrained, it is possible to compare the monitored water temperatures with Mg/Ca-inferred temperatures and predict the possibility of calcification over the monitoring period. We can calculate the Mg/Ca-inferred temperature using the temperature calibration of De Deckker *et al*. (1999), since the waters are of marine-like ionic composition (Table 2):

$$T\ (^{\circ}\mathrm{C}) = 2.69 + (5230 * [\mathrm{Mg/Ca}]_{ostracod}/[\mathrm{Mg/Ca}]_{water}) \tag{1}$$

4.1 Tracking the calcification of *C. torosa*

Temperatures for each collected individual have been calculated using Mg/Ca$_{water}$ values of 3.9, 4.2, 4.4 and 5.1 mol/mol, and equation (1). For the August 2016 collections, there is a bimodal distribution of inferred temperature, which provides some support for the model of Heip (1976) (Fig. 7). Under all Mg/Ca$_{water}$ scenarios, there appears to be two generations of *C. torosa*, calcifying in the spring (valves with Mg/Ca inferred temperatures of 20.6 to 34.5 ˚C at Mg/Ca$_{water}$ of 4.2 mol/mol for 04-Aug-2016; Table 4), and autumn (inferred temperatures of 7.0 to 9.2 ˚C). In April and early May 2017, water temperatures reach 21.6 and 31.6 ˚C, suggesting that, with similar temperatures in the spring of 2016 (16.7 to 22.8 compared with 17.9 to 22.8

in 2017; Met Office, 2012), adult valves collected in August 2016 could be recording spring temperatures.

There is also a bimodal distribution of inferred temperatures in April 2017 using the Mg/Ca$_{water}$ scenario of 3.9 mol/mol and in June 2017 under all Mg/Ca$_{water}$ scenarios. However, the upper-limits of calculated temperatures are untenable with values between 36.8 and 47.4 ˚C at Mg/Ca$_{water}$ of 3.9 to 4.4 mol/mol (Table 4), suggesting that these individuals calcified under higher Mg/Ca$_{water}$ concentrations, which were not recorded over the monitored period. It is therefore likely that these individuals calcified in the previous year when Mg/Ca$_{water}$ values could have reached 5.1 mol/mol (average seawater concentrations due to a direct marine connection at high tides – tidal connection is unpredictable, but has been recorded by camera traps), and thus lowering inferred temperatures to 32.1 to 36.9 ˚C (within the expected summer to spring temperatures for the region). Individuals from the previous calcification cycle becoming a larger proportion of the sample size may relate to A-1 valves calcifying after the collection date, and therefore there is a higher proportion of the previous year's adult population. This is confirmed by the Mg/Ca-inferred temperature of the majority of individuals collected in June 2017 closely mirroring monitored temperatures (Fig. 7). Furthermore, by June there is a lack of individuals with an Mg/Ca-inferred temperature below 16 ˚C (with Mg/Ca$_{water}$ of 4.2 mol/mol), suggesting that adults that calcified in the previous autumn and spring have died and are no longer in the living assemblage (i.e. there is a turnover in population to individuals that have calcified within the current year).

There is further evidence of the loss of adults from the population in the winter months. Water temperatures of 7 ˚C were measured in November 2016, May 2017, and April 2017 with Mg/Ca-inferred temperatures of 7 ˚C from individuals collected in August 2016, December 2016, February 2017 and September 2017. The lack of Mg/Ca-inferred temperatures below 7 ˚C from the December 2016 and February 2017 datasets could be related to the fact that 1) adult individuals calcifying in the spring of the previous year were no longer present in the population or 2) adults calcifying in September 2016 were a larger percentage of the population and thus a larger percentage of the sample. However, there is no consensus over how long *C. torosa* individuals live as adults. The culture experiments of P. Frenzel suggest an adult life-span of 6-12 months; the adults from cultures were harvested at 6 months after moulting with the majority of the population alive (P. Frenzel, personal communication, 2018). Furthermore, it is possible that female life-span is longer than that of males due to the brood care of eggs and small juveniles. It is unlikely that it is necessary for males to overwinter in order to mate with newly-moulted adult females in spring (confirmed by the larger percentage of adult females from December 2016 onwards once overwintering commences; Table 4).

Adults collected in February 2017 may therefore have calcified in the previous autumn, or earlier. Based on an adult life span of 6-12 months, it is possible that the lack of valves yielding an Mg/Ca-inferred temperature below 7 ˚C, when spring temperatures were on average lower, is based on a combination of the loss of the previous adult generation and the increase in percentage of adults calcifying at a later date. Population data and our interpretation of Mg/Ca-inferred temperatures presented here suggest that individuals may be present in the population for 12 to 18 months, but that there is a replacement of adults from June to September; the percentage of juvenile individuals with adhering material drops to 0 % from June (Table 3) and the number of A-1 individuals in the sample reduces between June and September (Fig. 4), suggesting moulting to adulthood commences in May to June.

It is reasonable to assume that there was no life-cycle development (release of broods or moulting) from December 2016 to April 2017, due to the overwintering of valves (Heip, 1976; Horne, 1983). However, based on the assumption that valves calcify in early to mid-autumn, we would expect the samples collected during December to April to have more individuals that reflect the temperature of autumn calcification (e.g. 2.1 to 17.1 ˚C for October to November) than spring calcification (e.g. 4.7 to 31.0 ˚C for March to May). Using the average Mg/Ca_{water} value of 4.2 mol/mol, the majority of valves collected in December 2016 and February 2017 appear to reflect the mean temperatures from October 2016 with average Mg/Ca-inferred ostracod values of 12.7 ˚C and an average measured water temperature of 12.8 ˚C (Fig. 7). Furthermore, for June an increase in valves recording temperatures between 10 and 20 ˚C may reflect the increase in water temperatures from May when A-1 valves would have been calcifying.

Despite the fact that valve Mg/Ca appears to reflect the mean temperatures from the preceding months, no significant relationship was established between Mg/Ca-inferred temperatures and water temperatures averaged for up to six months prior to collection (Table 6). This may be due to adults having calcified more than six months prior to collection or that the samples are composed of a mixed population containing individuals that calcified under different conditions. The exact timing of calcification is an important variable in determining a significant relationship between Mg/Ca-inferred temperatures and water temperatures. Although relatively little difference is observed between day and night temperatures in the pond, it is unknown if ostracods calcify at night, during the day or both. It has been shown for foraminifera that calcification occurs in light-limited environments and that diurnal patterns in Mg/Ca observed as bands of high and low Mg/Ca through the test have important implications for palaeothermometry (Fehrenbacher *et al*., 2017). Notwithstanding differences in and problems with vital effects (e.g. Weiner and Dove, 2003), the mechanisms of biomineralisation for ostracods and foraminifera are broadly similar based on the assumptions of mineral precipitation (e.g. Mucci and Morse, 1983) and, therefore, it is possible that ostracod Mg/Ca is, at times, recording night temperatures. More information on the controls on biomineralisation would, however, be needed to fully understand these patterns.

