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# Seasonal Mg/Ca-inferred temperatures of brackish water ostracods

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# ABSTRACT

The common brackish water ostracods Cyprideis torosa and Loxoconcha elliptica frequently occur together in high abundances in marginal marine environments. Seasonality of calcification differs between species and can have important implications for palaeotemperature reconstructions. There are existing palaeotemperature calibrations for both genera. However, the Loxoconcha spp. Mg/Ca temperature calibration has not thus far been applied to L. elliptica. The equation for Loxoconcha spp. does not rely on a known Mg/Cawater value, unlike the calibration for C. torosa, suggesting it may be possible to reconstruct temperatures without an estimation of Mg/Cawater, which is potentially particularly beneficial in environments that have highly dynamic Mg/Cawater. However, the calibration has only been applied in environments with marine-like Mg/Cawater. Demonstrating the applicability of the equation in marginal marine environments (with Mg/Cawater 3-5 mol/mol) and tracking the seasonal calcification of L. elliptica alongside C. torosa, therefore, has the potential to improve uncertainty in seasonal palaeotemperature reconstructions. Here, we compare previous monitoring of C. torosa with L. elliptica from the same collections. We demonstrate that the Mg/Ca temperature calibration for Loxoconcha spp. is appropriate to use with L. elliptica. Mg/Ca-inferred temperatures broadly track spring temperatures and suggest spring calcification. Cyprideis torosa Mg/Ca-inferred temperatures record the range of expected temperatures between spring and autumn. When analysing multiple single valves of L. elliptica and C. torosa simultaneously, the maximum Mg/ Ca<sub>C, torosa</sub> can, therefore, be used to reconstruct maximum summer temperatures, the minimum Mg/Ca<sub>C, torosa</sub> to reconstruct autumn temperatures, and the Mg/Ca<sub>L-elliptica</sub> to reconstruct the range in spring temperatures.

## 1. Introduction

Ostracods, small aquatic crustaceans, are common in most aquatic habitats with species inhabiting a range of ecological and climatic niches. Consequently, ostracod faunal assemblages are a common proxy to reconstruct a range of Quaternary environmental conditions. In marginal marine environments ostracod faunal records have been used to reconstruct temperature, salinity, and water depth. Diversity in brackish water environments is, however, often low with the common brackish water ostracods Cyprideis torosa and Loxoconcha elliptica frequently occurring together in high abundances (e.g. Anadón et al., 2002; Frenzel and Boomer, 2005; Bini et al., 2012; Marco-Barba et al., 2013a, 2013b), dominating the ostracod fauna at the marine end of estuarine systems (Penney, 1987). Alternatively, or in combination to faunal assemblages, the adult and A-1 (the moult stage prior to adulthood; ostracods in the Subclass Podocopa, such as C. torosa and L. elliptica, grow through nine instars; Horne, 2005) valves (used hereafter to differentiate when discussing individual valves, with 'shells' used more generally) are routinely used for geochemical (trace element and stable isotope) analysis, providing, in some instances, quantitative information on water temperature and composition. Despite their widespread use, relatively little is understood about the life cycle and calcification timing of many routinely analysed ostracod species, including those that frequently occur together. Seasonality of shell formation, however, differs between species and can have important implications for palaeoenvironmental, particularly palaeotemperature, reconstructions.

The Mg content of ostracod shells is commonly used as a palaeotemperature proxy. The uptake of Mg into ostracod shells is temperature dependent and in situations where the composition of the water shows minimal variation, temperature is the major control (Chivas et al., 1986; De Deckker and Forester, 1988; Holmes and Chivas, 2002). For Sr, Sr/Ca<sub>water</sub> appears to be the dominant control on Sr/Ca<sub>shell</sub>, and in some circumstances this covaries with salinity (Chivas et al., 1985). De Deckker et al. (1999) have, however, suggested a small temperature dependence on the incorporation of Sr into *Cyprideis autraliensis* shells.

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There are existing palaeotemperature calibrations for ostracods from the genus *Cyprideis* and *Loxoconcha*. A palaeotemperature calibration for *C. australiensis* is given by the equation of De Deckker et al. (1999):

$$T(^{\circ}C) = 2.69 + 5230^{*} \left( \left[ Mg/Ca \right]_{shell} / \left[ Mg/Ca \right]_{water} \right)$$
(1)

The 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). It has been demonstrated that *C. torosa* Mg/Ca-inferred temperatures can be used to reconstruct spring and autumn temperatures, with maximum summer water temperature also recorded (Roberts et al., 2020).

For *Loxoconcha*, a palaeotemperature calibration is given by the equation of Cronin et al. (2003, 2005):

$$T(^{\circ}C) = 0.644^{*} [Mg/Ca]_{ostracod} - 2.4284$$
 (2)

The regression model is derived from a compilation of *Loxoconcha* spp. Mg/Ca values, and has been successfully applied to *L. matagordensis*, *L. impressa* and *Loxoconcha* sp. However, to our knowledge, the temperature calibration of Cronin et al. (2003) has not, thus far, been applied to *L. elliptica*. This is likely due to the extensive use, and therefore comparative value, of *C. torosa* shell chemistry in palae-otemperature reconstructions; where the two species coexist, previous studies have exclusively analysed Mg/Ca<sub>C.torosa</sub> (e.g. Marco-Barba et al., 2013a).

Demonstrating the use of the Cronin et al. (2003) calibration and understanding the seasonal Mg/Ca-inferred temperature from L. elliptica shells, however, has the potential to increase understanding and accuracy of palaeotemperature reconstructions from marginal marine environments. The life cycle of the species is relatively well defined with Horne (1983) suggesting that the first generation of adults calcifies in the spring with one or more generations moulting to adulthood in the summer months. However, little is understood about whether Mg/Cainferred temperatures would record restricted periods (i.e. spring or summer temperatures), which may differ to other species and allow seasonal reconstructions. For C. torosa, ecological monitoring has shown that temperature is an important control on the life cycle of the species (e.g. Mezquita et al., 2000), but there is limited information for other abundant brackish water species. Tracking the seasonal calcification of L. elliptica alongside C. torosa, therefore, has the potential to provide seasonal palaeotemperature reconstructions.

The calibration of De Deckker et al. (1999) for C. australiensis is reliant on an understanding of the Mg/Cawater at the time of shell calcification. Adjusting the Mg/Cawater value used in the equation from 5.1 to 3.9 mol/mol (i.e. reducing marine influence), for example, shifts reconstructed temperature by up to 10.5 °C (Roberts et al., 2020). Conversely, the Cronin et al. (2003) calibration for Loxoconcha spp. does not rely on a known Mg/Cawater value, suggesting it may be possible to reconstruct temperatures without an estimation of Mg/Cawater. The successful application of the equation in marginal marine environments across a range of Mg/Cawater values could be particularly beneficial in environments that are highly dynamic, such as estuaries, where there can be large variations in Mg/Cawater values on diurnal timescales and the calcification environment thus remains unknown. However, the calibration has thus far only been applied in environments with marinelike Mg/Cawater that shows little variation (although Cronin et al., 2003 and 2005 do not provide any water values) and the calibration study did not assess the relationships between trace-element/Cashell and traceelement/Cawater, creating a potentially large uncertainty in the use of the calibration. In this study, we apply the Loxoconcha spp. Mg/Catemperature calibration to L. elliptica, assessing the relationships with trace-element/Cawater, and estimating the seasonal calcification of individuals. We compare these results with previous monitoring and Mg/ Ca-inferred temperatures of C. torosa, the results of which are discussed in full in Roberts et al. (2020) but, where appropriate, key results are highlighted and discussed here.

#### 2. Methods

### 2.1. Field methods

Water samples and living specimens of C. torosa and L. elliptica 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). The pond is situated above Mean High Water level, but is still within the intertidal zone. Ostracods were collected in a 250  $\mu m$ zooplankton net from the top 1 cm of sediment at location 'X' (Fig. 1). Sediment was then washed through a 250 µm sieve to remove any remaining fine sediment and dried in an oven at 50 °C. Adult carapaces of both species with soft tissue and appendages (indicating that the individuals were alive at the time of collection) were selected for geochemical analyses. 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 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<sub>3</sub> equivalent was determined using a Hach Digital Titrator, 1.6 N Sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) cartridge and Phenolphthalein and Bromcresol Green-Methyl Red indicators.

A Tinytag Aquatic 2 temperature logger with temperature range - 40 to +70 °C was deployed at a depth of  $\sim10$  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 at https://www.metoffice.gov.uk/research/climate/maps-and-data/historic-station-data. Temperature data were extracted for Kent International Airport, Manston weather station (WMO ID 3797) located 2 km inland from the study pond (Fig. 1).

# 2.2. Geochemical analyses

#### 2.2.1. Ostracods

Prior to geochemical analyses, soft tissue and any adhering dried sediment were removed from valves using needles, a fine paint brush wetted with methanol and ultra-pure 18.2  $\Omega$  deionised water under a binocular microscope. Valves were then sonicated in methanol and 18.2  $\Omega$  ultra-pure deionised water and dried at 50 °C prior to analysis. Single ostracod valves were dissolved in 500 µL of 1.07 M HNO<sub>3</sub> (trace metal grade) in an acid-leached (48 h in 80 °C 10% HNO3) 600 µL microcentrifuge tube. The Mg/Ca and Sr/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 4.1  $\pm$  0.2 mmol/mol and Sr/ Ca of 0.19  $\pm$  0.006 mmol/mol for 19 determinations across five runs, in good agreement with the mean values of 3.9 mmol/mol and 0.19 mmol/ mol quoted in Greaves et al. (2008). 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. No relationships between contamination indicators and Mg/Ca were observed, suggesting no bias in Mg/Ca ratios and thus Mg/Cainferred temperatures.

# 2.2.2. Waters

The methods for the analysis of waters are described in Roberts et al. (2020). In brief, major and minor cations (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and Sr<sup>2+</sup>) were analysed using a Varian 720 ES ICP-OES and major anions (Cl<sup>-</sup> and SO<sup>2+</sup><sub>4</sub>) were analysed using a Dionex Ion Chromatograph, both at UCL. Analysis of the standard river water SLRS-4 and the multianion standard solution PRIMUS were in good agreement with the published (Yeghicheyan et al., 2001) or certified values, respectively.



