The potential of marine bivalve *Spisula sachalinensis* as a marine temperature record

Tansy Branscombe[a,*], Rick Schulting[a], Julia Lee-Thorp[a], Melanie J. Leng[b]

[a]*School of Archaeology, University of Oxford, 1 South Parks Road OX1 3TG*
[b]*National Environmental Isotope Facility, British Geological Survey, Nicker Hill, Keyworth, Nottingham NG12 5GG and School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD*

Abstract

This paper presents a four year subannual isotope marine temperature record using modern *Spisula sachalinensis* specimens from Tomakomai (Hokkaido's Pacific coast, Japan). This species is commonly found in pre- and protohistoric shell middens and faunal assemblages from around the Seas of Japan and Okhotsk, so has significant potential as an indicator of (pre)historic marine and, by inference, climatic conditions. However, previous sclerochronological research on the species' growth has shown significant geographical variation in growth pattern, rendering palaeoclimatic interpretation difficult. To address this issue, this study applied sequential isotopic analysis on two sectioned modern valves, providing a proxy for sea surface temperature (SST) and allowing the direct identification of seasonality during shell growth. The sequences span four years of growth, as confirmed by visually identified growth patterns and the oxygen isotope results, which show clear annual cycles in δ^{18}O. δ^{13}C seasonality is less clear, but shows a weak inverse correlation with temperature potentially relating to primary productivity. Annual growth lines show that shell growth occurs during both warm and cooler SST, but is more rapid during the cooler seasons. This is consistent with warm-season growth minima seen in shells from Hakodate Bay by Kato and Hamai (1975), but not with their suggestion that it is associated with shells growing at the southern limits of their distribution. Comparison to average local SST shows that δ^{18}O-derived temperature falls within the expected range, but, contrary to expectations given preferential cool-season shell growth, appears biased towards warmer temperatures. Factors that could contribute to this are discussed. Overall, stable isotope analysis of *Spisula sachalinensis*

*Corresponding author

Email addresses: tansy.branscombe@arch.ox.ac.uk (Tansy Branscombe), rick.schulting@arch.ox.ac.uk (Rick Schulting), julia.lee-thorp@arch.ox.ac.uk (Julia Lee-Thorp), mjl@bgs.ac.uk (Melanie J. Leng)

is considered a useful complement to macro/microscopic sclerochronological research in building a holistic picture of shell growth, and has significant potential as a high resolution proxy for palaeoenvironmental studies of past SST.

Keywords:
Palaeoclimate, Northwest Pacific, Oxygen isotopes, Sclerochronology, Seasonality

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1. Introduction

The value of marine bivalve shells as temperature archives has long been recognised (e.g. Emiliani et al., 1964), with high resolution sampling and increasingly precise stable isotope mass spectrometry allowing the interpretation of data at subannual scales. Following the recognition that some species of clam, e.g. *Arctica islandica*, can live for more than 500 years (Butler et al., 2013; Schöne et al., 2005), researchers have noted the importance of long-lived molluscs as 'sentinel species' in marine climate research (Mann et al., 2013). The shells of such large clams grow incrementally allowing for correlation between annual growth increments and calendar dates (e.g. Khim et al., 2000). Furthermore, their shell oxygen and carbon stable isotope ratios ($\delta^{18}O_{shell}$ and $\delta^{13}C_{shell}$) reflect information about ambient water conditions. Water temperature and salinity in particular drive $\delta^{18}O_{shell}$, while $\delta^{13}C_{shell}$ has a more complicated relationship to water conditions involving animal metabolic effects, salinity and dissolved inorganic carbon (DIC)(McConnaughey and Gillikin, 2008). This means that the growth increments can be interpreted as subannual proxy records for these variables (Emiliani et al., 1964).

Spisula (*Mactra/Pseudocardium*) *sachalinensis* (Figure 1), or the Sakhalin surf clam, is a large bivalve found in the Northwest Pacific, notably in Japan, the Korean peninsula and the Russian Far East (including the eponymous Sakhalin Island). In Japanese this species is known as ubagai (ウバガイ) or hokkigai (ホッキ貝). It is easily recognised by its bright pink muscle and is eaten both raw and cooked. The majority of Japanese surf clam harvest occurs from the port city of Tomakomai on the south coast of Hokkaido (Figure 2), where clams are dredged using jet stream seine fishing. As of 1999, 50,000 tons of clam were caught from the Tomakomai coast annually, and it remains a specialty dish of the region (Sasaki and Ohta, 1999).

Previous research on the species has been guided by its commercial im-

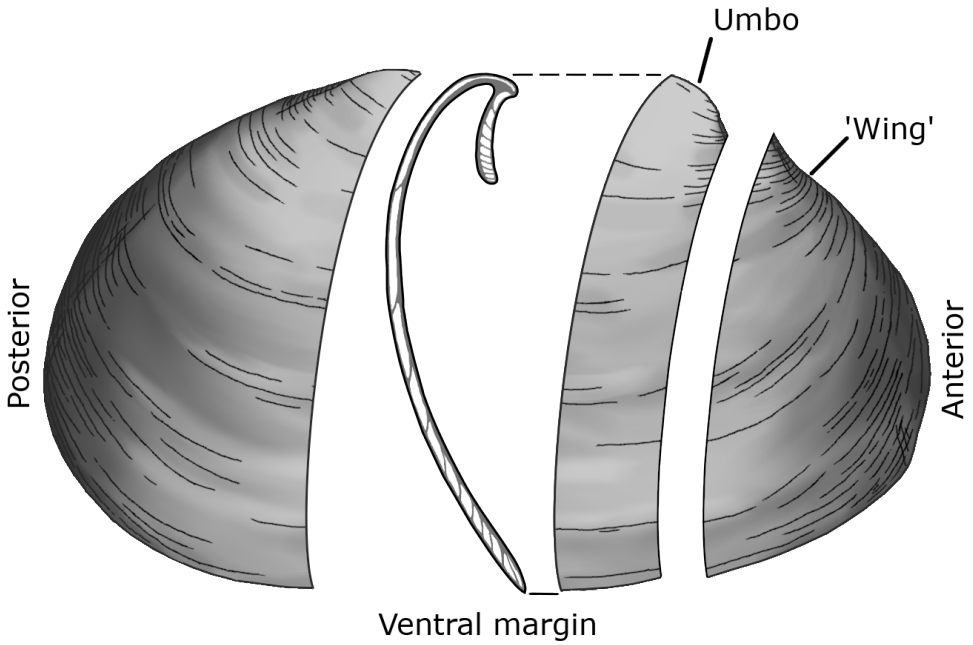

Figure 1: Schematic illustration of *Spisula sachalinensis* showing section along the axis of maximum growth

portance, focusing on its nutritional value and optimal growth and spawning conditions (e.g. Golikov and Scarlato, 1970; Kato and Hamai, 1975; Lee et al., 1996; Sakurai and Seto, 1998; Sasaki, 1981; Sasaki and Ohta, 1999; Tabakaeva and Tabakaev, 2017; Takahashi and Yamamoto, 1970).

The potential of this species for climate science has not gone unrecognised. For example Mann et al. (2013) list it as one of four major long-lived mactrid clam species, and note that the genus *Spisula* has been suggested as a good continental shelf sentinel species covering both the North Pacific and North Atlantic. The only isotopic study to specifically test the species as a climatic indicator, however, is that by Khim et al. (2000), confirming the expected relationship between $\delta^{18}O_{shell}$ and sea surface temperature in specimens from the east coast of Korea. They also discuss trends in $\delta^{13}C_{shell}$, suggesting that as in other bivalve species (e.g. Gillikin et al., 2006; McConnaughey and Gillikin, 2008) this is primarily a reflection of the $\delta^{13}C$ of DIC.