Despite the Mg/Ca$_{water}$ scenario used in equation (1), no valves have an Mg/Ca-inferred temperature below 7 ˚C during the period that temperatures were monitored, perhaps suggesting a minimum temperature control on calcification. Temperature controls on calcification have been suggested by several authors (Heip, 1976; Wansard *et al*., 2017). Originally the temperature control on calcification was proposed to be purely biological; the initial findings of Heip (1976) suggested that the first three instar stages tolerate lower temperatures to moult since they develop in brood care in the female valves, but a minimum ambient water temperature of 7.3 ˚C is required for individuals to be released from brood care, at least 16.3 ˚C for instars 4 and 5 to moult, and a minimum temperature of 9.3 ˚C to reach adulthood. The findings presented here suggest that there may be a combined biological and environmental control on calcification; the lack of Mg-inferred temperatures below 7 ˚C is likely due to a biologically linked minimum temperature control on calcification of A-1 to adult moult stage, which coincides with the environmental conditions at the time of overwintering of populations.

4.2 Implications for palaeotemperature reconstruction

The trace-element composition of *C. torosa* has been used extensively for palaeoenvironmental reconstruction for sites with marine-type waters (e.g. Anadón *et al.* 1987; Gasse *et al.* 1987; De Deckker *et al.* 1988a, b; Gibert *et al.* 1990; De Deckker & Williams 1993; Wansard 1996; Ingram *et al.* 1998; Holmes *et al.* 2010; Marco-Barba *et al.* 2013; Grossi *et al.* 2015). However, quantifying trace-element partitioning for *C. torosa* is problematic due its eurytopic nature and, therefore, it is often impossible to determine the composition of host water and temperature at the time of calcification.

With detailed temperature monitoring, this study at the coastal pond in Pegwell Bay extends the understanding of the Mg/Ca signal that may be represented in a fossil *C. torosa* record from marine-type waters. A minimum Mg-inferred temperature of 7 ˚C that coincided with the overwintering of valves suggests that in some environments winter temperatures (i.e. minimum temperature values) will not be recorded. Reconstructions will therefore have a strong bias towards warmer temperatures. The use of Mg/Ca-inferred temperatures to reconstruct minimum temperatures should therefore be avoided. Furthermore, based on three years of 5-day averaged temperatures Heip (1976) suggests a minimum temperature to moult to adulthood of 9.3 ˚C. In environments where spring and autumn temperatures are not substantially lower than 9 ˚C, any Mg/Ca-reconstructed temperature significantly below this may be an artefact of the calibration or a result of a lowered Mg/Ca_{water} value rather than a true temperature signal. Maximum temperatures (taking 34.2 ˚C in June 2017 as an analogue for expected maximum temperatures) are, however, recorded by individuals (e.g. those collected in April with an Mg/Ca temperature of 38.4˚C), suggesting that, there is no upper temperature limit on calcification in the pond at Pegwell Bay. However, in sites with substantial evaporation, the increase in Mg/Ca_{water} may mask the temperature dependence of calcification at higher temperatures. In marginal-marine waters, however, where multiple individual valves from the same stratigraphic interval are analysed for a palaeotemperature dataset, maximum temperatures are likely to be captured. With bulk multiple shell analyses (i.e. many shells combined together and analysed as one), the presence of multiple generations in a fossil assemblage is likely to limit the accurate reconstruction of minimum or maximum temperatures. Instead the analysis of a large number of single valves in likely to be more informative. The results presented here show that under conditions of constant Mg/Ca_{water}, the Mg/Ca of *Cyprideis* can be used to reconstruct water temperatures exceeding ~9.3 ˚C, the minimum temperature at which the species moults to adulthood (Heip, 1976). Good understanding of modern site systematics and hydrochemistry are, however, necessary to confirm this relationship for palaeo-datasets.

Sr/Ca_{shell} values show a small increase in June 2017 and September 2017 (Fig. 5), which could be reflecting high EC values recorded in June 2017, and therefore spring calcification (Fig. 8). However, there is little change in Sr/Ca_{shell} throughout the sampling period, whereas there is a large spread of Mg/Ca-inferred temperatures for each dataset; Sr/Ca_{shell} values vary by 2.19 mmol/mol compared to 29.84 mmol/mol for Mg/Ca_{shell}, implying that calcification temperature has large variations and that the $Sr/Ca_{ostracod}$ uptake is not thermodependent (c.f. De Deckker *et al*., 1999). Whilst there is no significant relationship between $Sr/Ca_{ostracod}$ and Sr/Ca_{water} in June 2017 ($R^2 = 0.12$, $p = 0.4$; Fig. 6), it is likely that Sr/Ca_{water} varies little despite large changes in EC (Table 2) or that there is a Sr/Ca_{water} control on calcification that is more limited than the temperature control. Whilst we have shown that the Mg/Ca_{shell} is controlled primarily by temperature in this environment, in a highly variable environment with higher diurnal variation than the pond at Pegwell Bay, such as estuaries, where *C. torosa* is often abundant, there may be small windows of time that water conditions are optimal for calcification and there is therefore potential for this to mask the temperature dependence of Mg/Ca_{shell}.

5. Conclusions

Annual monitoring can be used to broadly track the calcification of *C. torosa.* However, given the longevity of *C. torosa* adults and hydrological variability, it may be difficult to confirm that calcification took place during the period when temperatures were measured. Currently, studies are limited by a lack of knowledge on *C. torosa* adult longevity. Based on the Mg/Ca-inferred temperatures from adult valves, the results presented here support the suggestion that *C. torosa* calcification occurs in spring and autumn and support the model of two overlapping populations proposed by Heip (1976). Due to the range of temperatures recorded, we suggest that calcification can occur as late as November and occurs throughout the summer months, and there is potential for adult longevity of 12-18 months. Due to autumnal temperatures in the UK, we propose a minimum temperature control on calcification to adulthood of 7, lower than the 9.3 ˚C proposed by Heip (1976).

The exact timing of calcification for these samples remains unclear and conditions from the previous year may be recorded. Börner *et al*. (2013) raised concerns that individuals calcifying under different seasonal conditions may result in intra-sample noise, which may interfere with long-term patterns in palaeolimnological studies. Due to the often low resolution (at least five to ten years) and relative rarity of annually-laminated sediments (which often do not contain ostracods), it is likely that these seasonal signals would be lost in a fossil assemblage unless a particularly strong seasonal pattern were present; for the pond at Pegwell Bay, spring and autumn calcification is likely to produce similar Mg/Ca-inferred temperatures. When paired with modern hydrological systematics of the site, this intra-annual noise should not be an issue for the majority of paleolimnological studies especially where multiple valves are analysed to give an indication of the variability within the sample. To determine the number of individual valves required to capture the expected variability, a simple sample size calculator can be used that incorporates the desired error at a given level of significance and estimated variability using the t distribution (see Holmes, 2008 for further details). However, where palaeotemperatures are required to inform minimum temperatures, it is likely that the lowest Mg/Ca-inferred temperatures are reflecting autumn, and therefore unlikely absolute minimum temperatures. Where multiple individual valves are analysed there should be good indication of maximum and mean inferred temperatures, reflecting spring to autumn conditions.

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Tables

Table 1. Electrical conductivity, salinity, and average water temperature for each of the sampling days. Temperature is the average recorded over a 24-hour period, except for 4-Aug-2016 which is averaged from data logger deployment at 14:40.

Table 2. Water chemistry variables recorded on 18-Apr-2017, from high to low tide on 27-June-2017, and 28-Sep-2017 . Numbers appearing after the 12:00 sampling times (1,2 etc.) relate to the locations in Figure 4..

Table 3. Percentage male (M), female (F) and juvenile (juv.) individuals for each sampling month. The number of individuals with adhering detritus is also noted and broken down by M, F and juv.

Table 4. Ostracod Mg/Ca and Mg/Ca inferred temperature alongside measured water temperature. Water temperature is the average recorded over a 24-hour period, except for 4-Aug-2016 which is averaged from data logger deployment at 14:40.