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

## 3. Results

## 3.1. Water chemistry

The electrical conductivity of the pond was highest in June reaching an average of 75.2 mS cm<sup>-1</sup> with the lowest values of 40.2 mS cm<sup>-1</sup> recorded in December (Table 1; Fig. 2d). Sr/Ca<sub>water</sub> values are lower in April than in June (7.45 mmol/mol compared with an average of 10.25 mmol/mol). Mg/Ca<sub>water</sub> values were similar during the monitored period at 4427.89 mmol/mol in April and an average value of 4144.77 mmol/mol in June. Further water chemistry results are described in full in Roberts et al. (2020) and presented in Table 1.

# 3.2. Water temperature

Water temperatures ranged from -1.6 to 34.2 °C, with minimum temperatures recorded in January and maximum temperatures in June (Table 1; Fig. 2a). There was an average of  $\pm 1$  °C difference between day and night temperatures.

### 3.3. Ostracod shell chemistry

Variability in Mg/Ca<sub>C.torosa</sub> is strongly seasonal with gradually decreasing values recorded April to September (Fig. 2b) and the lowest average values of 7.88 and 8.24 mmol/mol recorded in December and February respectively (Table 2). The variability in Mg/Ca<sub>L.elliptica</sub> is less marked with lowest average values of 18.7 and 20.6 mmol/mol in August and June (Table 2) and little variation between December and April (Fig. 2c).

For both species, valves had high Sr/Ca<sub>shell</sub> (up to 4.23 mmol/mol in June), typical of a marine-influenced system (Table 2). For *C. torosa*, there is an indication of seasonal control with the lowest values recorded in December and February (Fig. 2c). In general, Sr/Ca<sub>*C.torosa*</sub> is similar throughout the year ( $\pm$  2.19 mmol/mol). Seasonality in Sr/Ca<sub>*L.elliptica*</sub> is more marked with highest values recorded in June (average of 3.49 mmol/mol) and gradually decreasing in September (average of 3.09 mmol/mol) (Fig. 2f).

Using the valves and water collected in June 2017, M/Ca<sub>C.torosa</sub> and M/Ca<sub>water</sub> show a positive, but statistically insignificant linear relationship ( $R^2 = 0.12$ , p = 0.4 for Sr/Ca and  $R^2 = 0.30$ , p = 0.2 for Mg/Ca) (Fig. 3a, b). For *L. elliptica* there is a positive, but statistically insignificant ( $R^2 = 0.1$ , p = 0.5), relationship between Mg/Ca<sub>L.elliptica</sub> and Mg/

#### Table 1

Water composition and chemistry recorded on sampling days and from high to low tide on 27-June-2017. Numbers appearing after the 12:00 sampling times (1,2 etc.) relate to the locations in Fig. 1 with the daily average and standard deviation in bold. Values marked with (\*) are above the scale for accurate conversion.

Date	Time / Location	Cation concentration (mg $L^{-1}$ )					Trace-element/Ca (mmol/mol)		Anion concentration (mg $L^{-1}$ )	
		Na <sup>+</sup>	$\mathbf{K}^+$	Ca <sup>2+</sup>	$\mathrm{Sr}^{2+}$	$Mg^{2+}$	Sr/Ca	Mg/Ca	$Cl^{-}$	$SO_4^{2-}$
18-Apr-2017		11,934.60	445.35	496.16	8.08	1332.25	7.45	4427.89	14,568.01	5132.92
27-June-2017	06:00	91,132.00	243.42	804.46	18.05	2060.38	10.26	4223.52	14,662.74	2117.87
	07:00	47,536.80	139.65	795.55	17.72	2024.14	10.19	4195.70	14,584.57	2164.07
	08:00	44,429.20	132.66	693.89	15.59	1760.88	10.28	4184.80	13,239.73	1942.17
	08:30	65,715.80	188.87	661.52	14.59	1646.83	10.09	4105.26	13,136.87	1736.55
	09:00	48,956.00	143.25	796.15	17.19	2032.71	9.87	4210.29	14,293.15	2121.86
	10:00	43,324.70	128.07	721.28	16.09	1811.96	10.21	4105.26	13,326.92	1967.73
	12:00-1	72,766.50	201.52	771.77	17.42	1925.81	10.32	4114.91	14,727.81	2059.38
	12:00-2	50,562.30	145.69	715.88	15.96	1780.74	10.20	4101.97	12,972.51	1840.22
	12:00-3	33,012.40	105.05	630.11	14.13	1555.84	10.25	4071.78	11,292.15	1803.05
	12:00-4	42,708.70	128.89	699.98	15.51	1718.52	10.14	4048.56	13,046.82	1806.51
	12:00-5	45,115.70	135.69	742.60	16.60	1843.63	10.23	4094.05	13,407.76	1928.17
	12:00-6	39,816.40	123.77	683.65	15.39	1695.61	10.29	4090.03	12,671.54	1871.37
	14:00	53,578.00	153.83	779.12	17.68	1988.87	10.38	4209.54	14,405.43	2080.02
	15:00	51,046.10	146.94	705.34	16.01	1802.53	10.38	4211.21	13,870.14	2003.66
	17:00	41,804.80	123.11	716.16	16.70	1808.51	10.67	4164.34	14,249.60	2349.22
	Average	51,433.69	149.36	727.83	16.31	1830.46	10.25	4144.77	13,592.52	1986.12
	Std Dev.	$\pm 14,783.53$	$\pm 35.80$	$\pm 52.48$	±1.17	±149.86	±0.17	±58.88	±938.76	$\pm 165.17$
28-Sept-2017		7987.84	330.99	379.55	6.00	896.62	7.23	3895.53	—	_

Date	Time / Location	Electrical conductivity (mS cm <sup>-1</sup> )	Salinity PSU	Water Temp. (°C)	Alkalinity as $CaCO_3$ equivalence (mg L <sup>-1</sup> )	
					$CO_3^{2-}$	HCO <sub>3</sub>
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	0	210
27-June-2017	06:00	70.5	48.3*	15.8	0	266
	07:00	75.0	51.9*	16.4	0	266
	08:00	75.9	52.6*	17.6	0	244
	09:00	77.8	54.2*	19.0	0	256
	10:00	76.9	53.4*	19.4	0	272
	12:00-1	77.8	54.2*	22.5	0	270
	12:00-2	76.7	53.3*	21.6		
	12:00-3	72.3	49.8*	21.5		
	12:00-4	70.2	48.1*	23.2		
	12:00-5	71.7	49.3*	21.9		
	12:00-6	71.9	49.4*	22.2		
	14:00	77.9	54.2*	23.3	0	260
	15:00	78.2	54.5*	24.7	14	254
	17:00	78.8	55.0*	22.6	0	248
	Average	75.2	~52.0*	20.8		259.6
	Std Dev.	±3.0	±2.3	±2.7		±9.8
28-Sept-2017		33.3	20.8	18.3		

Ca<sub>water</sub> (Fig. 3c). For Sr/Ca<sub>L.elliptica</sub>, there is a statistically significant positive relationship ( $R^2 = 0.7$ , p = 0.0) with Sr/Ca<sub>water</sub> (Fig. 3d). There is no systematic relationship between Mg/Ca<sub>shell</sub> and Sr/Ca<sub>shell</sub>.

## 4. Discussion

## 4.1. Mg/Caostracod relationship with Mg/Cawater

The importance of the relationship between Mg/Ca<sub>water</sub> and Mg/ Ca<sub>ostracod</sub> is often debated and some authors (e.g. Dettman and Dwyer, 2012) have argued that Mg/Ca<sub>water</sub> plays a minor role compared to temperature in controlling Mg partioning. In marine settings, there is little variation in the Mg/Ca<sub>water</sub> so water composition is of lesser importance anyway. Since Mg/Ca<sub>water</sub> is known to show little variation in Chesapeake Bay, where the *Loxoconcha* spp. calibration was established, the Cronin et al. (2003) calibration could effectively eliminate any effect of Mg/Ca<sub>water</sub>. However, *L. elliptica* is present in fresh- (or near fresh-) water with an optimum salinity range of 0 to 20 ‰ (Whittaker, 1972). Although the measured range in the pond at Pegwell Bay was 3.9 to 4.2 mol/mol, over a salinity range of 0 to 20 ‰ (the optimum conditions for *L. elliptica* presence and abundance) in marginal marine environments, the Mg/Ca<sub>water</sub> values could vary between ~1 mol/mol (fresh water) and 5 mol/mol (marine water). Uncertainties in the relationship with Mg/Ca<sub>water</sub> are, therefore, more critical for palae-otemperature reconstructions in such environments.

During the monitoring period at the pond in Pegwell Bay, there was no significant relationship found between Mg/Ca<sub>L.elliptica</sub> and Mg/Ca<sub>water</sub> (Fig. 3c), suggesting that in this environment temperature is the dominant control on Mg/Ca<sub>L.elliptica</sub>. Furthermore, the Sr/Ca<sub>L.elliptica</sub> values show little variation across a range of Mg/Ca<sub>L.elliptica</sub> values. The similarity in Sr/Ca<sub>shell</sub> values would suggest that water composition was similar at the time of calcification. Any variation in Mg/Ca<sub>shell</sub> would, therefore, indicate that temperature is a more dominant control on Mg partitioning. Consequently, we observe a range in Mg/Ca<sub>L.elliptica</sub> values even at the same water composition (inferred from the Sr/Ca<sub>L.elliptica</sub> values) at the time of calcification. If water was a dominant control, we



Fig. 2. Average water temperature (a), *Cyprideis torosa* Mg/Ca (b), *Loxoconcha elliptica* Mg/Ca (c), electrical conductivity (d), *Cyprideis torosa* Sr/Ca (e), *Loxoconcha elliptica* Sr/Ca (f). For (b), (c), (e), and (f) data from individual valves are represented by the grey circles and the mean is denoted by the black line.