Khim et al. (2000) focus on the utility of *S. sachalinensis* as a monitor for modern hydrological conditions, but do briefly mention possibilities for palaeoclimatic research.

Our study continues this research, but focuses on the region of Hokkaido as opposed to the Korean coast. Not only is this region notable for the modern commercial harvest of *S. sachalinensis* as previously outlined, it is also an important area for Japanese archaeology. In Hokkaido, unlike in central/-southern Japan, coastally-adapted indigenous hunter-gatherers remained the primary occupants into the late 19th century (Hudson, 2014; Ohnuki-Tierney, 1976; Seaton, 2017). The maritime adaptations of Jomon, Epi-Jomon, Okhotsk and Ainu groups - representing a span of c. 7000 years - mean that shell middens are a frequent element of the archaeological record in Hokkaido(Habu et al., 2011; Oikawa, 1995; Okada, 1998), and the surf clam is a common species found at sites in northern Japan and Hokkaido (Makino, 2011; Nagasaki, 1994; Okada, 1998). Use of the species as a palaeothermometer could provide an important sub-annual oceanographic and palaeoclimatic proxy, given the interdependence of oceanographic and atmospheric factors and their effect on regional SST (e.g. Abe et al., 2016; Hirose and ichi Fukudome, 2006; Imada et al., 2017; Seo et al., 2013; Takano et al., 2008).

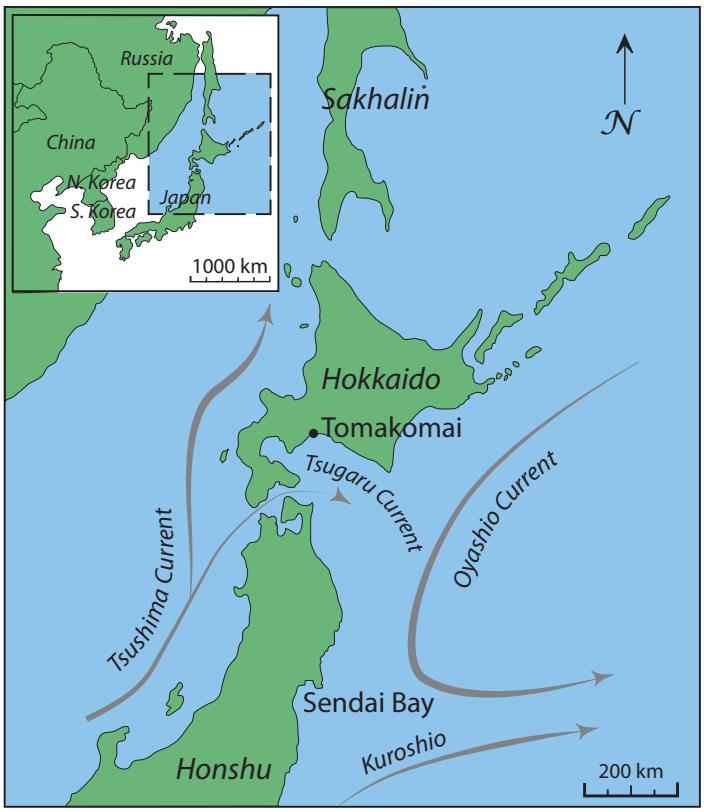

Figure 2: Map showing the location of Tomakomai within northern Japan, and the surrounding ocean currents.

For Honshu and the southern islands of Japan much of the historical period is well-documented climatically by instrumental measurements (back to the late 18th century (Demaree et al., 2013; Können et al., 2003)) and historical documents (back to the 11th century(Mikami, 2008)). However, as Hokkaido was not colonised by the Japanese state until after the Meiji restoration of 1868 there is relatively little historical climatic documentation that specifically relates to this region. Therefore, the use of shellfish (and other marine carbonates) have the potential to fill significant gaps in archaeological and palaeoclimatic knowledge, both due to their archaeological prevalence and the relative gap in historical climatic data specific to this island.

We aim to use high-resolution analysis of $\delta^{18}O_{shell}$ and $\delta^{13}C_{shell}$ to identify seasonality of growth through isotope analysis of a modern, known-age *S. sachalinensis*. A focus is placed on identifying the season of growth decrease/cessation, which has been a point of disagreement in previous research, as outlined below. We aim to investigate the relationship between $\delta^{18}O_{shell}$ and sea surface temperature, in order to demonstrate the potential of *S. sachalinensis* for palaeoclimatic reconstruction in the Northwest Pacific.

2. Growth and ecology

S. sachalinensis is a species that prefers sandy substrates in the littoral zone, growing rapidly for c. eight years, when shell length reaches its maximum size of around 10 cm in length and subsequent growth becomes negligible (Sasaki, 1981). Large shells are often 20-30 years old, with one individual from the Sea of Japan even reported to have reached 70 years (Selin, 2010). The species reaches sexual maturity at three years (Golikov and Scarlato, 1970), after which spawning takes place annually during the spring/early summer in Hokkaido (Kinoshita and Shibuya, 1920; Sakurai et al., 1992; Sasaki, 1981; Takamaru, 1976). In Primorje, Russian Far East, Golikov and Scarlato (1970) report spawning between late June and October. It seems likely that these differences relate to temperature variation between regions, as Lee et al. (1996) have shown that water temperature can be a limiting factor for egg development in *S. sachalinensis*.

Water depth estimates for living colonies vary from 1-5 m (Golikov and Scarlato, 1970), to 3-6 m (Sasaki and Ohta, 1999) and up to 12-15 m (Sasaki, 1989). Golikov and Scarlato (1970) discuss seasonal change in depth distribution, although the extent of this variation does not account for the wide

differences in depth range described in the literature. Sasaki (1989) suggests that a maximum depth of 15 m is common across a number of fishing spots, as a result of sediment conditions. Coarser-grained sand particles found at greater depths inhibit the shellfish's burrowing behaviour. Differences in sediment composition across the species' geographical distribution could therefore be responsible for different depth ranges given in the literature.

As is common in marine bivalves, growth is not constant throughout the year, resulting in the formation of annual growth increments and lines (Kato and Hamai, 1975; Sasaki, 1981). Growth increments show a prismatic structure and represent rapid shell growth. Growth lines are a nacreous layer (due to a difference in the orientation of the crystal structure or the organic compounds therein) and represent a period of slowed or ceased shell growth (Kato and Hamai, 1975). Both of these layers are composed of aragonite ($CaCO_3$). In *S. sachalinensis*, previous work suggests three potential triggers for the formation of annual growth lines: low winter SST (Yamamoto, 1947), high summer SST (Sasaki, 1981) and the end of the spawning season (Kato and Hamai, 1975). As with variation in spawning season, it seems likely that these multiple triggers relate to different environmental conditions across the species' geographic distribution; the warm-season growth lines reported by Sasaki (1981) occurred in shells near the southern end of the *S. sachalinensis* distribution in Sendai Bay, Honshu, whereas the cool-season lines reported by Yamamoto (1947) were from its northern distribution on Sakhalin. Given the expected relationship between $\delta^{18}O_{shell}$ and SST, stable isotope analysis of a modern specimen of *S. sachalinensis* from Tomakomai should confirm the species' regional growth pattern.

3. Materials and methods

The modern shells used in this study were collected from the coast of Tomakomai on Hokkaido's Pacific coast (Figure 2) in August 2019.

The shells were obtained from a Sapporo sushi restaurant, and had been briefly boiled (<20 minutes) by the restaurant to remove any remaining organic material before export to the UK (standard practice as required by the UK Department for Environment Food & Rural Affairs). As these shells were obtained from a restaurant rather than directly from the study site, we are not able to provide exact coordinates, however the restaurant confirm that they were harvested in Tomakomai on 3rd August 2019 at approximately 42°35′N, 141°36′E.