Table 5. Minimum, maximum and average monthly air and water temperature, and monthly rainfall for the monitoring period August 2016 to September 2017. Air temperature and precipitation data were downloaded from Met Office (2012)

Table 6. Pearson correlation coefficient for Mg/Ca vs average temperature of months preceding collection

Figures

Figure 1. Location of the coastal pond at Pegwell Bay. The inset map shows the location of samples taken on 27/6/2017. Samples were collected at 'X' for all sample dates.

Figure 2. Electrical conductivity and average water temperature for each sampling day. Note that there is no $\text{Mg/Ca}_{\text{water}}$ or $\text{Sr/Ca}_{\text{water}}$ data for the seasonal sampling.

Figure 3. Variations in trace-element/Ca, Cl^-, electrical conductivity and temperature across the pond at locations 1, 2, 3, 4, 5, 6 between 12:00 and 13:00 on 27/06/2017. Note the logarithmic scale. Ostracod samples were collected at 'X' for all sample dates

Figure 4. Length-height relationship for a subsample of 100 individuals of *Cyprideis torosa* for each collection in a) August, b) December, c) February, d) April, e) June and f) September. Black circles denote juvenile individuals, blue adult males and purple adult females

Figure 5. Ostracod Sr/Ca_{shell} (a) and Mg/Ca_{shell} (b) for samples collected on each sampling day. Data from individual valves are represented by the grey circles and the mean is denoted by the black line

Figure 6. Relationship between a) Mg/Ca_{shell} and Mg/Ca_{water} and b) Sr/Ca_{shell} and Sr/Ca_{water} using the values for water and ostracods collected in June 2017.

Figure 7. Water, air and ostracod Mg/Ca-inferred temperatures calculated under Mg/Ca_{water} scenarios of a) 3.9 mol/mol, b) 4.2 mol/mol, c) 4.4 mol/mol, and d) 5.1 mol/mol. Air and water temperature are displayed by the red and blue lines respectively. The frequency distributions of ostracod Mg-inferred temperature for each collection (the red bars) are placed along the x-axis date of collection. Air temperature was obtained from Met Office (2012)

Figure 8. Average Sr/Ca_{shell}, Mg/Ca_{shell}, electrical conductivity and average water temperature for each sampling day. The grey line relates to the right y-axis in each graph.

Table 1. Electrical conductivity, salinity, and average water temperature for each of the sampling days. Temperature is the average recorded over a 24-hour period, except for 4-Aug-2016 which is averaged from data logger deployment at 14:40.

Date	Electrical conductivity (mS cm^{-1})	Salinity PSU	Average water temperature (°C)
04-Aug-2016	55.2	36.6	20.9
01-Dec-2016	40.2	25.7	3.0
02-Feb-2017	45.1	29.2	8.3
18-Apr-2017	44.6	28.8	10.2
27-Jun-2017	75.2	~53*	17.2
28-Sept-2017	33.3	20.8	18.3

*above scale for accurate conversion

Table 2. Water chemistry variables recorded on 18-Apr-2017, from high to low tide on 27-June-2017, and 28-Sep-2017 . Numbers appearing after the 12:00 sampling times (1,2 etc.) relate to the locations in Figure 4.

Date	**Time / Location**	**Cation concentration (mg L^{-1})**					**Trace-element/Ca (mmol/mol)**		**Anion concentration (mg L^{-1})**	
		Na^+	**K^+**	**Ca^{2+}**	**Sr^{2+}**	**Mg^{2+}**	**Sr/Ca**	**Mg/Ca**	**Cl^-**	**SO_4^{2-}**
18-Apr-2017		11934.60	445.35	496.16	8.08	1332.25	7.45	4427.89	14568.01	5132.92
27-June-2017	**06:00**	91132.00	243.42	804.46	18.05	2060.38	10.26	4223.52	14662.74	2117.87
	07:00	47536.80	139.65	795.55	17.72	2024.14	10.19	4195.70	14584.57	2164.07
	08:00	44429.20	132.66	693.89	15.59	1760.88	10.28	4184.80	13239.73	1942.17
	08:30	65715.80	188.87	661.52	14.59	1646.83	10.09	4105.26	13136.87	1736.55
	09:00	48956.00	143.25	796.15	17.19	2032.71	9.87	4210.29	14293.15	2121.86
	10:00	43324.70	128.07	721.28	16.09	1811.96	10.21	4105.26	13326.92	1967.73
	12:00-1	72766.50	201.52	771.77	17.42	1925.81	10.32	4114.91	14727.81	2059.38
	12:00-2	50562.30	145.69	715.88	15.96	1780.74	10.20	4101.97	12972.51	1840.22
	12:00-3	33012.40	105.05	630.11	14.13	1555.84	10.25	4071.78	11292.15	1803.05
	12:00-4	42708.70	128.89	699.98	15.51	1718.52	10.14	4048.56	13046.82	1806.51
	12:00-5	45115.70	135.69	742.60	16.60	1843.63	10.23	4094.05	13407.76	1928.17
	12:00-6	39816.40	123.77	683.65	15.39	1695.61	10.29	4090.03	12671.54	1871.37
	14:00	53578.00	153.83	779.12	17.68	1988.87	10.38	4209.54	14405.43	2080.02
	15:00	51046.10	146.94	705.34	16.01	1802.53	10.38	4211.21	13870.14	2003.66
	17:00	41804.80	123.11	716.16	16.70	1808.51	10.67	4164.34	14249.60	2349.22
	Average	**51433.69**	**149.36**	**727.83**	**16.31**	**1830.46**	**10.25**	**4144.77**	**13592.52**	**1986.12**
	Std Dev.	**±14783.53**	**±35.80**	**±52.48**	**±1.17**	**±149.86**	**±0.17**	**±58.88**	**±938.76**	**±165.17**
28-Sept-2017		7987.84	330.99	379.55	6.00	896.62	7.23	3895.53		

Table 2. Continued

Date	Time / Location	Electrical conductivity (mS cm^{-1})	Water Temp. (°C)	Alkalinity as $CaCO_3$ equivalence (mg L^{-1})	
				CO_3^{2-}	HCO_3^-
18-Apr-2017		44.6	10.2	0	210
27-June-2017	**06:00**	70.5	15.8	0	266
	07:00	75.0	16.4	0	266
	08:00	75.9	17.6	0	244
	09:00	77.8	19.0	0	256
	10:00	76.9	19.4	0	272
	12:00-1	77.8	22.5	0	270
	12:00-2	76.7	21.6		
	12:00-3	72.3	21.5		
	12:00-4	70.2	23.2		
	12:00-5	71.7	21.9		
	12:00-6	71.9	22.2		
	14:00	77.9	23.3	0	260
	15:00	78.2	24.7	14	254
	17:00	78.8	22.6	0	248
	Average	**75.2**	**20.8**		**259.6**
	Std Dev.	**±3.0**	**±2.7**		**±9.8**
28-Sept-2017		**33.3**	**18.3**		

Table 3. Percentage male (M), female (F) and juvenile (Juv) individuals for each sampling month. The number of individuals with adhering detritus is also noted and broken down by M, F and juv.