would expect both Sr/Ca<sub>L.elliptica</sub> and Mg/Ca<sub>L.elliptica</sub> values not to vary. In addition, biological mechanisms, rather than environmental controls, have been argued to be a more dominant control on the uptake of Mg into the shell, limiting the influence on Mg/Ca<sub>water</sub> (Dettman and Dwyer, 2012). Indeed, Chivas et al. (1986) demonstrate that a 50-fold increase in Mg/Ca<sub>water</sub> results in only a doubling of Mg/Ca<sub>L.torosa</sub>. This has not been demonstrated for *Loxoconcha* spp., but evidence suggests that temperature is a major control on the Mg/Ca<sub>shell</sub> of *L. matagordensis* (Vann et al., 2004). Furthermore, it is possible to draw on further circumstantial evidence to provide support for a minimal control of Mg/Ca<sub>water</sub> between 3.9 and 4.2 mol/mol (the values recorded in the pond at Pegwell Bay). Within the UK, there are unpublished Mg/Ca values of

*L. rhomboidea* from the Feet Lagoon, where Mg/Ca<sub>water</sub> varies between 3.25 and 4.99 mol/mol (Eisenhauer, 1999). We can, therefore, also apply the Cronin et al. (2003) calibration here. Mg/Ca-inferred temperatures calculated from individuals collected in July 1995 from the Fleet Lagoon range from 17.4 to 28.7 °C, reflecting expected temperatures from May to July (Met Office, 2012). This coincides with the expected calcification timing of the species in the Fleet Lagoon (Whittaker, 1972), and provides support for the appropriate use of the calibration. Furthermore, in the Fleet Lagoon, despite a range a Mg/Ca<sub>Lrhomboidea</sub> values, Sr/Ca<sub>Lrhomboidea</sub> values vary by  $\pm 0.00$  mmol/mol (1  $\sigma$ ), which again suggests that water composition was similar at the time of calcification and Mg partitioning is controlled by temperature.