Age estimate of the two shells were obtained via preliminary identification of annual growth lines, which were later confirmed or adjusted based on the shells' $\delta^{18}O_{shell}$ profiles. One specimen (TBMOD1) is estimated to have been 28 years old at the time of collection, and the other (TBMOD2) is estimated at 26 years old. From this assessment, the four years sampled in this study are considered most likely to represent 1996-1999 (Figure 3).

Shells were sectioned using a geological trim saw with a 15.24 cm diameter diamond saw blade of 0.1 cm thickness. They were sectioned from the umbo to the shell edge by manually holding the shell in position along the axis of maximum growth, perpendicular to growth banding (Figure 1). After sectioning, the 'wing' on the anterior side was also cut away to leave a thick section. Sections were embedded with the cut surface corresponding to the axis of maximum growth face down in a plastic mould. using MetPrep 'Kleer-Set' polyester resin and hardener, and were left to cure overnight. Once fully cured, the side representing the axis of maximum growth was prepared using a series of silicon carbide grinding and polishing papers. These ranged from P180 up to P4000, producing a polished sampling surface and ensuring that no resin residue remained over the sampling area.

Resin blocks were mounted onto the sampling slide of a New Wave Research microdrill. This allows for high resolution computer-controlled drilling of the growth bands. A 0.3 mm diamond-coated dental drill bit was fitted to the micromill to sample incrementally across the shell section. A section of growth encompassing three annual growth bands was selected for each shell (Figure 3). This came from the middle of the shell's growth, when the shell is growing fast enough to provide wide increments but not from the earliest years of growth when the outer shell layer being targeted is thinner. The distance between samples (measured from the centre of one sample to the centre of the next) averaged 0.8 mm. The samples that coincided with nacreous growth lines were specifically noted. Samples were drilled over three passes at 100 μm depth per pass (300 μm total depth), with a scan speed of 100 μm/second. A total of 37 samples of aragonite powder were produced, and these each weighed around 50 μg.

Further samples were hand drilled from each shell to test for diagenetic alteration of the aragonite. These were analysed using FTIR-ATR, using the method outlined by Loftus et al. (2015), which differentiates aragonite and calcite polymorphs using the different vibrational characteristics of their molecular structure. Results showed no evidence of aragonite-to-calcite re-crystallisation, but the work of Moon et al. (2018) suggest that a lack of

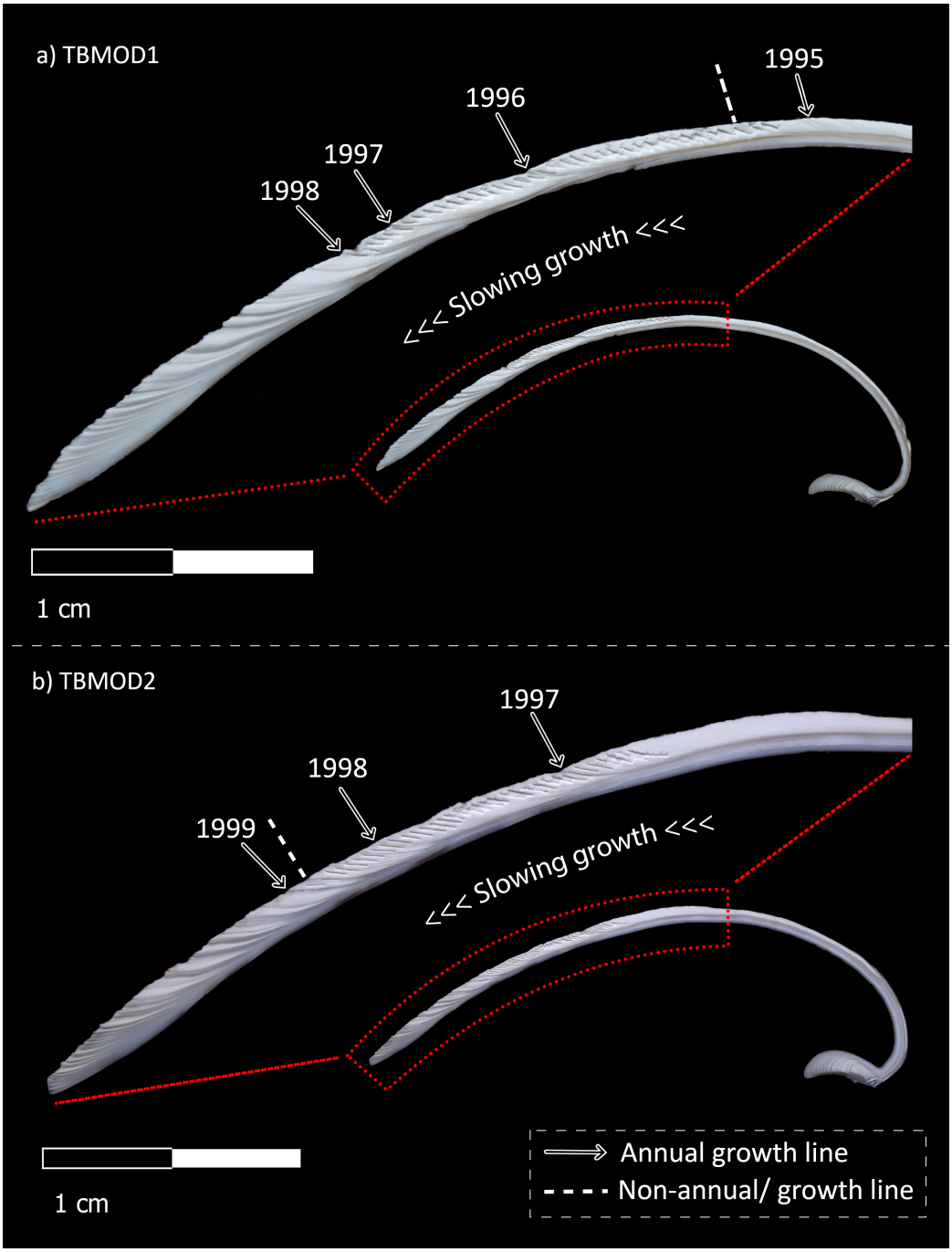

Figure 3: Image of sectioned *Spisula sachalinensis* valves showing completed micromill sampling and the location of growth lines. NB: the non-annual growth line marked by a dashed line in TBMOD1 has been partially obscured by the milling tracks.

calcite recrystallisation does not preclude diagenetic shifts in $\delta^{18}O_{shell}$.

Isotope ratio mass spectrometry was undertaken at the British Geological Survey in Keyworth, using an IsoPrime dual inlet mass spectrometer and Multiprep device. Sample isotope values are reported using the delta notation ($\delta^{18}O$, $\delta^{13}C$) in per mil, calculated on the VPDB scale using a laboratory standard calibrated against NBS-19. Nine calcite standards (KCM) were measured alongside every 31 samples. A calcite-acid fractionation factor, Craig correction (Craig, 1957), and drift correction (measured using standards at the start and end of the run) were applied to all measurements. The average analytical precision for KCM standards run at the same time was $\pm 0.05‰$ for $\delta^{18}O$ and $\pm 0.03‰$ for $\delta^{13}C$. An uncertainty of $\pm 0.05‰$ $\delta^{18}O$ would in turn correspond to an error of ± 0.24 °C in the reconstruction of water temperature at the time of formation, using the aragonite-SST equation:

$$SST = 20.6 - 4.34(\delta^{18}O_{carbonate} - (\delta^{18}O_{seawater} - 0.27)) \quad (1)$$

This is a slightly modified version of the original (Grossman and Ku, 1986) equation, where a -0.27‰ correction has been applied to the seawater $\delta^{18}O$ value ($\delta^{18}O_{seawater}$) to account for measurmenets on the modern VSMOW (Vienna Standard Mean Oceanic Water) scale rather than the now-defunct SMOW scale originally used (Dettman et al., 1999; Leng and Lewis, 2016). $\delta^{18}O_{seawater}$ was set at a constant $-0.85‰$, based on data from NASA's Global Seawater Oxygen-18 Database (Schmidt et al., 1999) (`https://data.giss.nasa.gov/o18data/`).