	% individuals					
	April	**Aug.**	**Dec.**	**Feb.**	**June**	**Sept.**
F	51	20	40	56	47	56
M	42	41	28	32	24	36
Juv.	7	39	32	12	29	8
Detritus	7	8	13	7	9	11
	F 4	**F** 4	**F** 6	**F** 2	**F** 8	**F** 10
	M 3	**M** 1	**M** 6	**M** 1	**M** 1	**M** 1
	Juv. 0	**Juv.** 3	**Juv.** 1	**Juv.** 4	**Juv.** 0	**Juv.** 0

Table 4. Ostracod Mg/Ca and Mg/Ca inferred temperature alongside measured water temperature. Water temperature is the average recorded over a 24-hour period, except for 4-Aug-2016 which is averaged from data logger deployment at 14:40.

Collected	**Water temp. (°C)**	**$Mg/Ca_{ostracod}$ (mmol/mol)**	**Average $Mg/Ca_{ostracod}$ (mmol/mol)**	**Mg/Ca temp. (°C) Mg/Ca_{water} 3.9 mol/mol**	**Mg/Ca temp. (°C) Mg/Ca_{water} 4.2 mol/mol**	**Mg/Ca temp. (°C) Mg/Ca_{water} 4.4 mol/mol**	**Mg/Ca temp. (°C) Mg/Ca_{water} 5.1 mol/mol**	**Average Mg/Ca temp. (°C) Mg/Ca_{water} 4.2 mol/mol**
04-Aug-2016	20.9	18.57	12.74	27.6	25.8	24.8	21.7	18.55
		4.68		9.0	8.5	8.3	7.5	
		3.58		7.5	7.1	6.9	6.4	
		5.24		9.7	9.2	8.9	8.1	
		17.78		26.5	24.8	23.8	20.9	
		14.39		22.0	20.6	19.8	17.4	
		3.48		7.4	7.0	6.8	6.3	
		25.57		37.0	34.5	33.1	28.9	
		21.34		31.3	29.3	28.1	24.6	
01-Dec-2016	9.2	12.75	7.88	19.8	18.6	17.8	15.8	12.50
		4.93		9.3	8.8	8.5	7.7	
		7.64		12.9	12.2	11.8	10.5	
		4.35		8.5	8.1	7.9	7.2	
		4.42		8.6	8.2	7.9	7.2	
		7.33		12.5	11.8	11.4	10.2	
		4.27		8.4	8.0	7.8	7.1	
		20.05		29.6	27.7	26.5	23.2	
		5.19		9.7	9.2	8.9	8.0	
02-Feb-2017	6.4	4.57	8.24	8.8	8.4	8.1	7.4	12.95
		4.24		8.4	8.0	7.7	7.0	
		5.48		10.0	9.5	9.2	8.3	
		5.92		10.6	10.1	9.7	8.8	
		15.37		23.3	21.8	21.0	18.4	
		10.03		16.1	15.2	14.6	13.0	
		12.46		19.4	18.2	17.5	15.5	
		9.72		15.7	14.8	14.2	12.7	
		5.94		10.7	10.1	9.8	8.8	
		8.66		14.3	13.5	13.0	11.6	

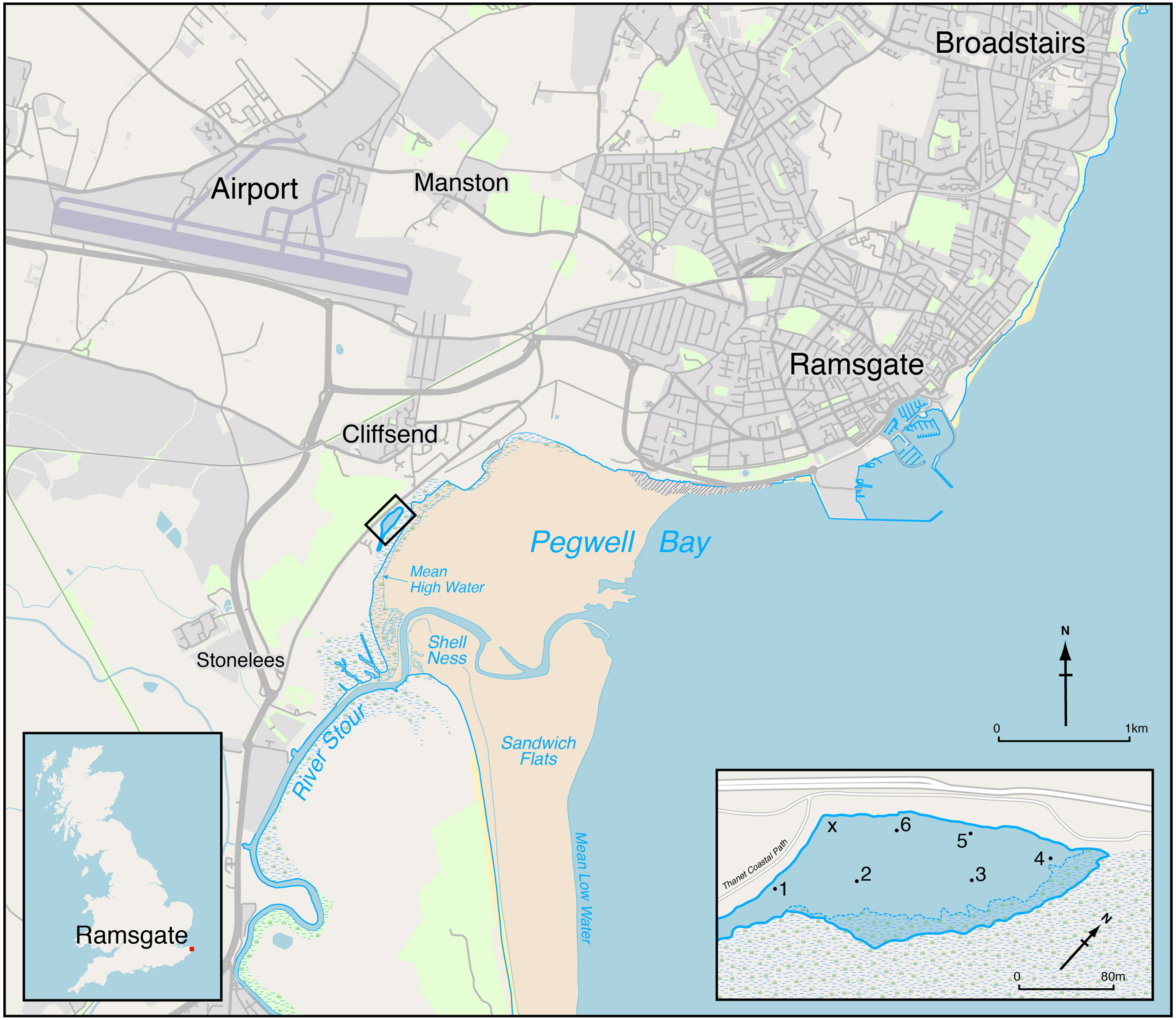
18-April-2017	17.6	17.56	23.26	26.2	24.6	23.6	20.7	31.65
		18.02		26.9	25.1	24.1	21.2	
		28.67		41.1	38.4	36.8	32.1	
		15.87		24.0	22.5	21.6	19.0	
		17.10		25.6	24.0	23.0	20.2	
		24.24		35.2	32.9	31.5	27.6	
		19.61		29.0	27.1	26.0	22.8	
		33.32		47.4	44.2	42.3	36.9	
		31.91		45.5	42.4	40.6	35.4	
		26.26		37.9	35.4	33.9	29.6	
27-Jun-2017	21.4	13.06	16.73	20.2	19.0	18.2	16.1	23.53
		33.12		47.1	43.9	42.1	36.7	
		21.34		31.3	29.3	28.1	24.6	
		11.80		18.5	17.4	16.7	14.8	
		23.50		34.2	32.0	30.6	26.8	
		15.11		22.9	21.5	20.6	18.2	
		19.63		29.0	27.1	26.0	22.8	
		11.01		17.5	16.4	15.8	14.0	
		14.18		21.7	20.3	19.5	17.2	
		10.67		17.0	16.0	15.4	13.6	
		10.68		17.0	16.0	15.4	13.6	
28-Sept-2017	16.7	9.89	9.77	16.0	15.0	14.5	12.8	14.87
		8.07		13.5	12.7	12.3	11.0	
		5.08		9.5	9.0	8.7	7.9	
		8.63		14.3	13.4	13.0	11.5	
		11.22		17.7	16.7	16.0	14.2	
		15.77		23.8	22.3	21.4	18.9	