## Table 2

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

ItempItempName immo/ imm	Collected	Water	Mg/Ca <sub>C.</sub>	Average Mg/	Mg/Ca temp.	Average Mg/Ca temp.	Mg/Ca <sub>L</sub>	Mg/Ca	Average Mg/Ca	Sr/Ca <sub>C.</sub>	Sr/Ca <sub>L</sub>
nemnemnemnemnemnemnemnemnemnemnemnem04.00008.578.572.542.541.551.901.639.583.643.64203.647.313.647.313.643.643.643.643.643.647.783.647.313.643.643.643.643.643.641.781.783.647.211.587.807.813.643.641.847.222.337.812.221.303.643.641.847.221.232.201.233.643.641.847.221.232.201.232.643.641.857.817.222.383.643.641.857.327.222.643.643.641.857.327.222.643.643.641.857.327.243.643.643.641.857.347.243.643.643.641.857.347.243.643.643.641.857.247.247.247.243.641.857.247.347.347.347.343.641.847.347.347.347.347.347.347.341.857.357.347.347.347.347.347.341.857.357.347.347.347.34 <td></td> <td>temp. (°C)</td> <td>torosa</td> <td>Ca<sub>C.torosa</sub></td> <td>(°C)</td> <td>(°C) Mg/Ca<sub>water</sub> 4.2 mol/</td> <td>elliptica</td> <td>temp. (°C)</td> <td>temp. (°C)</td> <td>torosa</td> <td>elliptica</td>		temp. (°C)	torosa	Ca <sub>C.torosa</sub>	(°C)	(°C) Mg/Ca <sub>water</sub> 4.2 mol/	elliptica	temp. (°C)	temp. (°C)	torosa	elliptica
04-Mag: 020160.0910.2712.742.56.218.5519.9010.389.58.472.9604-Mag: 12.914.688.5215.547.433.023.682.6412.947.1315.447.733.162.6412.947.1315.947.733.133.023.6813.947.1315.947.743.133.0214.9320.6115.897.802.243.023.487.743.133.023.272.577.8318.5612.502.2912.332.042.102.177.8818.5612.502.2912.332.643.1112.202.132.943.513.663.664.426.1112.902.25514.032.693.354.426.112.2012.01010.512.843.273.121.1822.4813.142.643.322.774.426.112.2912.3514.032.693.357.012.246.112.843.613.623.623.131.242.2514.032.693.353.523.141.322.2613.042.643.323.152.241.183.822.773.443.613.141.251.262.261.262.263.353.152.241.183.322.773.44			(mmol/	(mmol/mol)	Mg/Ca <sub>water</sub>	mol	(mmol/			(mmol/	(mmol/
04.Meg8.08.5712.742.5.8218.5519.0010.389.588.72.72.862013.645.237.337.343.643.643.643.547.132.437.347.343.643.643.782.782.43315.527.823.263.003.783.787.927.907.927.907.943.169.001.347.927.981.2507.907.943.169.011.347.927.981.2207.907.927.903.729.101.347.927.981.2207.907.927.903.729.101.347.927.988.837.257.927.927.943.729.101.347.917.927.931.367.927.943.739.147.917.947.927.947.957.947.957.949.157.947.947.957.947.957.947.957.949.167.947.947.947.947.947.947.949.167.947.947.947.947.947.947.949.167.947.947.947.947.947.947.949.167.947.947.947.947.947.947.949.171.947.947.947.947.947.94			mol)		4.2 mol/mol		mol)			mol)	mol)
016     8.23     1.54     7.81     3.64     3.64       1.54     2.54     2.61     2.64     1.279     3.68     2.01       1.43     2.021     2.64     1.529     7.82     2.25     3.00       3.54     0.021     1.589     7.82     2.25     3.00       3.57     2.57     2.43     2.22     1.39     2.04     3.04       2.13     0.257     7.89     3.65     1.250     2.28     1.31     2.88     3.27       7.41     1.250     7.89     1.51     7.82     2.98     3.28     3.28       7.44     1.250     2.28     1.31     2.88     3.27     3.88       7.42     2.20     1.250     1.81     2.88     3.28     3.28       7.42     2.20     2.28     1.31     2.88     3.29       7.44     1.20     2.28     1.24     3.81     3.83     3.83       7.42     2.29     2.28     1.24     3.81     3.83     3.93       7.42     2.44     1.52     2.44     3.61     3.43     3.63       7.41     2.47     3.61     3.44     3.61     3.43     3.63       7.41     3.44     3.61	04-Aug-	20.9	18.57	12.74	25.82	18.55	19.90	10.38	9.58	3.47	2.96
1.48         3.42         3.49         3.49         3.49         3.49         3.49           1.78         2.37         3.49         3.79         3.69         3.69           1.78         2.43         1.63         7.279         3.69         3.69           3.48         7.02         1.69         7.82         3.25         3.60           3.48         7.02         1.69         7.82         3.60         3.67           2.13         2.27         7.80         1.89         2.29         1.80         2.60         3.67           2.13         7.85         1.85         1.260         2.280         1.29         3.60         3.68           3.43         1.85         1.250         7.63         1.60         7.62         3.68           7.33         1.182         2.01         1.61         7.63         3.68         3.73           7.34         1.82         2.76         3.60         1.61         9.89         2.73         3.68           7.05         8.24         8.31         1.25         3.60         1.64         3.63         3.73           7.14         1.18         3.60         1.61         9.89         1.73	2016		1.00		0.50		15.04	<b>7</b> 00		0.00	0.60
n         3.3         1.3         1.4			4.68		8.52		15.94	7.83		3.02	3.68
n 1778         14.48         1.14			5.58 5.24		7.15		15.49	7.54		3.45	2.04
n+39         1.439         7.02         15.09         7.80         2.63         3.03           25.57         3.53         2.20         1.89         7.80         2.63         3.27           25.67         7.88         12.50         2.60         1.63         3.07         3.28           26.01         1.53         2.57         7.88         12.50         1.63         3.07         3.08           4.73         8.83         1.20         1.03         2.64         3.72           4.33         8.11         1.02         2.68         1.02         2.63         3.63           4.74         8.13         2.55         3.63         3.64         3.63         3.64           5.7         7.82         8.63         1.25         3.63         3.63         3.63           6.7         7.85         8.64         9.75         3.63         3.63         3.63           6.7         7.85         8.64         9.75         3.63         3.63         3.63           6.7         7.85         8.64         9.75         3.63         3.75         3.63         3.75           6.7         7.85         8.64         9.5         9.75 <td></td> <td></td> <td>17 78</td> <td></td> <td>24.83</td> <td></td> <td>14.85</td> <td>7 14</td> <td></td> <td>3.15</td> <td>2.90</td>			17 78		24.83		14.85	7 14		3.15	2.90
ide70215897.002749.180-bec2.253.4.532.202.4061.1893.02			14 39		20.61		15.92	7.82		3.25	3.00
01-Dec 2010         25.7 (1.2)         36.3 (2.2)         22.2 (2.0)         1.2 (2.0)         20.4 (2.0)         20.4 (			3.48		7.02		15.89	7.80		2.74	3.18
01-bec 0.01-bec 0.01-bec 0.01-bec12.4 0.0212.5012.6012.2012.0122.8 0.0132.032.7 0.027.6412.5012.5010.102.983.517.6412.002.5514.052.983.617.6411.822.5514.052.693.617.748.012.5514.052.693.617.828.012.5514.052.693.617.939.153.0619.493.612.693.617.949.059.1519.109.941.672.643.917.941.572.1819.109.912.732.403.917.941.951.919.912.732.463.397.921.062.543.393.553.169.913.223.551.031.1819.149.913.233.553.163.123.243.241.041.041.943.243.253.163.163.123.163.263.261.051.261.261.261.261.263.263.363.163.163.163.163.163.161.051.261.261.261.261.263.263.16 <td< td=""><td></td><td></td><td>25.57</td><td></td><td>34.53</td><td></td><td>22.23</td><td>11.89</td><td></td><td>2.04</td><td>3.04</td></td<>			25.57		34.53		22.23	11.89		2.04	3.04
obsec solo9.21.277.881.851.2502.801.2501.2102.843.214.938.932.921.232.843.273.833.264.358.111507.423.363.683.684.358.111507.423.363.687.331.18222.481.201.433.6120.457.7534.3019.003.633.7362.464.578.612.9534.3019.003.6362.7617.753.42019.103.613.623.7362.474.578.617.981.251.202.743.7362.471.579.169.912.732.743.737.847.857.867.869.912.743.733.747.847.857.867.867.867.867.867.867.847.847.867.867.867.867.867.867.847.847.867.867.867.867.867.867.847.867.867.867.867.867.867.867.847.867.867.867.867.867.867.867.847.867.867.867.867.867.867.867.847.867.867.867.867.867.867.867.847.867.867.867.86 <t< td=""><td></td><td></td><td>21.34</td><td></td><td>29.27</td><td></td><td>24.06</td><td>13.07</td><td></td><td>3.02</td><td>3.27</td></t<>			21.34		29.27		24.06	13.07		3.02	3.27
2010     2,292     1,233     2,44     3,23       7,64     1,20     2,010     10,51     2,83     3,23       4,42     8,19     2,555     14,03     2,69     3,31       4,47     8,19     2,83     1,61     2,84     3,15       4,27     8,01     2,83     1,61     2,84     3,15       4,27     8,01     2,83     1,61     2,84     3,15       5,19     0,15     3,83     1,80     2,89     1,23     3,37       6,21     5,38     0,51     1,31     9,99     1,27     2,64     3,33       5,48     0,51     1,316     9,91     2,54     3,91     3,37       5,48     0,51     1,316     9,91     2,54     3,91       5,48     0,51     1,316     9,91     2,54     3,91       5,49     1,18     1,316     9,41     3,92     2,54     3,92       16,50     1,262     1,48     1,49     2,42     3,92     3,92       16,41     1,69     1,48     1,49     2,42     3,92     3,92       16,51     1,69     1,26     1,40     2,58     3,16       16,54     1,61     1,26     1,26 </td <td>01-Dec-</td> <td>9.2</td> <td>12.75</td> <td>7.88</td> <td>18.56</td> <td>12.50</td> <td>22.80</td> <td>12.25</td> <td>13.10</td> <td>2.88</td> <td>3.51</td>	01-Dec-	9.2	12.75	7.88	18.56	12.50	22.80	12.25	13.10	2.88	3.51
1.9.1       4.9.3	2016										
n     7.64     1.220     0.10     10.51     2.94     3.36     3.36       4.42     8.19     2.555     14.33     2.69     3.31       4.27     8.01     2.888     16.17     2.84     3.61       5.19     7.82     9.16     3.83     3.51     3.36     3.51       6.4     7.53     8.24     8.30     1.95     19.49     3.33     3.77       6.4     7.5     8.24     8.38     12.95     19.40     1.03     3.63       7.61     7.83     9.35     19.16     9.40     1.23     2.64     3.37       7.61     7.98     11.65     19.16     9.17     2.67     3.31       7.63     11.65     19.16     9.16     3.23     3.65       7.64     10.61     19.16     9.02     3.22     3.65       7.64     10.31     10.61     2.24     19.80     3.22     3.65       9.72     10.33     13.84     2.191     1.68     2.62     3.12       2.16     13.24     2.34     2.69     3.12     3.12       9.72     10.3     3.46     2.97     3.22     3.63       18.4     1.67     2.24     3.84     2			4.93		8.83		22.92	12.33		2.84	3.27
4.35       8.11       15.91       7.82       3.6       3.63         7.33       1.82       2.65       1.43       2.69       3.43         7.33       1.82       2.48       1.05       2.09       3.15         02-Feb.       6.4       8.7       8.01       3.69       3.37         2017       0.15       0.15       3.03       19.49       3.53       3.37         2017       4.4       7.98       19.12       9.09       1.273       2.63       3.01         2017       4.34       9.51       1.006       2.50       12.06       2.54       3.30         10.03       1.518       19.83       1.034       3.02       2.66       3.62         9.72       1.80       2.24       1.199       3.35       3.52         10.03       1.348       2.24       1.199       3.03       3.52         2017       1.66       1.328       3.165       1.237       3.64       3.62         2017       2.54       3.365       2.250       1.62       3.63       3.62         201       1.55       3.26       2.45       3.65       1.65       2.23       3.64       3.77			7.64		12.20		20.10	10.51		2.98	3.22
4.42         8.19         2555         14.03         2.09         3.43           4.27         8.01         2.848         16.17         2.91         3.5           6.4         5.7         2.05         3.60         3.60         3.63         3.73           5.15         9.15         3.40         19.49         3.63         3.73           2017         7.8         3.43         1.25         12.65         1.273         2.46         3.63           5.18         0.51         7.98         2.468         1.394         2.67         3.30           15.37         0.51         1.06         1.016         9.91         2.54         3.30           15.37         1.5.8         0.34         1.02         2.54         3.32         3.50           16.4         15.18         1.94         9.09         3.23         3.53         3.52           15.4         1.9.14         9.09         3.25         3.02         3.62         3.62           16.4         1.9.09         2.24         1.8.5         2.62         3.62         3.62           16.4         1.9.0         1.6.5         1.4.9         3.63         3.62			4.35		8.11		15.91	7.82		3.36	3.68
7.33     11.82     22.48     12.05     2.91     3.15       20.05     27.65     34.20     19.60     3.33     3.37       20.17     8.24     8.3     12.95     19.12     9.89     12.73     2.44     3.61       20.17     8.24     8.3     12.95     19.12     9.89     12.73     2.67     3.01       20.17     2.54     10.06     2.50     12.06     2.54     3.39       5.92     10.06     2.50     12.06     2.54     3.39       5.92     10.06     2.50     12.06     2.23     3.56       10.03     15.18     19.81     10.34     3.22     3.62       9.72     14.80     2.24     1.89     3.37     3.35     3.52       9.72     14.80     2.24     1.89     2.27     3.04     3.22       9.72     14.80     2.24     1.89     2.24     3.09     3.22     3.02       2017     16.7     2.32     3.06     3.79     3.16     3.24     3.16       2017     11.65     2.24     3.09     3.22     3.00     3.22     3.00       21.91     1.92     2.99     12.62     1.16     3.24     3.01    <			4.42		8.19		25.55	14.03		2.69	3.43
