

RESEARCH ARTICLE

Carbon and Oxygen Isotope Records from *Tridacna derasa* Shells: Toward Establishing a Reliable Proxy for Sea Surface Environments

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Citation: Yamanashi J, Takayanagi H, Isaji A, Asami R, Iryu Y (2016) Carbon and Oxygen Isotope Records from *Tridacna derasa* Shells: Toward Establishing a Reliable Proxy for Sea Surface Environments. PLoS ONE 11(6): e0157659. doi:10.1371/journal.pone.0157659

Editor: Geerat J. Vermeij, University of California, UNITED STATES

Received: January 12, 2016

Accepted: June 2, 2016

Published: June 21, 2016

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: This work was supported in part by KAKENHI (Grant-in-Aid for Scientific Research; <http://www.jsps.go.jp/english/e-grants/index.html>) (grant numbers 21340152 and 26302008 to YI) from the Japan Society for the Promotion of Science (JSPS). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

We report the carbon ($\delta^{13}\text{C}$) and oxygen ($\delta^{18}\text{O}$) isotope records of three modern *Tridacna derasa* shells from Ishigaki-jima, southwestern Japan. The high-resolution $\delta^{13}\text{C}$ profiles of samples from the inner shell layer on cross-sections fall within similar narrow ranges and display no regular variations or trends, such as an ontogenetic trend or abrupt short-term drops likely to be related to reproductive activity. This suggests that the calcification site of this species is not likely affected by photosynthetic CO_2 uptake or CO_2 incorporation during respiration. The $\delta^{18}\text{O}$ profiles show distinct seasonal cycles. The intraspecific variability in the $\delta^{18}\text{O}$ values is small in parts of the shell precipitated in the adult stage, but is not negligible in the juvenile and senescent stages. The differences in the monthly and seasonally resolved $\delta^{18}\text{O}$ values among shells are less than 0.51‰ and 0.76‰, respectively. The shell $\delta^{18}\text{O}$ values are nearly identical or close to the $\delta^{18}\text{O}$ values for aragonite precipitated in oxygen isotope equilibrium with ambient seawater ($\delta^{18}\text{O}_{\text{EA}}$). The largest differences between the shell $\delta^{18}\text{O}$ and $\delta^{18}\text{O}_{\text{EA}}$ values calculated from the monthly and seasonally resolved data correspond to an overestimate of the seawater temperature by as much as 1.7°C and 2.3°C, respectively. However, these differences are smaller in the adult stage (<0.25‰) than in the other stages. This small difference allows an accurate reconstruction of the seawater temperature with an error of <1.1°C. Consequently, we recommend that multiple shell records be obtained because of the non-negligible intraspecific variations in their $\delta^{18}\text{O}$ values. Growth banding, composed of alternating narrow white bands and wide light-grey bands, is discernible on cross-sections of the inner shell layer. The $\delta^{18}\text{O}_{\text{shell}}$ data indicate that they were formed in winter and the other seasons, respectively.

Introduction

High-resolution paleoenvironmental records are required from various localities over the globe to understand past climate dynamics and predict future climate change. Marine carbonate-

Competing Interests: The authors have declared that no competing interests exist.

secreting organisms, such as corals and mollusks, are sensitive to the ambient environment and preserve various types of environmental information in their skeleton in the form of physical (e.g., increment width) or geochemical variations [1–3]. Of these records, the oxygen isotope ratios ($\delta^{18}\text{O}$) of biogenic carbonates has widely been used to reconstruct paleoenvironments because it commonly reflects both sea-surface temperatures (SSTs) and the $\delta^{18}\text{O}$ values of the ambient seawater ($\delta^{18}\text{O}_{\text{sw}}$) in which the carbonates were secreted [4–7]. Numerous paleoenvironmental studies have investigated the biogenic carbonates of foraminifers [8, 9], corals [10–14], mollusks [15–19], and brachiopods [20–24].

The tridacnids (Subfamily Tridacninae Lamarck, 1819 [25]) are some of the largest bivalves, with shell length being up to 1 m at a maximum, in geological history and have been a prominent member of Indo-Pacific coral reef communities since the Eocene [26]. All tridacnids live in the euphotic zone and are associated with unicellular algal symbionts (zooxanthellae). This association gives rise to unusually high calcification rates, attributed to light-enhanced calcification [27, 28]. The tridacnids form dense aragonitic shells with annual lines and daily growth bands in their inner shell layer [29, 30], which allow the reconstruction of paleoenvironmental change, even on a subdaily time scale [31]. The isotopic data collected previously indicate that tridacnids precipitate their shells in oxygen isotopic equilibrium with seawater [32, 33]. Patzöld et al. (1991) [34] showed that the biogenic (daily growth banding) and geochemical ($\delta^{18}\text{O}$ values) records in the inner shell layer are more suitable for paleoenvironmental reconstruction than those of the outer shell layers or hinge, so the former have been used for paleoenvironmental studies [2, 30, 35–38]. Therefore, we also studied the isotopic records in the inner shell layer.