Table 5. Minimum, maximum and average monthly air and water temperature, and monthly rainfall for the monitoring period August 2016 to September 2017. Air temperature and precipitation data were downloaded from Met Office (2012)

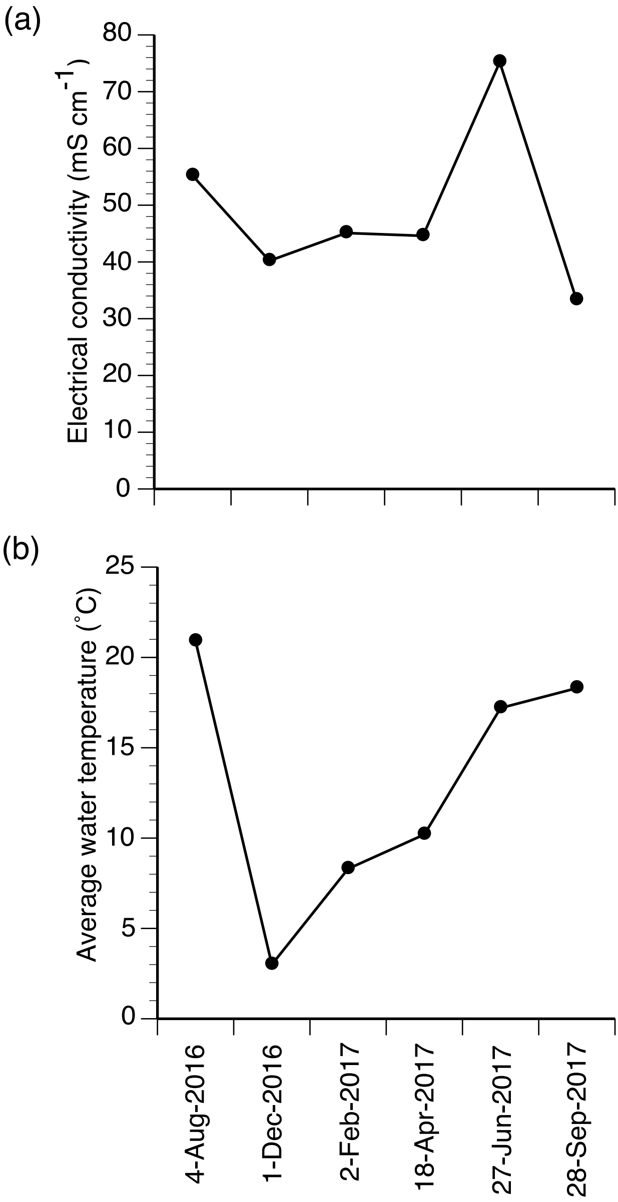
	Air temp. (˚C)			**Water temp. (˚C)**			**Precipitation (mm)**
Month/Year	**Max.**	**Min.**	**Average**	**Max.**	**Min.**	**Average**	
08/2016	23.3	14.4	18.5	27.4	13.0	19.4	18.0
09/2016	22.5	14.4	17.7	26.6	14.7	19.6	76.2
10/2016	15.0	9.4	11.9	17.1	9.8	12.8	34.8
11/2016	10.1	4.4	7.4	12.6	2.1	7.8	103.4
12/2016	9.3	3.8	6.8	10.1	0.4	5.7	9.2
01/2017	6.3	0.7	3.5	7.7	-1.6	3.3	48.2
02/2017	9.2	4.5	6.6	11.3	1.2	6.4	26.4
03/2017	13.0	6.0	9.2	16.4	4.7	10.2	17.2
04/2017	13.7	5.9	9.4	21.6	5.5	13.9	10.8
05/2017	17.9	9.9	13.5	31.0	8.1	17.6	57.8
06/2017	22.4	13.1	17.4	34.2	11.8	21.4	37.8
07/2017	22.8	14.5	18.0	30.9	13.9	20.6	74.2
08/2017	21.4	13.3	16.9	25.9	15.6	20.2	85.6
09/2017	18.3	11.1	14.3	22.9	12.3	16.8	37.0

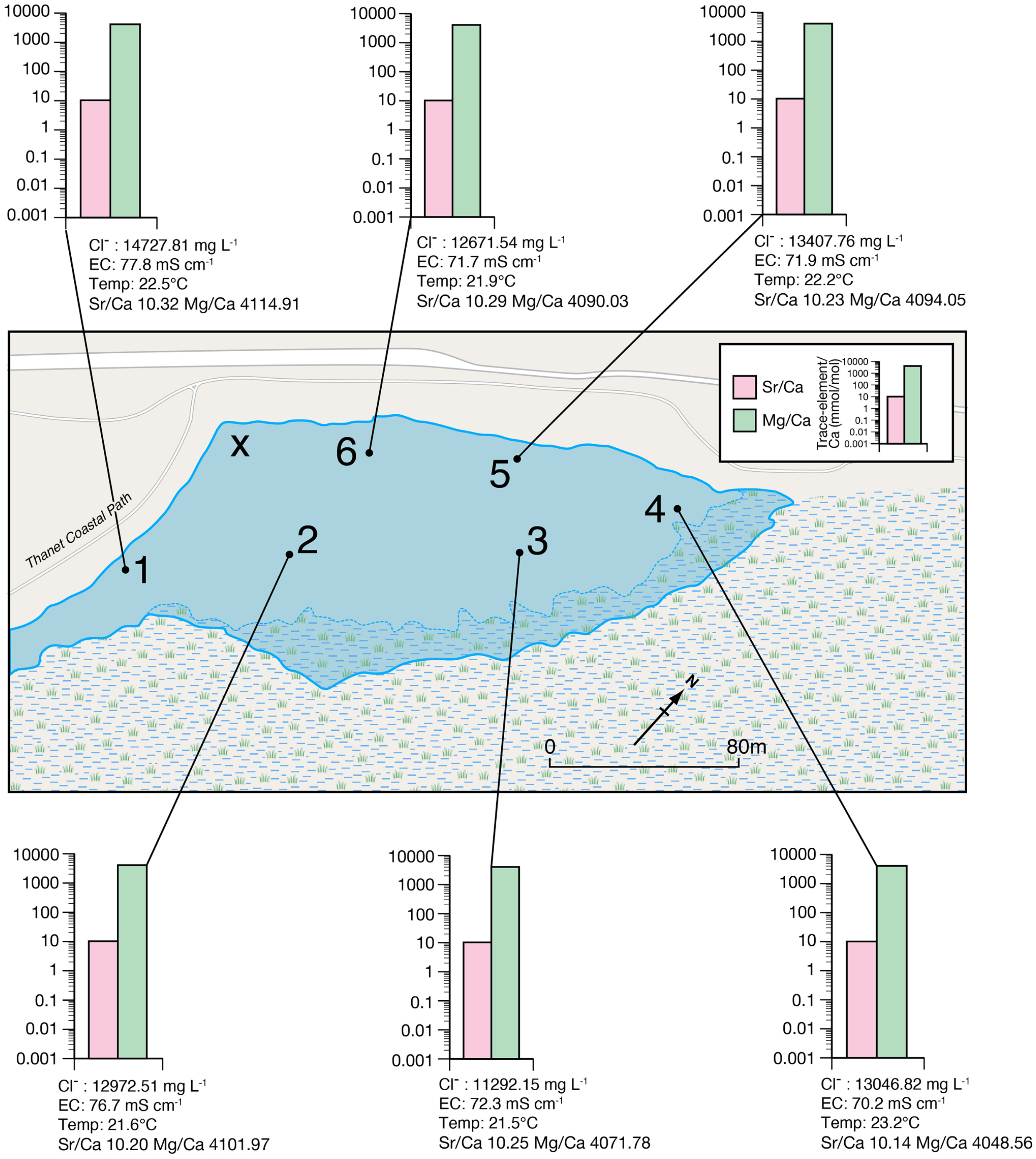
Table 6. T-test for Mg/Ca-inferred temperatures vs average temperature of months preceding collection