The modern observed SST range for the Tomakomai coast was accessed using the Physical Oceanography Distributed Active Archive Centre (PO-DAAC) satellite imagery database. Specifically, we used Level 4 data from the Canadian Meterological Center, which provides daily global SST measurements at a 0.2 degree resolution, combining infrared imagery with in-situ readings from ships and buoys to provide an estimate of foundation temperature, 'defined as the temperature of the water column just below the diurnal warm layer' (Marullo et al., 2016, pg. 8357). While several global satellite SST datasets exist within the Group for High Resolution Sea Surface Temperature (GHRSST), this particular dataset was the only one at Level 4 resolution to provide the required data through the 1990s. The *ghrsst* Python package developed for this work (Stuart, 2020) was used to access daily SST observations from the Tomakomai coast, from January 1992 to December

2002. From this, monthly averages were calculated (Table 1) to provide a general picture of the observed temperature range contemporary with the sampled period of shell growth. The range in average monthly temperature is 17.43 °C, while the maximum average annual temperature range (the difference between the average February minimum and August maximum) is 18.93 °C.

Table 1: Summary of average monthly observed SST (°C) for the Tomakomai coast for the period of 1992-2002. Daily readings were used to produce a minimum, maximum and mean reading for each month, which were then averaged over all years of available data. Raw data at: `https://podaac-opendap.jpl.nasa.gov/opendap/hyrax/allData/ghrsst/data/GDS2/L4/GLOB/CMC/CMC0.2deg/v2/contents.html`

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Average Min	3.40	2.27	2.38	3.16	5.79	10.55	14.72	19.21	17.82	13.43	9.26	5.68
Average	4.66	2.97	3.01	4.38	8.01	12.72	17.14	20.40	19.47	15.66	11.11	7.41
Average Max	6.03	3.01	3.71	5.86	10.36	14.87	19.36	21.20	21.20	17.79	13.26	9.27

4. Results

Of the 78 samples analysed for isotope composition, seven failed to produce enough gas for isotopic measurement. Isotope data are shown in Table 2. $\delta^{18}O_{shell}$ shows a range of 3.46‰, while $\delta^{13}C_{shell}$ range is 1.55‰. Linear regression analysis shows a weak and statistically insignificant negative correlation between $\delta^{18}O_{shell}$ and $\delta^{13}C_{shell}$ ($r = -0.18$, $p = 0.14$). $\delta^{18}O_{shell}$ results equated to a temperature range of 15.01 °C, with a minimum of 5.40 °C and a maximum of 20.41 °C (Table 2).

The stable isotope data (Figure 4) show clear peaks and troughs in $\delta^{18}O_{shell}$ in three cycles, matching the expected three-year sampling periods. In general the $\delta^{18}O_{shell}$ peaks appear broader than the corresponding troughs, leading to a 'cuspate' rather than sinusoidal wave appearance, as defined by Goodwin et al. (2003). As samples move towards the ventral growth edge (Figure 1) the periodicity of the wave decreases, corresponding to shortening growth increments.

Figure 5 shows SST calculations from $\delta^{18}O_{shell}$ overlain on observed SST variation for Tomakomai, illustrating how the $\delta^{18}O_{shell}$-derived temperatures fit against observed annual SST range for the local coast. In fig. 5, the

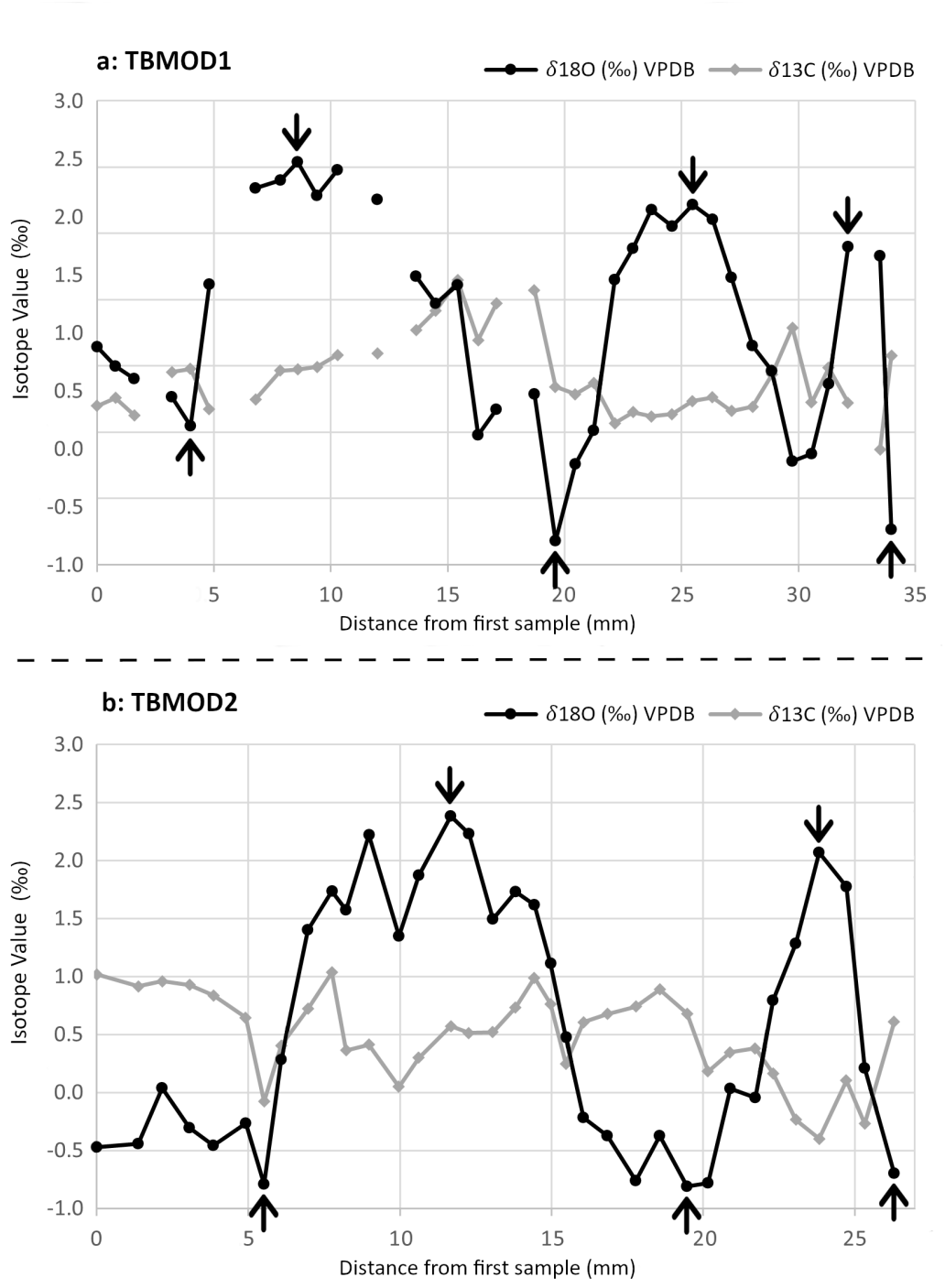

Figure 4: Graph plotting oxygen and carbon stable isotope results. Peaks and troughs in $\delta^{18}O_{shell}$ marked with arrows. Earliest/closest to umbo shell growth on the left.