However, some issues remain to be resolved when using tridacnid $\delta^{18}\text{O}$ values as reliable paleoenvironmental proxies. One of the most critical issues is that previous studies of tridacnid $\delta^{18}\text{O}$ values predominantly dealt with isotopic data from a single shell of a single taxon, so neither the inter- nor intraspecific variations were fully considered [2, 30, 35–37]. In this article, we first report the intraspecific (= intershell) variations in carbon ($\delta^{13}\text{C}_{\text{shell}}$) and oxygen ($\delta^{18}\text{O}_{\text{shell}}$) isotope ratios of samples from the inner shell layer on cross-sections of modern *Tridacna derasa* (Röding, 1798) [39]. The studied materials were three shells collected from Ishigaki-jima, Ryukyu Islands, southwestern Japan (Figs 1 and 2) [40]. We then compare the $\delta^{18}\text{O}_{\text{shell}}$ profiles of this species with each other and with oceanographic data around Ishigaki-jima (Figs 3–6). In this way, we show the extent to which the $\delta^{18}\text{O}_{\text{shell}}$ values of *T. derasa* reliably record oceanographic conditions, especially SSTs.

Materials and Methods

Study site and climate regime

The Ryukyu Islands are located to the southwest of mainland Japan and are composed of several tens of islands and islets (Fig 1A). These islands are arranged in a curved row, known as the Ryukyu Island Arc. Most of the islands are rimmed by well-developed fringing coral reefs, although they are located near the northern limit of the coral reef province in present-day oceans.

Ishigaki-jima is located in the southwestern part of the Ryukyus (24°19′–37′N, 124°4′–21′E; Fig 1A). The climate on the island is subtropical (Table 1). The monthly mean atmospheric temperature measured at the Ishigaki Meteorological Observatory ranges measured at from 18.6°C (January) to 29.5°C (July), with an annual mean of 24.3°C. Semidiurnal tides are clearly evident throughout the islands, with a maximal range of 1.9 m at spring tide and 1.0 m at neap tide. The annual rainfall reaches 2100 mm, with rainy months in May–June and August–October. The prevailing wind is SSE in summer and NNW in winter. A monitoring record from the