	T-test	p-value
Collection month	1.03	>0.3
1 month before	0.56	>0.6
2 months before	0.22	>0.8
3 months before	-0.20	>0.8
6 months before	-1.12	>0.3



Map data © OpenStreetMap contributors, CC-BY-SA, opendatacommons.org


(a)
Electrical conductivity (mS cm^{-1})
0
10
20
30
40
50
60
70
80
(b)
Average water temperature (°C)
0
5
10
15
20
25
4-Aug-2016
1-Dec-2016
2-Feb-2017
18-Apr-2017
27-Jun-2017
28-Sep-2017



Cl⁻ : 14727.81 mg L⁻¹
EC: 77.8 mS cm⁻¹
Temp: 22.5°C
Sr/Ca 10.32 Mg/Ca 4114.91

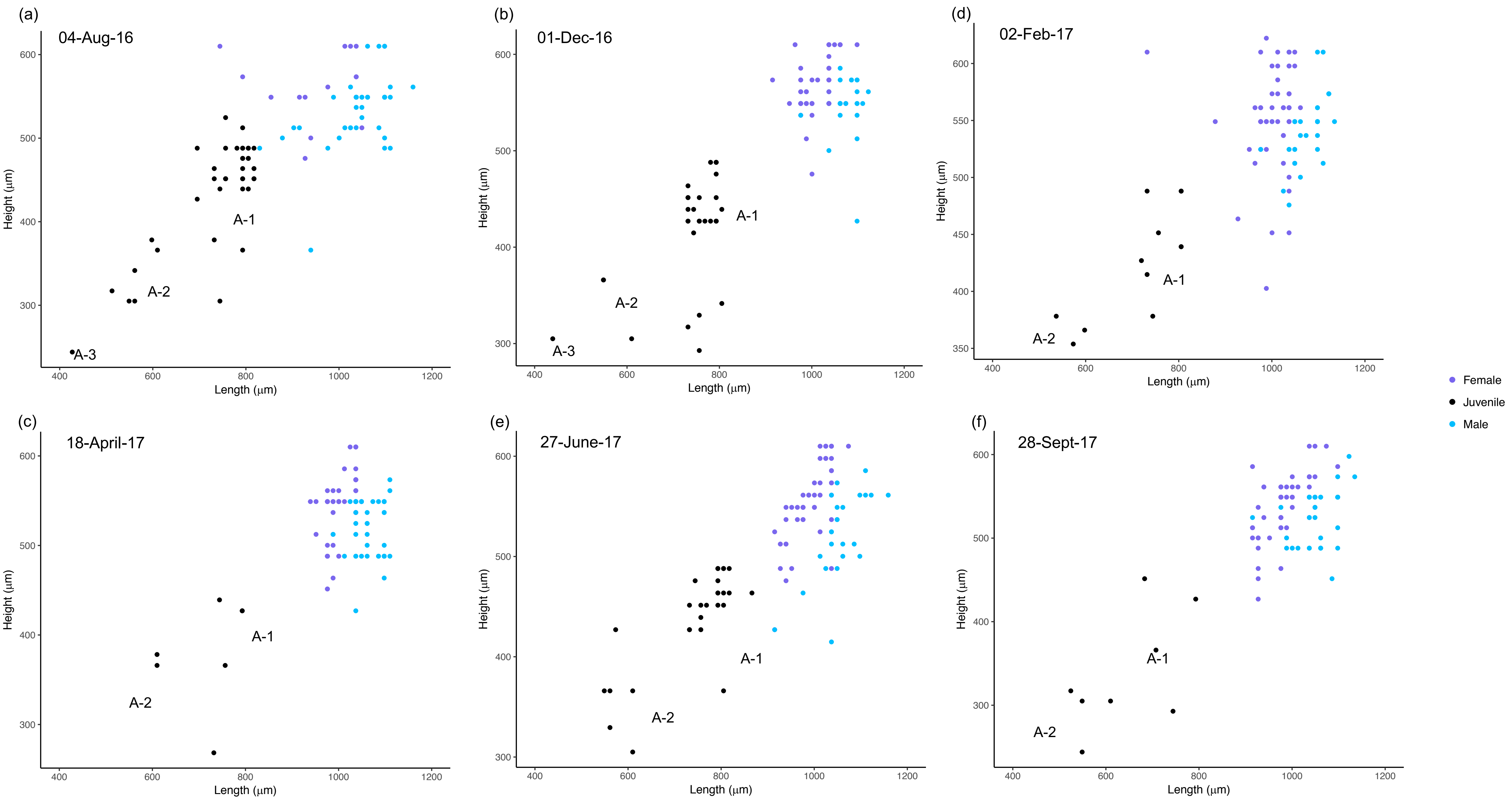
Cl⁻ : 12671.54 mg L⁻¹
EC: 71.7 mS cm⁻¹
Temp: 21.9°C
Sr/Ca 10.29 Mg/Ca 4090.03