Table 2: Stable isotope data from shells TBMOD1 and TBMOD2 alongside $\delta^{18}O_{shell}$-derived sea surface temperature (SST). Failed samples are greyed out. SST calculations have an uncertainty of ±0.24 °C due to analytical precision based on the aragonite-SST conversion defined in eq. (1).

Number	Sample ID	$\delta^{18}O$ (‰) VPDB	$\delta^{13}C$ (‰) VPDB	SST (°C)	Comments
1	TBMOD1-101	+0.64	+0.20	13.0	
2	TBMOD1-102	+0.50	+0.26	13.6	
3	TBMOD1-103	+0.40	+0.13	14.0	
4	TBMOD1-104	NA	NA	NA	Growth stoppage
5	TBMOD1-105	+0.26	+0.45	14.6	
6	TBMOD1-106	+0.05	+0.48	15.5	
7	TBMOD1_1	NA	NA	NA	
8	TBMOD1_2	+1.12	+0.17	10.9	
9	TBMOD1_3	NA	NA	NA	Growth stoppage
10	TBMOD1_4	+1.84	+0.25	7.8	
11	TBMOD1_5	+1.90	+0.46	7.5	
12	TBMOD1_6	+2.04	+0.47	6.9	
13	TBMOD1_7	+1.78	+0.49	8.0	
14	TBMOD1_8	+1.98	+0.58	7.2	
15	TBMOD1_9	NA	NA	NA	
16	TBMOD1_10	+1.75	+0.59	8.1	
17	TBMOD1_11	NA	NA	NA	
18	TBMOD1_12	+1.18	+0.77	10.6	
19	TBMOD1_13	+0.97	+0.92	11.5	
20	TBMOD1_14	+1.11	+1.15	10.9	
21	TBMOD1_15	-0.02	+0.69	15.8	
22	TBMOD1_16	+0.17	+0.97	15.0	
23	TBMOD1_17	NA	NA	NA	
24	TBMOD1_18	+0.28	+1.07	14.5	
25	TBMOD1_19	-0.82	+0.34	19.3	Growth stoppage
26	TBMOD1_20	-0.24	+0.28	16.8	
27	TBMOD1_21	+0.01	+0.37	15.7	
28	TBMOD1_22	+1.15	+0.07	10.8	
29	TBMOD1_23	+1.38	+0.15	9.7	
30	TBMOD1_24	+1.68	+0.12	8.5	
31	TBMOD1_25	+1.55	+0.14	9.0	
32	TBMOD1_26	+1.71	+0.23	8.3	
33	TBMOD1_27	+1.60	+0.26	8.8	
34	TBMOD1_28	+1.17	+0.16	10.7	
35	TBMOD1_29	+0.65	+0.19	12.9	
36	TBMOD1_30	+0.46	+0.43	13.7	
37	TBMOD1_31	-0.22	+0.79	16.7	

38	TBMOD1_32	-0.16	+0.22	16.5	Growth stoppage
39	TBMOD1_33	+0.37	+0.48	14.2	
40	TBMOD1_34	+1.40	+0.22	9.7	
41	TBMOD1_35	NA	NA	NA	
42	TBMOD1_36	+1.33	-0.13	10.0	
43	TBMOD1_37	-0.74	+0.58	18.9	Growth stoppage
44	TBMOD2-1	-0.47	+1.02	17.8	
45	TBMOD2-2	-0.44	+0.91	17.7	
46	TBMOD2-3	+0.04	+0.96	15.6	
47	TBMOD2-4	-0.30	+0.93	17.1	
48	TBMOD2-5	-0.45	+0.84	17.7	
49	TBMOD2-6	-0.26	+0.65	16.9	
50	TBMOD2-7	-0.79	-0.08	19.2	Growth stoppage
51	TBMOD2-8	+0.28	+0.41	14.5	
52	TBMOD2-9	+1.40	+0.72	9.7	
53	TBMOD2-10	+1.73	+1.04	8.2	
54	TBMOD2-11	+1.57	+0.36	8.9	
55	TBMOD2-12	+2.22	+0.41	6.1	
56	TBMOD2-13	+1.35	+0.05	9.9	
57	TBMOD2-14	+1.87	+0.30	7.6	
58	TBMOD2-15	+2.38	+0.57	5.4	
59	TBMOD2-16	+2.23	+0.51	6.1	
60	TBMOD2-17	+1.50	+0.52	9.2	
61	TBMOD2-18	+1.73	+0.74	8.2	
62	TBMOD2-19	+1.62	+0.99	8.7	
63	TBMOD2-20	+1.11	+0.77	10.9	
64	TBMOD2-21	+0.48	+0.25	13.7	
65	TBMOD2-22	-0.21	+0.61	16.7	
66	TBMOD2-23	-0.37	+0.68	17.4	
67	TBMOD2-24	-0.76	+0.74	19.0	
68	TBMOD2-25	-0.37	+0.89	17.4	
69	TBMOD2-26	-0.81	+0.68	19.2	Growth stoppage
70	TBMOD2-27	-0.78	+0.18	19.1	Growth stoppage
71	TBMOD2-28	+0.04	+0.35	15.6	
72	TBMOD2-29	-0.04	+0.38	15.9	
73	TBMOD2-30	+0.80	+0.16	12.3	
74	TBMOD2-31	+1.29	-0.23	10.2	
75	TBMOD2-32	+2.07	-0.40	6.8	
76	TBMOD2-33	+1.78	+0.10	8.0	
77	TBMOD2-34	+0.21	-0.27	14.8	
78	TBMOD2-35	-0.70	+0.61	18.8	
79	TBMOD2-36	-1.08	-0.09	20.4	Growth stoppage
	MIN	-1.08	-0.40	5.4	
	MAX	+2.38	+1.15	20.4	
	RANGE	3.46	1.55	15.0	
	AVERAGE	+0.70	+0.45	12.7	

$\delta^{18}O_{shell}$-derived SST results have been normalised to account for this slowing growth (and therefore decreasing width in annual growth increments) using a piecewise constant dilation factor. The distance between samples within a growth increment was multiplied by *maximum increment width/width of particular growth increment*. This means that each year of growth appears equal in length, whilst maintaining the original relative spacing between samples within each increment. This was then placed over the satellite-observed SST curve for the relevant years to provide the best visual fit.

5. Discussion

5.1. How well does $\delta^{18}O_{shell}$ data match modern SST observations?

Overall, it is clear that estimates of SST based on $\delta^{18}O_{shell}$ follow a broadly sinusoidal pattern, and none fall outside of the observed range for the collection location. This reinforces the conclusion of Khim et al. (2000), that $\delta^{18}O_{shell}$ in *S. sachalaninensis* can be interpreted as a proxy for subannual cycling of marine temperatures. The results in fig. 4 show shortening period in the later years of shell growth, in keeping with expectations from the ecological literature for *S. sachalinensis*, which suggests significantly slowed growth after individuals reach ~eight years (Sasaki, 1981).

Due to the nature of shell growth, as well as the sampling methodology used, some 'dampening' of the true seasonal signal (Goodwin et al., 2003) is to be expected in such proxies; although micromill sampling resolution is high compared to other techniques, it will still result in some averaging over the true extremes in $\delta^{18}O_{shell}$, which is exacerbated by decreasing increment width as a shell's ontological age increases. We can see this 'dampening' effect in these results, with a calculated temperature range of 15.01 °C, while the range for average observed SST range was higher at 17.43 °C. The overall average temperature across the observed satellite data was 10.62 °C, while the average of the $\delta^{18}O_{shell}$-derived SST calculations is two degrees higher at 12.7 °C. Nonetheless, we can observe a good fit between observed and calculated temperatures, particularly in the overall shape of the temperature curve and around the seasonal peaks in SST. As will be discussed in Section 4.3, this bias towards warmer temperatures and poorer fit around SST minima is unexpected given the seasonality of growth line formation.