4.27     8.01     28.48     16.17     2.64     3.61       5.19     9.15     3.62     3.62     3.53     3.73       02.76b     6.4     8.27     3.63     3.73       02.76b     10.01     10.29     12.95     3.03     3.73       02.76b     5.48     9.15     3.01     9.15     3.01     3.03       5.48     9.51     12.95     10.6     9.91     2.54     3.90       5.48     9.51     12.60     2.64     3.60     2.64     3.60       15.57     21.83     25.41     3.04     3.02     2.66       12.46     18.21     19.14     9.90     3.22     3.65       13.91     10.09     2.24     3.80     3.52     3.42       5.4     10.09     2.24     1.91     1.62     2.77     2.77       18.90     17.5     23.26     3.165     2.60     1.262     3.00     3.22       18.91     17.6     23.26     3.165     2.66     3.00     3.22       18.91     1.6     2.266     3.30     3.22     3.00     3.22       18.91     1.6     2.266     3.24     3.30     3.22     3.24       18.91			7.33		11.82		22.48	12.05		2.91	3.15
20.0527.6534.209.1003.362.7302-Feb 20176.44.578.248.3812.9519.129.8912.732.463.3720172.548.3412.9519.129.8912.732.463.3720174.247.9824.6813.472.543.395.9210.0622.5012.062.543.395.9210.0622.5012.063.223.6210.0315.1819.810.340.322.549.7214.8022.2411.893.323.379.7410.343.16522.9111.682.772.9718.0717.5023.2623.6531.652.9111.682.273.343.31201717.5023.2623.5331.652.9111.682.023.103.223.353.22201713.4821.9111.682.023.103.223.373.373.333.32201713.333.402.7612.923.003.223.333.323.32201713.622.9613.482.9111.682.903.223.363.32201713.633.443.343.313.343.313.343.343.343.343.343.343.343.343.343.343.343.343.343.343.343.343.343.34 <th< td=""><td></td><td></td><td>4.27</td><td></td><td>8.01</td><td></td><td>28.88</td><td>16.17</td><td></td><td>2.84</td><td>3.61</td></th<>			4.27		8.01		28.88	16.17		2.84	3.61
0-2-Feb 02-Feb 2017              			20.05		27.65		34.20	19.60		3.36	2.73
02-4e00.40.478.240.5812.0519.129.9912.132.403.03201711.849.782.663.142.673.015.549.511.91.669.912.543.395.5210.062.541.3943.022.5610.0315.1819.819.903.223.029.7214.802.6314.502.772.975.9410.092.6314.502.753.44201717.562.32.624.5531.652.6612.623.02201717.562.32.62.51.42.7614.502.753.44201717.62.32.62.51.42.7614.503.022.96201717.62.32.631.652.6612.623.023.22201717.62.32.631.652.6714.903.033.22201717.102.982.50714.913.033.2215.872.2462.7614.913.043.333.4224.2432.882.50714.993.043.383.643.3314.182.942.55714.913.043.3824.2432.882.56714.993.043.383.4225.413.063.532.163.662.873.143.3244.1819.521.053.043.3825.7114.94<	00 5-1	<i>(</i> )	5.19	0.04	9.15	10.05	34.03	19.49	10.70	3.53	3.37
2017       4,24       7,98       24,68       13,47       2,67       3,01         5,48       9,51       12,16       9,91       2,54       3,39         15,37       21,83       22,61       13,94       3,22       3,26         15,37       12,83       19,84       1,9,94       3,22       3,26         12,46       18,21       19,81       1,99       3,22       3,22         12,46       18,21       19,81       1,99       3,22       3,02         5,94       10,09       2,244       1,89       3,22       3,02         2017       17,6       2,26       3,165       2,01       1,62       3,7       3,7         18-April       17,6       2,32       2,14       2,07       2,07       3,01       3,22         2017       2,514       2,66       1,23       3,60       3,92       3,63       3,93       3,22         18-April       1,62       2,26       2,267       1,23       3,60       3,93       3,22         2017       2,44       3,28       2,66       2,87       3,14       3,60       3,93         21,40       3,28       2,267       1,23	02-FeD-	6.4	4.57	8.24	8.38	12.95	19.12	9.89	12.73	2.46	3.63
1.2.5         1.2.5         1.2.5         1.2.5         2.5.4         3.5.7         2.5.4         3.5.7           5.92         10.06         2.5.0         12.06         2.5.4         3.5.0           10.03         15.18         19.36         0.3.4         3.0.2         2.5.4         3.0.2         2.5.4         3.0.2         2.5.4         3.0.2         2.5.4         3.0.2         2.5.4         3.0.2         2.0.2         2.2.2         3.0.2         2.2.2         3.0.2         2.2.2         3.0.2         2.2.2         3.0.2         2.2.2         3.0.2         2.2.2         3.0.2         2.2.2         3.0.2         2.2.2         3.0.2         3.2.2         3.0.2         3.2.2         3.0.2         3.2.2         3.0.2         3.2.2         3.0.2         3.2.2         3.0.2         3.2.2         3.0.2         3.2.2         3.0.2         3.2.2         3.0.2         3.2.2         3.0.2         3.2.2         3.0.2         3.2.2         3.0.2         3.2.2         3.0.2         3.2.2         3.0.2         3.2.2         3.0.2         3.2.2         3.0.2         3.2.2         3.0.2         3.0.2         3.0.2         3.0.2         3.0.2         3.0.2         3.0.2         3.0.2         3.0.2         3.0.2 </td <td>2017</td> <td></td> <td>4 24</td> <td></td> <td>7 98</td> <td></td> <td>24.68</td> <td>13 47</td> <td></td> <td>2.67</td> <td>3.01</td>	2017		4 24		7 98		24.68	13 47		2.67	3.01
19.92     10.06     22.50     12.06     22.50     22.50       15.37     21.83     25.10     13.04     3.02     2.961       12.46     18.21     19.43     9.90     3.22     3.52       5.97     14.80     22.24     1.89     2.50     2.77     2.97       5.94     10.09     22.24     1.89     2.60     2.77     2.97       5.94     10.09     2.23     1.68     2.75     3.44       2017     17.50     23.26     24.55     31.65     23.34     12.62     3.00     3.22       2017     18.07     2.246     2.76     14.19     3.34     3.01     3.22       2044     2.398     2.260     14.51     3.44     3.01     3.22       17.10     2.398     2.260     1.232     3.34     3.10       3.32     2.44     2.967     14.11     3.44     3.01       19.61     2.712     2.52     10.15     3.68     2.87     3.14       2.72     3.33     2.44     3.53     2.16     3.7     3.22       2.74     2.36     3.53     2.17     1.65     2.87     3.14       2.75     3.34     3.16     3.7 <t< td=""><td></td><td></td><td>5.48</td><td></td><td>9.51</td><td></td><td>19.16</td><td>9.91</td><td></td><td>2.54</td><td>3.39</td></t<>			5.48		9.51		19.16	9.91		2.54	3.39
15.37       21.83       25.41       13.94       3.23       3.56         10.03       15.18       19.48       10.34       3.22       3.26         9.72       14.80       22.24       11.89       3.23       3.55         9.72       14.80       22.24       11.89       3.35       3.52         9.74       10.99       26.29       14.50       2.77       2.97         8.66       13.48       21.91       11.68       2.75       3.44         18.4pril.       12.62       11.65       2.68       3.07       3.22         18.4pril.       23.65       31.65       2.97       3.20       3.22       3.26         18.4pril.       12.62       3.26       3.22       3.22       3.22       3.22       3.22         19.61       2.77       2.34       2.26       3.30       3.22       3.22       3.22       3.22       3.22       3.22       3.24       3.20       3.22       3.22       3.22       3.22       3.23       3.24       3.20       3.22       3.22       3.22       3.22       3.22       3.22       3.22       3.24       3.24       3.20       3.24       3.24       3.24       3.			5.92		10.06		22.50	12.06		2.54	3.50
Partial and the second seco			15.37		21.83		25.41	13.94		3.23	3.56
12.46       18.21       19.14       9.90       3.22       3.25       3.25         5.54       10.09       22.24       11.89       3.35       3.35         18.4 pril       17.65       2.326       14.80       2.24       11.89       2.75       3.21         18.4 pril       17.65       2.326       2.326       3.45       3.165       2.60       2.62       3.41         2017       11.65       2.62       3.63       3.62       3.22       3.61       3.22       3.61       3.22       3.61       3.22       3.61       3.22       3.61       3.22       3.24			10.03		15.18		19.83	10.34		3.02	2.96
9.72     14.80     22.24     11.89     3.35     3.52       5.94     10.09     26.29     14.50     2.77     2.97       8.66     13.48     21.91     11.68     2.62     31.50     2.87     3.81       2017     1     11.65     2.62     31.65     23.26     12.60     3.20     3.20       3017     18.92     23.26     23.26     23.65     23.65     14.90     2.62     3.00     3.20       18.02     28.67     38.40     27.05     14.90     3.20     3.20     3.20       18.57     22.46     22.76     12.23     3.00     3.20     3.20       17.10     23.98     25.67     14.11     3.34     3.10       33.32     44.18     24.24     3.33     3.01     3.80       33.32     44.18     24.34     14.15     6.64     2.69     3.34       31.01     24.24     35.36     3.58     3.04     3.80     3.80       31.91     24.24     35.36     3.58     3.04     3.80     3.81       31.91     24.24     35.36     3.58     3.65     3.65     3.65       31.91     24.24     35.36     3.58     3.64     3.			12.46		18.21		19.14	9.90		3.22	3.02
5.9410.926.2914.502.772.7918.6613.4823.441.682.702.79201717.5623.2624.5531.6523.3412.6012.6231.0201717.6023.2625.1421.8611.652.683.7928.6738.4027.0514.993.203.2215.8722.4622.0614.113.343.1024.2432.9825.6714.113.343.1024.2432.8822.9012.323.303.8231.9142.4332.825.6714.113.343.1031.9142.4323.5821.663.583.043.3831.9142.4323.5311.553.043.3831.9142.4323.5321.661.652.902.9827.7 unt21.416.732.933.583.8031.9142.4323.531.653.933.583.8031.9142.433.162.75315.303.163.78201731.12.15021.8211.623.093.583.8031.9143.9321.6111.423.313.9032.9415.6121.9411.833.923.933.9332.9415.6121.943.313.913.9133.9533.9633.9233.933.923.933.9334.9415.971			9.72		14.80		22.24	11.89		3.35	3.52
8.613.4821.9111.682.753.4418.4017.623.2623.2623.5531.6523.3412.6012.623.753.41201718.0225.1421.8611.652.683.703.203.203.2018.0222.4622.7612.233.603.793.7917.1023.9825.6714.113.343.1024.2432.8325.6714.113.643.8033.3244.1325.5214.012.693.4433.3242.4314.156.682.873.1425.2710.153.043.383.4931.9142.4314.156.682.873.1426.635.823.5821.8611.652.873.14201713.0218.9623.5321.8611.5710.823.79201731.1243.9321.8611.5710.823.783.8831.1820.3623.5312.083.133.092.6631.1820.3421.8611.5710.823.133.1328.59116.715.9728.6713.133.013.6628.59116.715.9723.6113.673.583.6536.6310.6714.8723.8212.713.623.1136.6315.9920.9111.043.433.002.6336.6315.9923.61 <td></td> <td></td> <td>5.94</td> <td></td> <td>10.09</td> <td></td> <td>26.29</td> <td>14.50</td> <td></td> <td>2.77</td> <td>2.97</td>			5.94		10.09		26.29	14.50		2.77	2.97
18-April 201717.617.623.2624.5531.6523.3412.6012.623.103.27201718.0225.1421.8611.652.683.0728.6738.4027.0514.993.203.2215.8722.4622.7614.113.343.1024.2432.8825.6714.113.643.8224.2432.8825.5214.012.693.3433.244.1819.5210.153.043.3826.2635.3821.8611.652.902.9827.Jun-21.416.7318.9623.5312.6616.572.90201711.8016.7319.9623.5317.638.933.583.8021.3429.2614.266.773.213.433.20201711.8017.3815.303.163.783.8021.3429.2614.266.773.213.4321.3429.2614.266.773.213.4321.419.6317.388.933.583.8021.5121.5021.8211.623.992.6615.6115.9721.8211.623.503.2510.6715.9721.8211.693.313.3010.6715.9723.513.643.123.6420.715.9114.8724.923.643.6215.6115.9920			8.66		13.48		21.91	11.68		2.75	3.44
2017       2017     18.02     25.14     21.86     11.65     26.86     3.07       28.67     38.40     27.05     14.99     3.20     3.22       15.87     22.46     22.76     12.23     3.60     3.79       24.24     32.88     22.90     12.32     3.30     3.82       33.32     14.11     3.44     3.35     3.14     3.36     3.36       33.32     44.18     14.15     6.68     2.87     3.14       26.26     35.38     21.86     11.65     2.90     2.92       26.26     35.38     21.86     11.65     2.90     2.92       26.26     35.38     21.86     11.65     2.90     2.98       26.26     35.38     21.74     11.57     10.82     3.07     3.72       2017     3.10     16.73     18.96     23.53     21.74     11.57     10.82     3.07     3.72       2017     3.11     21.50     14.28     6.77     3.21     3.43       11.80     13.96     22.53     15.30     3.16     3.78       21.54     14.92     21.50     24.53     15.20     3.16     3.78       11.80     21.91     21.61     11.49     3.31 <td>18-April-</td> <td>17.6</td> <td>17.56</td> <td>23.26</td> <td>24.55</td> <td>31.65</td> <td>23.34</td> <td>12.60</td> <td>12.62</td> <td>3.10</td> <td>3.12</td>	18-April-	17.6	17.56	23.26	24.55	31.65	23.34	12.60	12.62	3.10	3.12
18.0225.1421.8611.652.683.0728.6738.0427.0514.993.203.2215.8722.4622.7612.233.603.7917.1023.9822.9012.233.603.8224.2432.8622.9012.323.603.8219.6127.1225.5214.012.693.3431.9142.4319.5210.153.043.8131.9142.4314.156.682.902.9027-Jun-21.416.738.932.163.72201711.723.1923.5321.6411.652.9025.5211.6114.9317.638.933.583.80201731.1243.9323.5317.638.933.583.80201811.8017.3827.5315.303.163.78201711.8017.3827.5315.303.163.78201811.6012.923.133.303.603.65201913.9627.1418.639.573.503.2515.1115.9718.349.384.233.12201810.6715.9920.9111.043.662.63201916.799.899.7715.1923.8212.913.642.63201916.799.899.7714.8724.9913.093.642.63201416.69<	2017										