Cl⁻ : 13407.76 mg L⁻¹
EC: 71.9 mS cm⁻¹
Temp: 22.2°C
Sr/Ca 10.23 Mg/Ca 4094.05

Cl⁻ : 12972.51 mg L⁻¹
EC: 76.7 mS cm⁻¹
Temp: 21.6°C
Sr/Ca 10.20 Mg/Ca 4101.97

Cl⁻ : 11292.15 mg L⁻¹
EC: 72.3 mS cm⁻¹
Temp: 21.5°C
Sr/Ca 10.25 Mg/Ca 4071.78

Cl⁻ : 13046.82 mg L⁻¹
EC: 70.2 mS cm⁻¹
Temp: 23.2°C
Sr/Ca 10.14 Mg/Ca 4048.56


(a)
04-Aug-16
A-1
A-2
A-3
(b)
01-Dec-16
A-1
A-2
A-3
(d)
02-Feb-17
A-1
A-2
(c)
18-April-17
A-1
A-2
(e)
27-June-17
A-1
A-2
(f)
28-Sept-17
A-1
A-2
Height (μm)
Length (μm)
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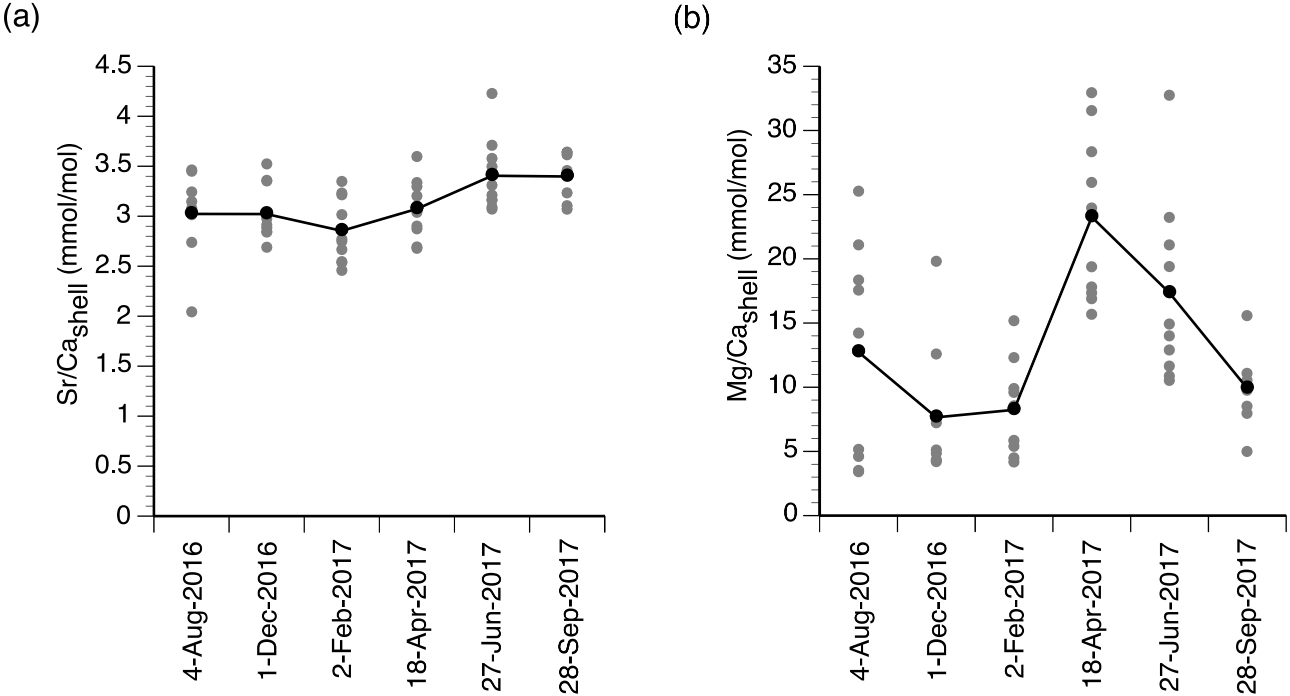
600
800
1000
1200
300
350
400
450
500
550
600
● Female
● Juvenile
● Male