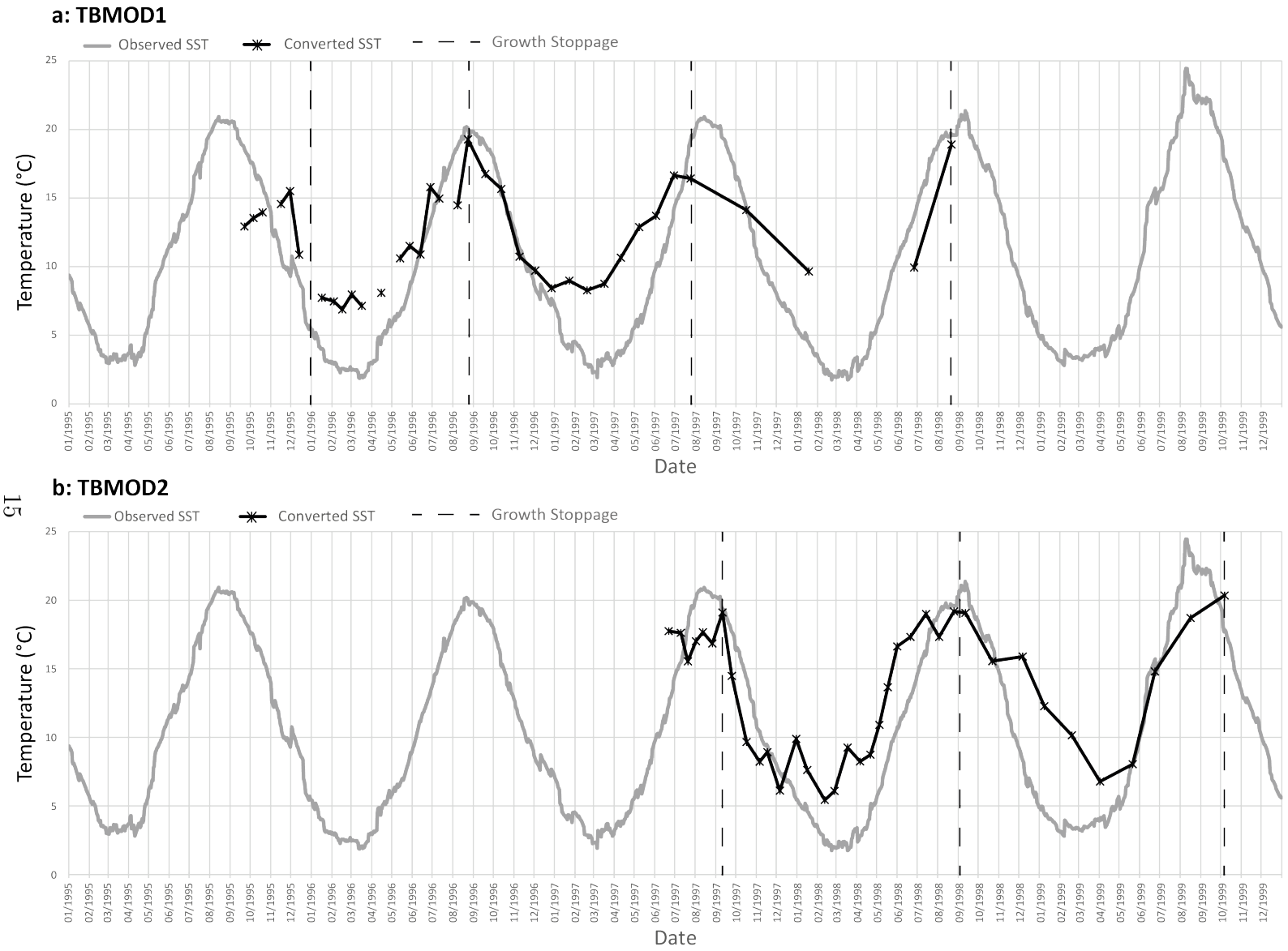

Figure 5: Temperature estimation based on $\delta^{18}O_{shell}$ results, overlain on average annual SST range for Tomakomai (table 1) where the annual period has been piece-wise linearly transformed to account for decreasing growth rate.

5.2. Interpretation of $\delta^{13}C_{shell}$ Results

Interpreting variation in $\delta^{13}C_{shell}$ values in marine carbonates is complex, because it involves several interacting biological and chemical processes such as surface productivity and dissolved carbon content, which in turn depend on the particular oceanographic context in terms of (e.g.) thermocline depth, prevailing currents, upwelling and salinity (Arthur et al., 1983a,b; Gillikin et al., 2006; McConnaughey and Gillikin, 2008; **?**). In this case, $\delta^{13}C_{shell}$ results show significantly less cyclicity than $\delta^{18}O_{shell}$, with a total amplitude of just 1.55‰ (Figure 4), and a very weak negative correlation between $\delta^{13}C_{shell}$ and $\delta^{18}O_{shell}$. This is similar to the findings of Khim et al. (2000), who also see a smaller overall range and relative stability in $\delta^{13}C_{shell}$ compared to $\delta^{18}O_{shell}$ for this species. They argue that this is related to the shallow growth-depth of *S. sachalinensis*, which live primarily above the thermocline and therefore do not record any major seasonal cycling in DIC. Although we lack growth-depth information for the shells sampled here, we consider that the situation is likely to be similar. The small degree of seasonal cycling that is seen (slightly higher $\delta^{13}C_{shell}$ in the summer/autumn) could relate to increased primary productivity and phytoplankton biomass in the warmer season (Agboola et al., 2013). Overall though, the lack of in situ instrumental records (e.g. DIC, salinity) from Tomakomai Bay, as well as detailed species-specific information on the metabolism and physiological incorporation of $\delta^{13}C$, prevents us from drawing more definitive conclusions on the potential of $\delta^{13}C_{shell}$ as a palaeoenvironmental indicator in *S. sachalinensis*.

5.3. Potential confounding factors to $\delta^{18}O_{shell}$ interpretation

One of the main aims of this work was to identify the seasonality of growth minima in *S. sachalinensis* from Tomakomai, as regular interruptions in shell growth result in biased (towards the season of fastest growth) or incomplete SST reconstructions. The ecological literature suggests that growth lines can be triggered variously by excessively warm water (Sasaki, 1981), cold water (Yamamoto, 1947) or by the end of the spawning season (Kato and Hamai, 1975) which falls in spring/early summer for Hokkaido (Kinoshita and Shibuya, 1920; Sakurai et al., 1992; Sasaki, 1981; Takamaru, 1976). In this project, nacreous growth lines were identified during sampling so that the samples representing these minima could be identified in the results. Based on the relationship we have established between $\delta^{18}O_{shell}$ and SST, we can use these results to identify when growth lines form in the seasonal growth cycle.

The dashed lines in fig. 5 show the locations of growth lines identified during sampling. These data confirm that growth lines occurred on an annual basis, matching expectations from the literature. In all cases where the growth line sample yielded δ^{18}O data, that sample or one directly adjacent provided the annual peak in SST. This indicates that the growth lines form in later summer/autumn when SST is highest (table 1). This matches the findings of Sasaki (1981), who attributes this aestival growth interruption to shells located at the southern limits of the species' distribution. Our results show that an aestival growth minimum is not restricted to the southernmost distribution, and extends at least 500 km north of Sendai Bay to southern Hokkaido (the region of most importance for modern commercial harvesting).