28.6738.4027.0514.993.203.2215.8722.6422.6612.323.343.1024.2432.8822.9012.323.303.8219.6127.1225.5214.113.043.3633.3244.1819.5210.153.043.3831.9142.4314.156.682.873.1426.2653.3821.6611.652.902.9627.Jun-21.413.0616.7318.9623.5321.7411.5710.823.073.72201733.1249.9323.5321.7411.5710.823.073.72201733.1229.2614.286.773.213.4311.8017.73827.5315.303.163.7823.5031.9622.5312.083.173.2915.1121.5021.8211.623.092.6615.9315.9111.623.313.303.3016.6715.9718.849.384.233.1210.6815.9718.349.384.233.12201716.719.9910.103.642.6328.5ept-16.79.899.7715.1914.8724.0913.0911.5628.611.2216.6713.943.642.633.643.31201716.719.9010.083.072.963.1417.14<			18.02		25.14		21.86	11.65		2.68	3.07
15.87     22.46     12.23     3.00     3.79       17.10     23.98     22.66     12.23     3.00     3.34       24.24     32.88     22.90     12.32     3.30     3.82       19.61     27.12     25.52     14.01     2.69     3.34       33.32     41.18     19.52     10.15     3.04     3.38       31.91     42.43     14.15     6.68     2.87     3.14       20.70     35.38     21.86     11.65     2.90     2.98       2017     10.82     3.07     3.21     3.43       2017     11.80     16.73     19.96     23.53     17.63     8.93     3.16     3.78       2017     11.80     17.38     27.53     15.30     3.16     3.78       21.41     19.05     11.62     3.16     3.78       23.50     31.96     22.53     12.08     3.16     3.78       23.50     31.96     22.53     12.08     3.16     3.78       23.50     15.97     21.80     3.16     3.78       15.91     15.97     18.63     9.57     3.50     3.25       11.01     16.40     21.97     11.52     3.10     3.16       28.			28.67		38.40		27.05	14.99		3.20	3.22
1.1023.9825.6714.11.3.443.1024.2432.8822.9012.323303.8219.6127.1225.5214.012.693.3433.3244.1819.5210.153.043.8833.9142.4235.3821.7411.570.0822.9827.Jun-21.413.0016.7318.9623.5321.7411.5710.823.073.72201733.1243.9317.638.933.583.803.683.8021.3429.2614.286.773.213.433.163.7823.5031.9627.5315.303.163.783.583.8023.5031.9627.5315.303.163.783.513.583.6025.5411.0116.4021.9711.623.092.663.173.2915.1121.5021.8211.623.902.663.313.3016.6715.9718.639.573.513.513.5116.6715.9718.349.384.233.1216.6715.9718.349.384.233.12201716.79.899.7715.0114.8724.0913.0911.563.643.13201716.723.3312.723.243.243.143.143.143.14201716.79.899.0123.5312.72 <t< td=""><td></td><td></td><td>15.87</td><td></td><td>22.46</td><td></td><td>22.76</td><td>12.23</td><td></td><td>3.60</td><td>3.79</td></t<>			15.87		22.46		22.76	12.23		3.60	3.79
24.2432.8622.9012.323.303.6219.6127.1225.5214.012.693.3433.3244.1819.5210.153.043.3831.9142.4314.156.682.873.1426.6235.3821.7411.652.902.982017113.0616.7318.9623.5321.7411.652.90201721.413.0616.7318.9623.5312.7410.823.78201723.5117.3827.5315.303.163.7823.5031.9622.5315.303.163.7823.5031.9622.5311.623.092.6619.6327.1418.639.573.092.6619.6427.1418.639.573.013.0114.1820.3421.6111.493.313.3011.0116.4021.9711.723.713.1511.0115.9718.349.384.233.1210.6815.9718.349.384.233.12201716.712.7423.8212.913.623.145089.0114.8723.8212.913.623.145089.0123.5312.723.243.2450813.4419.9010.383.072.9611.1513.4419.9010.383.653.6811.15 <td< td=""><td></td><td></td><td>17.10</td><td></td><td>23.98</td><td></td><td>23.07</td><td>14.11</td><td></td><td>3.34</td><td>3.10</td></td<>			17.10		23.98		23.07	14.11		3.34	3.10
27-Jun- 2017       21.61       2.0.2       1.0.1       2.0.3       3.38         31.91       42.43       14.15       6.68       2.87       3.14         26.26       35.38       21.86       11.65       2.90       2.98         2017       21.4       13.06       16.73       18.96       23.53       21.74       11.57       10.82       2.90       2.98         2017       21.4       13.06       16.73       18.96       23.53       21.74       11.57       10.82       3.07       3.72         2017       33.12       43.93       7.753       8.93       3.58       3.88       3.89         23.50       31.96       22.53       15.03       3.16       3.78       3.99       3.66       3.87         23.50       31.96       22.53       12.08       3.17       3.29       3.16       3.78       3.16       3.78         15.11       21.50       21.64       11.62       3.09       2.66       3.99       2.66       3.99       2.66       3.91       3.61       3.71       3.15         14.18       20.34       21.69       11.62       3.61       3.71       3.15       3.16       3.62			19.61		27.12		25.50	12.32		2.60	3 34
$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$			33 32		44 18		19 52	10.15		3.04	3 38
$\begin{array}{c c c c c c c c c c c c c c c c c c c $			31.91		42.43		14.15	6.68		2.87	3.14
27-Jun- 201721.413.0616.7318.9623.5321.7411.5710.823.073.7233.1243.9317.638.933.583.8021.3429.2614.286.773.213.4311.8017.3827.5315.303.163.7823.5031.9622.5312.083.173.2915.1121.5021.8211.623.092.6619.6327.1418.639.573.503.2510.6420.3421.6111.493.313.3010.6715.9718.349.384.233.1210.689.7715.9114.8720.9111.043.663.68201716.715.9114.8723.5312.723.623.14201711.2216.6713.4419.9011.363.072.9611.1216.7722.3315.947.833.072.9611.1216.6713.4419.947.833.072.9611.2216.6715.973.5312.723.623.14201715.9114.8723.8212.913.623.14201715.9114.8723.8212.913.623.14201811.2216.6713.4419.9011.563.653.6811.2216.6715.947.8315.943.653.68201911.5415.49 <td< td=""><td></td><td></td><td>26.26</td><td></td><td>35.38</td><td></td><td>21.86</td><td>11.65</td><td></td><td>2.90</td><td>2.98</td></td<>			26.26		35.38		21.86	11.65		2.90	2.98
2017       33.12       43.93       17.63       8.93       3.58       3.80         21.34       29.26       14.28       6.77       3.21       3.43         11.80       17.38       27.53       15.30       3.16       3.29         23.50       31.96       22.53       12.08       3.16       3.29         15.11       21.50       21.82       11.62       3.09       2.66         19.63       27.14       18.63       9.57       3.50       3.25         11.01       16.40       21.97       11.72       3.71       3.15         14.18       20.34       21.61       11.49       3.31       3.30         10.67       15.97       18.03       9.38       4.23       3.16         28-Sept-       16.7       9.89       9.77       15.01       14.87       24.09       13.09       11.56       3.64       3.13         2017       16.7       9.89       9.77       15.01       14.87       24.09       13.09       11.56       3.64       3.14         201       16.7       15.91       14.87       23.82       12.91       3.62       3.14         201       10.74 <t< td=""><td>27-Jun-</td><td>21.4</td><td>13.06</td><td>16.73</td><td>18.96</td><td>23.53</td><td>21.74</td><td>11.57</td><td>10.82</td><td>3.07</td><td>3.72</td></t<>	27-Jun-	21.4	13.06	16.73	18.96	23.53	21.74	11.57	10.82	3.07	3.72
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	2017										
$\begin{array}{c c c c c c c c c c c c c c c c c c c $			33.12		43.93		17.63	8.93		3.58	3.80
$\begin{array}{c c c c c c c c c c c c c c c c c c c $			21.34		29.26		14.28	6.77		3.21	3.43
23.5031.9622.5312.083.173.2915.1121.5021.8211.623.092.6619.6327.1418.639.573.503.2511.0116.4021.9711.723.713.1514.1820.3421.6111.493.313.3010.6715.9718.349.384.233.1210.6815.9920.9111.043.462.63201716.79.899.7715.0114.8724.0913.0911.563.643.13201712.7423.8212.913.623.143.073.253.143.073.2511.2216.6715.947.833.653.683.653.6811.2216.6715.947.543.112.64			11.80		17.38		27.53	15.30		3.16	3.78
$\begin{array}{c c c c c c c c c c c c c c c c c c c $			23.50		31.96		22.53	12.08		3.17	3.29
19.63       27.14       18.63       9.57       3.50       3.25         11.01       16.40       21.97       11.72       3.71       3.15         14.18       20.34       21.61       11.49       3.31       3.30         10.67       15.97       18.34       9.38       4.23       3.12         10.68       15.97       20.91       11.04       3.46       2.63         28-Sept-       16.7       9.89       9.77       15.01       14.87       24.09       13.09       11.56       3.64       3.13         2017         23.62       12.91       3.62       3.14         28-Sept-       16.7       9.89       9.01       23.53       12.72       3.24       3.24         2017        13.44       19.90       10.38       3.07       2.96         11.22       16.67       15.94       7.83       3.65       3.68         15.77       22.33       15.49       7.54       3.11       2.64			15.11		21.50		21.82	11.62		3.09	2.66
11.01       16.40       21.97       11.72       3.71       3.15         14.18       20.34       21.61       11.49       3.31       3.30         10.67       15.97       18.34       9.38       4.23       3.12         10.68       15.99       20.91       11.04       3.66       2.63         28-Sept       16.7       9.89       9.77       15.01       14.87       24.09       13.09       11.56       3.64       3.13         2017       7       15.01       14.87       24.09       13.09       11.56       3.64       3.13         2017       7       15.01       14.87       24.09       13.09       11.56       3.64       3.13         2017       7       15.01       14.87       24.09       13.09       11.56       3.64       3.13         2017       7       15.01       14.87       23.82       12.91       3.62       3.14         5.08       9.01       23.53       12.72       3.24       3.24         11.22       16.67       15.94       7.83       3.65       3.68         11.22       15.77       22.33       15.49       7.54       3.11       2.64 <td></td> <td></td> <td>19.63</td> <td></td> <td>27.14</td> <td></td> <td>18.63</td> <td>9.57</td> <td></td> <td>3.50</td> <td>3.25</td>			19.63		27.14		18.63	9.57		3.50	3.25
14.16     20.34     21.01     11.49     3.31     3.30       10.67     15.97     18.34     9.38     4.23     3.12       10.68     15.99     20.91     11.04     3.46     2.63       28-Sept     16.7     9.89     9.77     15.01     14.87     24.09     13.09     11.56     3.64     3.13       2017     10.68     12.74     23.82     12.91     3.62     3.14       5.08     9.01     23.53     12.72     3.24     3.24       8.63     13.44     19.90     10.38     3.07     2.96       11.22     16.67     15.94     7.83     3.65     3.68       15.77     22.33     15.49     7.54     3.11     2.64			11.01		10.40		21.97	11.72		3./1 2.21	3.15 2.20
10.67       15.97       16.34       9.36       4.23       3.12         10.68       15.99       20.91       11.04       3.46       2.63         28-Sept-       16.7       9.77       15.01       14.87       24.09       13.09       11.56       3.62       3.14         2017       8.07       12.74       23.82       12.91       3.62       3.14         5.08       9.01       23.53       12.72       3.24       3.24         8.63       13.44       19.90       10.38       3.07       2.96         11.22       16.67       15.94       7.83       3.65       3.68         15.77       22.33       15.49       7.54       3.11       2.64			14.18		20.34		21.01 18.34	0.38		3.31 4.22	3.3U 3.10
28-Sept-       16.7       9.77       15.01       14.87       24.09       13.09       11.56       3.64       3.13         2017       8.07       12.74       23.82       12.91       3.62       3.14         5.08       9.01       23.53       12.72       3.24       3.24         8.63       13.44       19.90       10.38       3.07       2.96         11.22       16.67       15.94       7.83       3.65       3.68         15.77       22.33       15.49       7.54       3.11       2.64			10.67		15.97		20.01	9.36		4.23	2.63
2017     8.07     12.74     23.82     12.91     3.62     3.14       5.08     9.01     23.53     12.72     3.24     3.24       8.63     13.44     19.90     10.38     3.07     2.96       11.22     16.67     15.94     7.83     3.65     3.68       15.77     22.33     15.49     7.54     3.11     2.64	28-Sent-	16.7	9.89	9 77	15.99	14 87	20.91	13.09	11 56	3.40	2.03
8.07         12.74         23.82         12.91         3.62         3.14           5.08         9.01         23.53         12.72         3.24         3.24           8.63         13.44         19.90         10.38         3.07         2.96           11.22         16.67         15.94         7.83         3.65         3.68           15.77         22.33         15.49         7.54         3.11         2.64	20-56-	10.7	5.05	2.11	10.01	1 1.07	27.07	10.09	11.00	3.04	5.15
5.089.0123.5312.723.243.248.6313.4419.9010.383.072.9611.2216.6715.947.833.653.6815.7722.3315.497.543.112.64	/		8.07		12.74		23.82	12.91		3.62	3.14
8.6313.4419.9010.383.072.9611.2216.6715.947.833.653.6815.7722.3315.497.543.112.64			5.08		9.01		23.53	12.72		3.24	3.24
11.2216.6715.947.833.653.6815.7722.3315.497.543.112.64			8.63		13.44		19.90	10.38		3.07	2.96
15.77 22.33 15.49 7.54 3.11 2.64			11.22		16.67		15.94	7.83		3.65	3.68
			15.77		22.33		15.49	7.54		3.11	2.64