(a)
Sr/Ca$_{shell}$ (mmol/mol)
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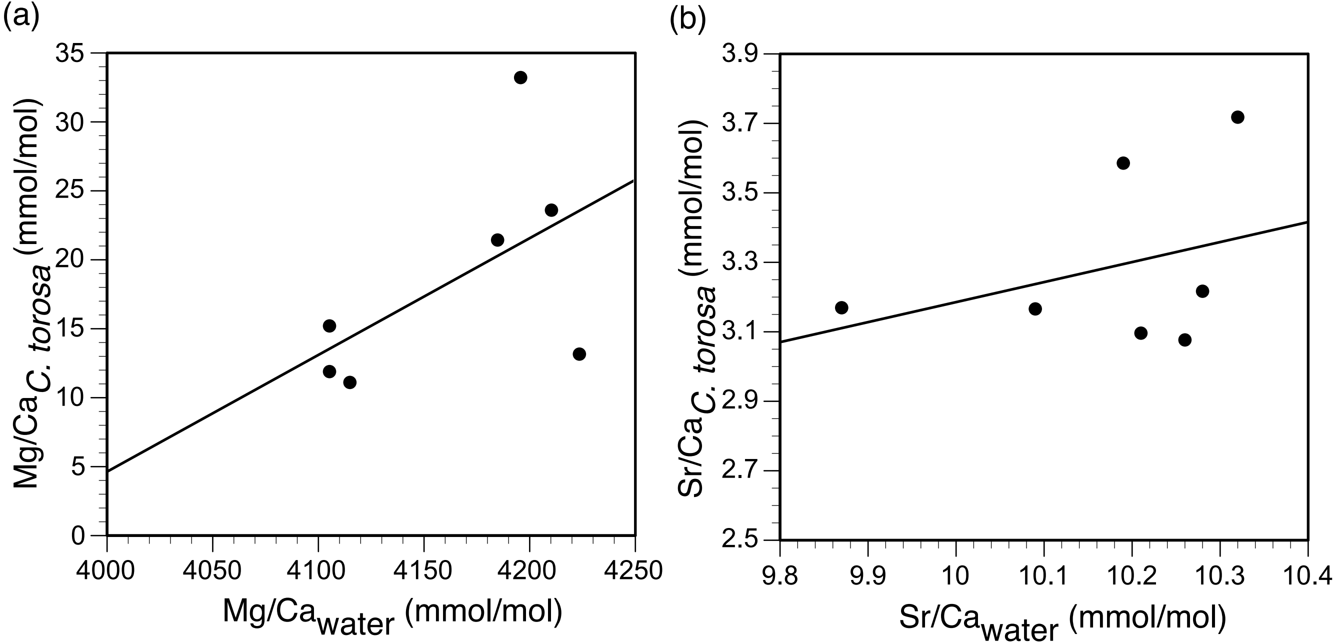
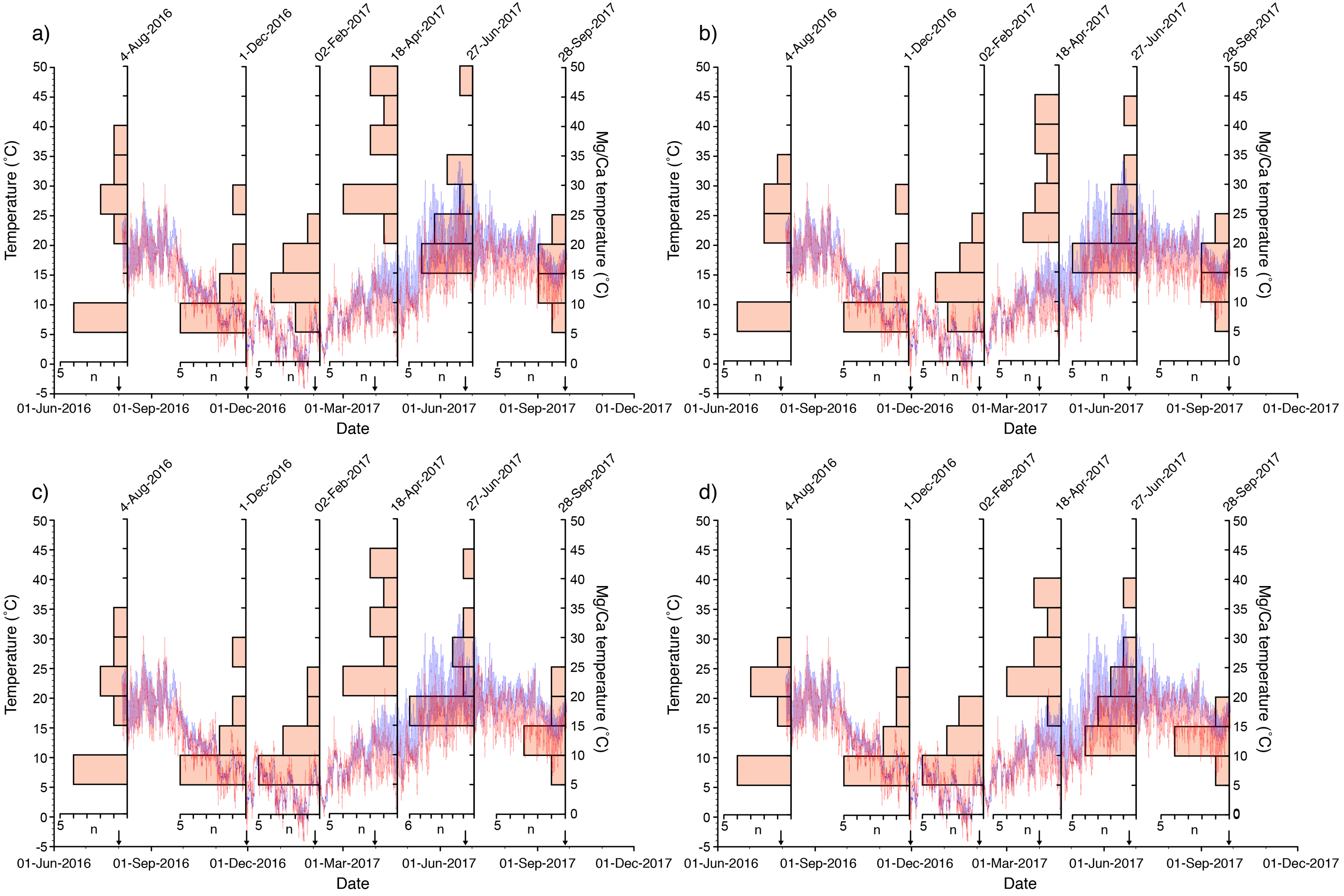
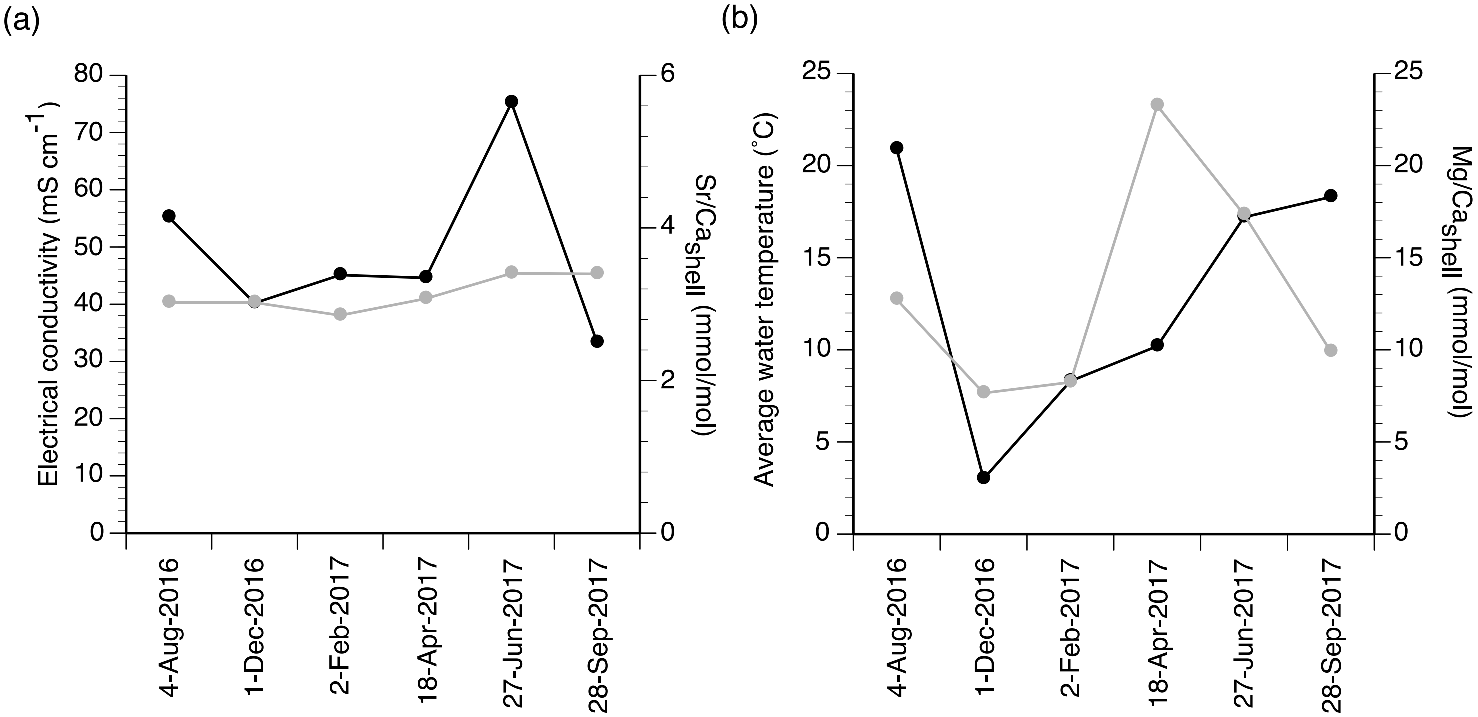
4
3.5
3
2.5
2
1.5
1
0.5
0
4-Aug-2016
1-Dec-2016
2-Feb-2017
18-Apr-2017
27-Jun-2017
28-Sep-2017
(b)
Mg/Ca$_{shell}$ (mmol/mol)
35
30
25
20
15
10
5
0
4-Aug-2016
1-Dec-2016
2-Feb-2017
18-Apr-2017
27-Jun-2017
28-Sep-2017


(a)
35
30
25
20
15
10
5
0
Mg/Ca$_{C.\ torosa}$ (mmol/mol)
4000
4050
4100
4150
4200
4250
Mg/Ca$_{water}$ (mmol/mol)
(b)
3.9
3.7
3.5
3.3
3.1
2.9
2.7
2.5
Sr/Ca$_{C.\ torosa}$ (mmol/mol)
9.8
9.9
10
10.1
10.2
10.3
10.4
Sr/Ca$_{water}$ (mmol/mol)


a)
b)
c)
d)
4-Aug-2016
1-Dec-2016
02-Feb-2017
18-Apr-2017
27-Jun-2017
28-Sep-2017
Temperature (°C)
Mg/Ca temperature (°C)
50
45
40
35
30
25
20
15
10
5
0
-5
n
6
01-Jun-2016
01-Sep-2016
01-Dec-2016
01-Mar-2017
01-Jun-2017
01-Sep-2017
01-Dec-2017
Date


(a)
Electrical conductivity (mS cm^{-1})
80
70
60
50
40
30
20
10
0
Sr/Ca$_{shell}$ (mmol/mol)
6
4
2
0
4-Aug-2016
1-Dec-2016
2-Feb-2017
18-Apr-2017
27-Jun-2017
28-Sep-2017
(b)
Average water temperature (°C)
25
20
15
10
5
0
Mg/Ca$_{shell}$ (mmol/mol)
25
20
15
10
5
0
4-Aug-2016
1-Dec-2016
2-Feb-2017
18-Apr-2017
27-Jun-2017
28-Sep-2017