For each shell sampled, there was one growth line identified that did not fit this warm-season growth pattern. These are labeled in fig. 3. On the basis of the isotope results, we suggest that these represent non-annual growth interruptions. Although regular growth lines are expected to form due to predictable annual factors (e.g. seasonal SST, spawning), it is well known that other stressful events can also trigger the formation of growth lines in mollusc species. For example, Schöne (2008) notes that significant storms, or the exposure of shells during neap tides, can also cause this response. In this case, it is possible that a non-annual environmental stressor is responsible for the growth line at sample three, which was then (mis)interpreted as an annually formed growth line. While it is not obvious from the satellite-observed record, it is worth noting that spring 1995 to spring 1996 was a La Niña event recorded in Japanese waters, indicating colder than average SST (http://ds.data.jma.go.jp/tcc/tcc/products/elnino/ensoevents.html). A potential connection between non-annual growth interruptions and ENSO events in this region warrants further investigation. In cases with subannual sampling and clear seasonal cycling, we don't anticipate misinterpretation of non-annual growth interruptions will be an issue for palaeoenvironmental reconstructions based on isotope evidence, as the combination of macroscopic sclerochronology and $\delta^{18}O_{shell}$ allows for annual and exceptional growth lines to be differentiated.

If researchers were to target annual samples based on growth line analysis of this species then misinterpretation of growth lines could result in errors where certain years are inadvertently sampled twice. This also poses a problem for the accurate aging of individual *S. sachalinensis* specimens, for example in this study the assignment of calendar years to the growth increments was carried out using a count of growth lines seen on the shell.

While the isotope results from the region of shell sampled meant that the non-annual lines identified could be factored into this assignment, it is certainly possible that further non-annual lines in the region of shell not sampled could also have been misinterpreted which would shift the calendar age of the sampled region. While none of the discussion in this study rests upon exactly accurate identification of the increments' calendar dates (excepting the possible connection between the non-annual growth line and 1995/6 La Niña event mentioned above), but in future research this is certainly an issue which should be examined closely and we suggest that where possible isotope analysis should supplement visual examination of growth lines to accurately identify whether they represent an annual growth stoppage.

Based on the positions of the growth lines in the $\delta^{18}O_{shell}$ cycle (aside from the exception outlined above), the shells grew preferentially in the cooler part of the year, with growth slowing or stopping completely during the warmest SSTs in late summer/autumn. By comparison to observed satellite data fig. 5, it appears that growth stoppage occurs during approx. August/September/October. This might suggest that $\delta^{18}O_{shell}$ would not reflect SST extremes during the warmer period of the year. As previously discussed, we would ordinarily expect some degree of 'dampening' of the seasonal temperature extreme from $\delta^{18}O_{shell}$, meaning that it is difficult to tell whether growth slows or stops completely during the formation of the growth line. In either case, we would expect that SST reconstruction would be biased towards the cooler part of the year. In practice, our results show annual SST minima consistently falling outside of the observed range and a minimum calculated SST at 6.9 °C compared to a satellite-observed minimum of 2.27 °C. This suggests that the isotope SST results are slightly offset from the observed satellite data towards warmer temperatures, which in turn means it is fiddicult to give a more precise estimate of the exact timing and duration of the growth stoppages. In explaining this offset, there are several potential contributing factors that we should consider, all of which are also more generally relevant in terms of the 'dampening' and palaeoclimatic interpretation of seasonal marine carbonate isotope proxies, as discussed below.

5.3.1. Estimating $\delta^{18}O_{seawater}$

As the other variable alongside $\delta^{18}O_{shell}$ in the aragonite palaeotemperature equation, misestimation of $\delta^{18}O_{seawater}$, as well as seasonally variable $\delta^{18}O_{seawater}$ has a significant impact on the accuracy of SST reconstructions. In modern cases such as this, the NOAA database can provide a regional

measurement, although this is not necessarily a close geographical match, and does not account for variation relating to seasonal currents or depth. In our data, for the difference between observed and calculated minimum SST to be accounted for by a misestimation of $\delta^{18}O_{seawater}$, the $\delta^{18}O_{seawater}$ used would have needed to be at least ~0.25‰ lower (at -1.1‰). This would bring the average $\delta^{18}O_{shell}$-derived SST down to 10.68 °C and in line with the average SST observed from satellite data (10.62 °C).A $\delta^{18}O_{seawater}$ value of -1.1‰ would be out of the expected range for the wider region; according to NASA data, within a c. 10 degree (lat/long) radius of Tomakomai bay, the minimum observed $\delta^{18}O_{seawater}$ was -0.89‰. Furthermore, based on salinity data from the Japan Oceanographic Data Center (`https://data.giss.nasa.gov/cgi-bin/o18data`) and the salinity:$\delta^{18}O_{seawater}$ equation for the Tsushima current provided by Kodaira et al. (2016), we see that minimum $\delta^{18}O_{seawater}$ is not reached until May, and total seasonal variation is only c. 0.4‰. As the placement of the growth lines indicate warm-season growth interruptions, we would expect that the SST recorded in shell growth to be biased towards colder-season growth with an average temperature less than that of the observed data. To achieve this, an even larger reduction in $\delta^{18}O_{seawater}$ would be needed.

Freshwater fluvial input is another consideration, and has the potential to affect coastal $\delta^{18}O_{seawater}$ values without necessarily impacting the measurements of the NOAA database which have been taken further from the shore. Tomakomai bay receives fluvial input from several minor rivers, the largest of which is the Yufutsu river, but unfortunately no $\delta^{18}O$ measurements appear to have be published relating to these inputs. Measurements of precipitation from Tomakomai provide an average annual $\delta^{18}O$ of -9.7‰(Li, 2017). If shells were harvested from an area of the bay particularly affected by riverine input then this could result in more negative $\delta^{18}O_{seawater}$ values than the -0.85‰used in the palaeotemperature equation here which would help to explain the temperature offset. Unfortunately, without more precise knowledge of the shell harvest location or in-situ measurements of near-shore $\delta^{18}O_{seawater}$ we are unable to confirm that this is the case. This is therefore an avenue which should be further explored in future studies of this species and region.

5.3.2. Diurnal temperature variation

If extremes of temperature can inhibit shell growth on a seasonal basis, it follows that this could also affect growth on a daily basis if diurnal variation is regularly bringing seawater temperature outside of the preferred range.

This could also contribute to biases or 'dampening' in the proxy record. For the Tomakomai coast, diurnal warming (ΔSST) is expected to be small, with models predicting <1 °C ΔSST (Noh et al., 2011). This small variation is confirmed by comparing combined day- and night-time satellite SST data. Therefore, even if *S. sachalinensis* consistently precipitated preferentially during the warmest part of the day during the cooler months, this would not account for the offset in $\delta^{18}O_{shell}$-derived winter temperatures.

5.3.3. Growth depth

In the other available paper addressing stable isotope results for *S. sachalinensis*, the extent to which the full SST range was recorded varied between sampled individuals (Khim et al., 2000). The authors compare $\delta^{18}O_{shell}$-derived SST range to observed SST (using the Grossman and Ku (1986) equation), although they do not present the specific SST calculations or specify the $\delta^{18}O_{seawater}$ value used. Nevertheless, they did not posit any species-wide offset in estimated SST and were confident in the use of *S. sachalinensis* as a SST proxy. Non-equilibrium precipitation is rare in marine molluscs (McConnaughey and Gillikin, 2008; Twaddle et al., 2016), and it would be surprising in this species given that isotopic equilibrium has been shown for its Atlantic sibling, *S. solidissima* (Jones et al., 1983). Instead, Khim et al. (2000) suggest that the differences they observe could relate to the depth at which shells precipitated, although conflicting evidence exists as to whether shallower growth-depth corresponds to increased or decreased annual $\delta^{18}O_{shell}$ range (Geary et al., 1992; Krantz et al., 1987).