Additionally, it would be expected that the equation would provide erroneous results if Mg/Ca<sub>water</sub> were an important consideration. For example, for *C. torosa*, there are two possible Mg/Ca temperature calibrations; the De Deckker et al. (1999) calibration used throughout this paper, and applicable in water of marine-like composition, and the Wansard (1996) calibration that can be used in more dilute waters, with lower Mg/Ca<sub>water</sub>. Like the Cronin et al. (2003) equation, the Wansard

(1996) equation does not include Mg/Ca<sub>water</sub>; it is given as T (°C) = 3.3 + (1971.0 \* [Mg/Ca]<sub>ostracod</sub>). However, even without knowledge of the Mg/Ca<sub>water</sub>, inappropriate use of the De Deckker et al. (1999) or Wansard (1996) equation for *C. torosa* produces results that are evidently incorrect; for individuals that have calcified in water of Mg/Ca that is too low (for De Deckker et al., 1999) or too high (for Wansard, 1996) Mg/Ca-inferred temperatures using these equations give untenable



Fig. 3. Relationship between  $Mg/Ca_{ostracod}$  and  $Mg/Ca_{ostracod}$  and  $Sr/Ca_{ostracod}$  and  $Sr/Ca_{water}$  for *Cyprideis torosa* (a,b) and *Loxoconcha elliptica* (c,d) collected in June 2017. The regression equation and  $R^2$  are shown for statistically significant relationships only.

results. For instance, using the Wansard (1996) calibration calculates Mg/Ca-inferred water temperature values of up to 68 °C for the pond at Pegwell Bay. Due to uncertainties in calcification timing in field experiments, the exact temperature at the time of calcification is unlikely to be known. However, since the Cronin et al. (2003) calibration calculates reasonable water temperature that would be expected for the region across the range of Mg/Ca<sub>water</sub> values presented here, it is likely to be appropriate for use on *Loxoconcha* spp. individuals that have calcified in environments where Mg/Ca<sub>water</sub> is expected to vary between  $\sim$ 3 mol/mol to 5 mol/mol. Whilst it cannot be confirmed that there would not be an effect of Mg/Ca<sub>water</sub> at lower values (< 3 mol/mol), there is evidence to suggest that the Cronin et al. (2003) calibration is appropriate for use on material collected from the pond at Pegwell Bay.

### 4.2. Estimating seasonal calcification

In temperate brackish water environments, there is a single generation of *C. torosa* per year, but with two peaks of adults (Horne, 1983). Calcification from A-1 to adult occurs biannually in spring and late summer to early autumn (Heip, 1976). Adults and A-1 juveniles overwinter together with the A-1 individuals producing new adult populations in the spring; the generations overlap so that the population comprises both overwintered adults and newly-matured adults, 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; these individuals moult to adulthood by the autumn (Heip, 1976; Horne, 1983). For *L. elliptica*, each year there are multiple generations of newly-matured adults from the spring to the summer, resulting in peak abundances during these months. The calcification period ends with the onset of low autumn temperatures (Theisen, 1966; Horne, 1983). Adults and juveniles of the last generation overwinter producing adults in the following spring (Theisen, 1966; Horne, 1983).

To estimate the calcification period of the individuals collected from the pond in Pegwell Bay, we can use the existing palaeotemperature calibrations for *Cyprideis* and *Loxoconcha* to compare with monitored temperatures. For *C. torosa*, we can calculate the Mg/Ca-inferred temperature using the temperature calibration of De Deckker et al. (1999; equation 1). We calculate temperatures using the average Mg/Ca<sub>water</sub> value of 4.2 mol/mol, which was recorded across the calcification period in the pond (Roberts et al., 2020). Altering the Mg/Ca<sub>water</sub> changes the inferred temperatures, but the seasonal pattern remains the same (see Roberts et al., 2020 for more details). For *L. elliptica*, we can calculate the Mg/Ca-inferred temperature using the temperature calibration of Cronin et al. (2003; equation 2).

The distribution of C. torosa Mg/Ca-inferred temperatures is

discussed in detail in Roberts et al. (2020) but the results are summarised here for context. Since it is reasonable to assume that there is no lifecycle development from late autumn to spring, but *C. torosa* A-1 juveniles moulting to adulthood in the autumn (Horne, 1983), it would be expected that samples collected during December to April would have more individuals that calcified in the autumn (reflecting temperatures of 2.1 to 17.1 °C for October to November) than spring calcification (reflecting temperatures of 4.7 to 31.0 °C for March to May). The majority of valves collected in December 2016 and February 2017 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. 4a). Additionally, there is a biomodal distribution of Mg/Ca-inferred temperatures in August 2016 and June 2017 (Fig. 4a), reflecting the presence of newly calcified adults alongside those that have overwintered.

As with C. torosa, late juvenile and some adult L. elliptica individuals are expected to overwinter (Horne, 1983). Reproduction and A-1 to adult moulting occurs during the spring and summer. However, unlike C. torosa, there is no bimodal distribution in calcification of L. elliptica (Fig. 4b) for the August 2016 and June 2017 collections, suggesting no overlapping of populations with distinct seasonal Mg/Ca-inferred temperatures. A lack of valves with Mg/Ca-inferred temperatures above 13.1 °C in these months suggests that Calcification occurs in spring (maximum monitored water temperatures in March 2017 were 16.1 °C). It appears that there is an increase in valves reflecting higher temperatures in December 2016, February 2017, and June 2017 (Fig. 4b). However, the Mg/Ca-inferred temperatures for these valves still fall within spring temperatures at 16.1 °C, 19.5 °C and 15.3 °C respectively (reflecting maximum monitored temperatures in March of 16.1 °C and April of 21.6 °C). The lack of valves with an Mg/Ca-inferred temperature in the 15 to 20 °C frequency class distribution in April 2017 may be related to random error in sampling.

The average value of the valves collected in December to April, during overwintering, all reflect temperatures of 7.8 to 19.6 °C (Table 2). Monitored water temperatures are not available for the calcification period prior to August 2016, but if water temperatures from 2016 are consistent with the observed trend with air temperatures, which are available for 2016 from Met Office (2012), then we would also expect monitored temperatures of ~5 to 34 °C in 2016 between March and September. Mg/Ca-inferred temperatures of 7.8 to 19.6 °C, therefore, suggest possible calcification from April to September. However, the Mg/Ca<sub>Lelliptica</sub> values all reflect lower temperatures, peaking at

19.6 °C, suggesting that spring is a more likely calcification period. Spring calcification is further suggested by a lack of Mg/Ca-inferred temperatures above 15.3 °C in April to September 2017 (Table 2), suggesting a turnover in population to a higher percentage of those that are newly calcified in spring 2017 rather than 2016 (monitored temperatures in March were 4.7 to 16.4 °C). Furthermore, there is an increase in the number of valves of both species closely matching monitored temperatures in April (5.5 to 21.6 °C) and June (11.8 to 34.2 °C).