As this study is based on data from just two shells, and without detailed information on growth-depth, it is difficult to discuss the potential effects of this factor. Still, it is logical that growth-depth would significantly impact calculated SST, as variable temperature stratification in the top 10 metres of the water column may generate differences between growth-depth water temperature and foundation SST (Göttsche and Olesen, 2017) as modelled from satellite data. For example, Sakaizawa et al. (2018) show that for the Tomakomai coast the temperature at 5 m depth in February is ~0.5 °C warmer than the surface temperature. This magnitude of difference would go some, but not all the way to explaining the differences seen between $\delta^{18}O_{shell}$-derived SST results and observed cool-season temperatures. Increased species-specific understanding of the relationship between growth depth and $\delta^{18}O_{shell}$ would be also useful, especially for studies which discuss modern ecology or oceanography.

5.3.4. Sample treatment

Another possible explanation for anomalies seen in the proxy record could be the treatment of the samples after the shell collection that might lead to temperature offsets. Boiling of the shells is a potential concern for stable isotope analysis, as it has the potential to encourage isotopic exchange and so alter the original $\delta^{18}O_{shell}$ value. Milano et al. (2016) showed that for the aragnoitic portion of gastropod *Phorcus turbinatus*, boiling at 100 °C did not produce significant isotopic exchange. They did see isotopic exchange in hotter temperatures (e.g. 300+ °C) and roasting conditions, but shifts in $\delta^{18}O_{shell}$ were also accompanied by visible changes in shell microstructure. Müller et al. (2017) suggested that $\delta^{18}O_{shell}$ may be slightly lowered by boiling, but do acknowledge that the differences seen between theirs and Milano et al.'s (2016) results were likely primarily due to experimental design. On the other hand, Moon et al. (2018) found that for *Spisula solidissima*, a shift in $\delta^{18}O_{shell}$ can occur even with 'negligible' mineralogical change and no calcite recrystallisation. This study looked at dry heat rather than boiling, but nonetheless raises the issue of heat-induced shifts in $\delta^{18}O_{shell}$, even for shells which appear to preserve seasonal cycling. For the shells used in this study, the length of boiling was <20 minutes, so significantly under the 40 minutes used by Müller et al. (2017), and no changes in shell microstructure are visible to the naked eye.

Moon et al. (2018) suggest that isotopic exchange is possible even without visible change in shell microstructure, and according to Müller et al. (2017), boiling for 15 minutes can result in a change of -0.5‰ in $\delta^{18}O_{shell}$. In this study FTIR-ATR (attenuated total reflectance Fourier transform infrared spectroscopy) was used to specifically check for recrystallisation of shell aragonite and no evidence was found. This means that extreme diagenetic alteration (of aragonite into calcite) has not occurred, but there is still the possibility that a small but significant systemic shift in $\delta^{18}O_{shell}$ could have occurred. Correcting for a -0.5‰ shift, as per Müller et al. (2017) would result in the $\delta^{18}O_{shell}$-derived SST results shifting down towards colder temperatures with an overall average temperature of 9.60 °C. This would be more in keeping with the observed SST.

5.4. Implications for palaeothermometry

One aim of this research was to assess the utility of *S. sachalinensis* as a palaeothermometer in archaeological studies. As outline in the introduction, the importance of marine diet over the past 7000 years of Hokkaido's history

means that marine carbonates are a common element of archaeological assemblages, and the unique hunter-gatherer development in Hokkaido means that there are gaps in our palaeoenvironemntal understanding of this region as compared to much of the rest of Japan,

In terms of carbon isotopes, our results show a slight but statistically insignificant covariance between high $\delta^{18}O_{shell}$/low $\delta^{13}C_{shell}$, with slightly higher $\delta^{13}C_{shell}$ values in summer and autumn potentially related to primary productivity. However, due to the lack of clear seasonal cycling and the complex relationship between $\delta^{13}C_{shell}$, oceanography and climate in similar species, we can't currently suggest a way that $\delta^{13}C_{shell}$ in *S. sachalinensis* can be employed as an effective subannual palaeoclimatic indicator.

By contrast, there is a strong case for the use of stable isotope analysis on *S. sachalinensis* as a palaeothermometer. Clear cyclicity can be seen in $\delta^{18}O_{shell}$, and based on comparison to observed SST and the position of growth lines, we are confident that ambient water temperature is the main driver. $\delta^{18}O_{shell}$ therefore has the potential to provide information on annual and sub-annual climate which should add important climatic and oceanographic context to the archaeological record.

However, as previously discussed and as expected for proxies of this kind, $\delta^{18}O_{shell}$ in this species does not necessarily appear to record the complete annual range of SST. There are a number of factors relating to the specific growth conditions, local oceanography and sample treatment that should be considered as far as possible within a palaeoclimatic or archaeological context. For example, exact specimen growth depth will always be an unknown quantity when dealing with archaeological material, but assuming a relatively consistent depth range of 1-15 m for *S. sachalinensis* as well as the fact that prehistoric gatherers would have been more likely to collect preferentially at shallow depths, this uncertainty can be fairly well constrained.

The growth pattern of the species, with rapid growth allowing subannual incremental sampling until eight to nine years of age, means that *S. sachalinensis* could be used to produce a SST proxy at either an inter- or intra-annual resolution. Furthermore, if young, fast-growing, shells are found in an archaeological context it should be possible to use growth-edge samples to estimate season of collection and/or site occupation. This technique was first proposed by Shackleton (1973), and has since been a common feature of stable isotope research in archaeomalacology (e.g. Bosch et al., 2018; Burchell et al., 2013; Jew et al., 2013; Mannino et al., 2007). However, it relies on fast-growing increments at the shell edge so would only be appropri-

ate for archaeological *S. sachalinensis* ontogenetically younger than ~eight years. Given archaeological biases towards larger, more meat-rich specimens, suitable shells are not likely to be commonly encountered except in cases of local resource over-exploitation.

Clumped isotope measurements as a palaeoclimatic proxy within *Spisula sachalinensis* may be a means to circumvent some of the issues identified here. This approach has been successfully used to provide seasonal climate reconstructions from Cretaceous bivalves (de Winter et al., 2020), and has the benefit of being independent from estimations of past $\delta^{18}O_{seawater}$ (Eiler, 2007; Henkes et al., 2013), which we have already noted among the confounding factors of temperature interpretation. This will be especially relevant for prehistoric and deep-time bivalves where historical records of seawater composition can't be relied upon, but could also be useful in cases of extreme seasonal variation in $\delta^{18}O_{seawater}$ which can be hard to incorporate into palaeoclimate models.

6. Conclusions

These results confirm the inverse relationship between $\delta^{18}O_{shell}$ and SST in *Spisula sachalinensis*, as previously identified for long-lived clam species in other regions, and indicate that shells from Tomakomai form a warm-season growth line. When compared to satellite-recorded SST, $\delta^{18}O_{shell}$-derived temperatures fall within the expected range for the region and show clear seasonal cycling. This demonstrates the potential utility of this species as a SST archive in both modern and palaeoclimatic contexts. Possible biases in this record include sample treatment, growth depth and $\delta^{18}O_{seawater}$, but further investigation is required to determine the extent to which these affect SST estimation.

This study also highlights complexities in *S. sachalinensis* growth that suggest the need for caution during macro/microscopic sclerochronological assessment. For example, not only is there is evidence for variation in growth line formation both between geographically distant shells (e.g. at the southern or northern limits of distribution) but we have also shown that this variation can occur within a single shell (e.g. the anomalous cool-season line observed in TBMOD1). Given these issues, we suggest that stable oxygen isotope analysis in combination with visual macro- and microscopic assessment of shell growth is the most reliable route to *S. sachalinensis* palaeothermometry.

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