Unlike C. torosa, with continuously shifting population dynamics resulting in a population turnover during the monitored period (Roberts et al., 2020), there are always individuals of L. elliptica present in the population that likely calcified in the spring. This implies longevity of adults for at least 12 months, with those calcifying in the spring still present in the population in the following spring. However, according to Horne (1983), we would also expect multiple generations of L. elliptica to moult to adulthood over the summer months, and to overlap with these individuals. Whilst there is evidence for the turnover in population to those that are newly calcified, the Mg/Ca-inferred temperatures do not provide evidence for summer calcification (Table 2). For example, for the September 2017 collection, there is no increase in the number of valves with Mg/Ca-inferred temperatures reflecting calcification in summer 2017, which would be expected as newly-calcified adult individuals progressively become a larger percentage of the population; maximum Mg/Ca-inferred temperatures are 13.1 °C, suggesting spring or minimum temperatures in July 2017 of 13.9 °C. Horne (1983) confirms that in some circumstances population dynamics cannot distinguish discrete generations of L. elliptica. Furthermore, there remain significant unknowns in the precise timing of ostracod shell calcification. However, low temperatures are likely not related to unknown Mg/ Cawater values since lower values (<3 mol/mol) would increase calculated temperatures. A lack of valves recording high summer temperatures, therefore, could suggest that no individuals moulted during this period, or alternatively suggest that individuals were calcifying at times outside of peak temperatures. In addition, the individuals present in the samples were exclusively A-1 and adult moult stages. Horne (1983) report that only A-2 to adult instars overwinter, suggesting the population in the Pegwell Bay pond may be exclusively composed of overwintered individuals. However, a lack of juvenile instars below A-3 in the collections does not confirm a lack of reproduction during the summer; the lack of A-3 valves is likely due to smaller individuals passing through the mesh size of the net used during sample collection.



**Fig. 4.** Water, air and ostracod Mg/Ca-inferred temperatures for *Cyprideis torosa* (a) and *Loxoconcha elliptica* (b). Mg/Ca-inferred temperatures for *C. torosa* are calculated using the average Mg/Ca<sub>water</sub> for the pond at Pegwell Bay of 4.2 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). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

This information cannot, therefore, verify a lack of life cycle development over the summer months, but the lack of summer Mg/Ca-inferred temperatures in addition to discrete populations being indistinguishable in some environments could be evidence for single generations in some marginal marine environments, and requires further investigation.

For both *C. torosa* and *L. elliptica*, Sr/Ca<sub>ostracod</sub> values are typical of individuals from waters of marine-like ionic composition. Highest values were recorded in June and lowest values in February (*C. torosa*) and September (*L. elliptica*). However, when considering the variability in Sr/Ca<sub>*L.elliptica*</sub> for each collection, the values all fall within the same range (Table 2), and therefore the individuals may still have calcified within the same season. Due to tidal connections, Sr/Ca<sub>water</sub> values vary on diurnal timescales (Table 1) in coastal ponds and therefore individuals calcifying within a short time period can have markedly different Sr/Ca values. Even with this in mind, in addition to a significant relationship between Sr/Ca<sub>water</sub> and Sr/Ca<sub>ostracod</sub> for *L. elliptica* (Fig. 3d), there is an indication that Sr/Ca<sub>ostracod</sub> reflects the electrical conductivity of the pond (Fig. 5b) and highlights the potential of Sr/Ca<sub>*L.elliptica*</sub> as a palaeosalinity indicator.

## 4.3. Implications for palaeotemperature reconstructions

Despite the widespread distribution of L. elliptica in Europe, its potential as a paleothermometer proxy has been overlooked, primarily due to the extensive use of C. torosa, with which it often occurs. However, quantifying the trace-element partitioning for C. torosa is problematic since it inhabits highly variable environments, and therefore, it is often impossible to determine the composition of host water (and temperature) at the time of calcification. This is particularly problematic for palaeotemperature reconstructions since the Mg/Ca calibration of De Deckker et al. (1999) requires an estimation of Mg/Ca<sub>water</sub> at the time of calcification. Altering the Mg/Cawater used in eq. (1) across the range of expected values for the pond at Pegwell Bay adjusts the calculated temperature sensitivity of Mg-uptake, resulting in an alteration of temperatures up to 10.5 °C, creating large unknowns in reconstructed temperatures. We have demonstrated that the Mg/Ca calibration for Loxoconcha spp. can be used without precise knowledge of Mg/Cawater in environments with Mg/Cawater of 3-5 mol/mol to provide spring to summer temperatures. With knowledge of seasonal calcification there is, therefore, potential to constrain temperatures without knowledge of water composition using Mg/Ca<sub>L.elliptica</sub> in environments where the two species coexist.

A lack of valves with an Mg/Ca-inferred temperature below 6 °C, suggests a temperature control on calcification and coincides with early spring temperatures (minimum temperatures of 5.5 °C were recorded in April 2017 and reflected in valves collected in June 2017). This is in agreement with the minimum Mg/Ca-inferred temperatures for *C. torosa* of 7 °C. Whilst these temperatures for *C. torosa* coincide with the overwintering of adults (Roberts et al., 2020), it is not expected for *L. elliptica* to calcify during the autumn, and therefore Mg/Ca values more likely reflect minimum spring temperatures, which in this locality are similar to autumn temperatures. For *C. torosa*, it was hypothesised to be a biologically linked control on calcification from A-1 to adult (Heip, 1976; Roberts et al., 2020), which is also probable for *L. elliptica*. For both species, Mg/Ca values cannot be used to reconstruct minimum winter temperatures and reconstructed values significantly below 6 °C are likely an artefact of the calibration.

For C. torosa, there is no upper limit on calcification temperature with individuals collected in April recording an Mg/Ca temperature of 38.4 °C (the maximum monitored temperature was 34.2 °C in June 2017). However, for L. elliptica a maximum Mg/Ca temperature of 19.6 °C is recorded in February 2017, reflecting either average summer temperatures or maximum spring temperatures (the maximum monitored temperature in spring 2017 was 21.6 °C). The lack of valves recording peak summer temperatures suggests that the Mg/Ca of L. elliptica cannot be used to reconstruct maximum temperatures in environments similar to the pond at Pegwell Bay. Even considering the reported error of ~2.9 °C (Cronin et al., 2003), the values still reflect maximum spring temperatures. Maximum Mg/Ca-inferred temperatures are possibly related to a biologically linked control on calcification, but since we would expect the biomineralisation processes in different species of brackish water ostracods to be broadly similar and this control is not evident in C. torosa, the maximum temperature most likely reflects maximum spring temperature. Furthermore, there is no evidence at Pegwell Bay of substantial evaporation causing an increase in Mg/ Cawater that would mask the temperature dependence of calcification at higher temperatures.

With detailed seasonal monitoring and an understanding of modern site systematics, there is potential to identify seasonal temperatures in the palaeorecord if multiple individual valves (rather than multiple valves analysed as a bulk sample) of both *C. torosa* and *L. elliptica* are analysed simultaneously. For example, the maximum Mg/Ca values of *C. torosa* can be used to reconstruct maximum summer temperatures, the minimum Mg/Ca<sub>c.torosa</sub> to reconstruct autumn temperatures, and the



Fig. 5. Average Mg/Ca<sub>shell</sub>, Sr/Ca<sub>shell</sub>, water temperature, and electrical conductivity for *Cyprideis torosa* (denoted by circles and the light grey solid line) and *Loxoconcha elliptica* (denoted by squares and the dashed dark grey line). The solid black line relates to the left y-axis in each graph.

Mg/Ca<sub>L.elliptica</sub> to reconstruct the range in spring temperatures. Furthermore, without knowledge of the Mg/Ca<sub>water</sub>, Mg/Ca<sub>L.elliptica</sub> values can be interpreted with fewer uncertainties than Mg/Ca<sub>L.elliptica</sub> and/or, in environments with similar spring and autumn temperatures, uncertainties in the selection of Mg/Ca<sub>water</sub> values can be limited by comparing the Mg/Ca-inferred temperatures of the two species. It is important to note, however, that this is true for environments with a similar range in Mg/Ca<sub>water</sub> as the pond at Pegwell Bay.

## 5. Conclusions

The Mg/Ca temperature calibration of Cronin et al. (2003) is appropriate to use with L. elliptica in marginal marine environments with Mg/Cawater of 3-5 mol/mol. The successful application of this calibration is potentially significant for paleotemperature reconstructions across Europe in locations where there is limited knowledge of, or high variability in, Mg/Cawater. There is no significant relationship observed between Mg/Cawater and Mg/CaL.elliptica, suggesting temperature as a major control. This reduces the uncertainty due to the lack of Mg/Cawater value in the calibration. For a more robust understanding of the relationship between Mg/Cawater and Mg/CaLelliptica, we recommend future research on L. elliptica Mg/Ca as a palaeotemperature proxy uses culture experiments to establish relationships under known conditions. In environments similar to the pond at Pegwell Bay, L. elliptica Mg/Ca-inferred temperatures could be used to constrain spring temperatures. When combined with C. torosa Mg/Ca-inferred temperatures, which record the range of temperatures from spring to autumn, this has the potential to disentangle seasonal temperature, resulting in spring, autumn and summer temperatures in the palaeotemperature record. Whilst there is no evidence that the uptake of Sr in L. elliptica is temperature dependent, potentially due to the dominant effect of Sr/Ca<sub>water</sub> masking any temperature dependence, the data presented here provide evidence of the potential of Sr/Ca<sub>L.elliptica</sub> to reconstruct Sr/Ca<sub>water</sub> and, thus, salinity in environments similar to the pond at Pegwell Bay.

# CRediT authorship contribution statement

**L.R. Roberts:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft. **J.A. Holmes:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing.

## **Declaration of Competing Interest**

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

## Data availability

Data will be made available on request.

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