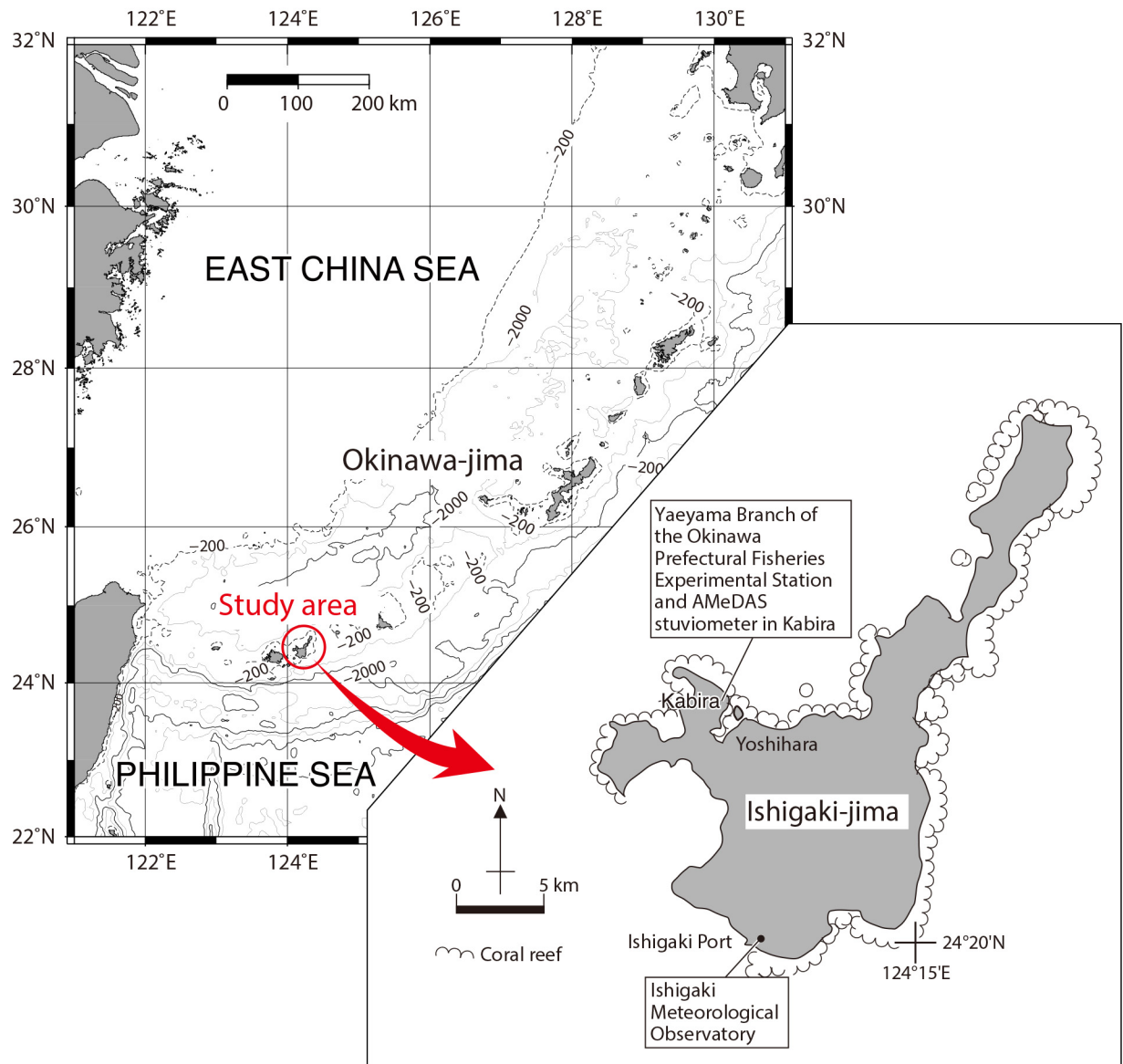


Fig 1. Location and map of the study site. (A) Study area in Ishigaki-jima (Ryukyu Islands). Bathymetric data are from ETOPO1 Global Model [40]. (B) Map of Ishigaki-jima.

doi:10.1371/journal.pone.0157659.g001

sea surface at Ishigaki Port during 1998–2004 showed an average sea surface salinity (SSS) of 34.3, generally ranging from 33.6 to 35.0 and associated with short-term drops of <2 caused by heavy rainfall [41]. The Integrated Global Ocean Services System (IGOSS) data (1° resolution gridded data, centered at 24°30'N and 124°30'E) for 1993–2011 suggest that the SSTs varied between 22.3°C and 31.2°C, with an annual mean of 26.5°C. The highest and lowest monthly mean SSTs were 30.6°C in August and 22.6°C in February, respectively [42].

Tridacna derasa

There are currently eight described species within the genus *Tridacna* Bruguière, 1797 [43, 44]. They are among the most conspicuous marine invertebrates on coral reefs due to their large

KTd-1



KTd-4



KTd-5

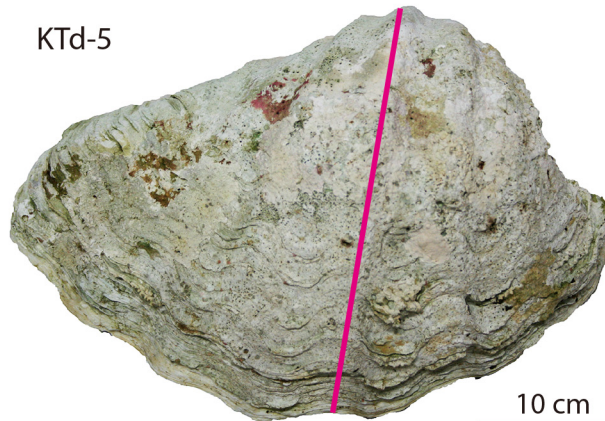


Fig 2. Photographs of the studied *Tridacna derasa* shells. The shells (left valves) were cut vertically (pink lines) along their maximum growth axes.

doi:10.1371/journal.pone.0157659.g002

size and brilliantly colored mantle that contains photosynthesizing symbionts [44]. Of these species, *T. derasa* shells were examined in this study. *Tridacna derasa*, *T. gigas*, *T. crocea*, *T.*

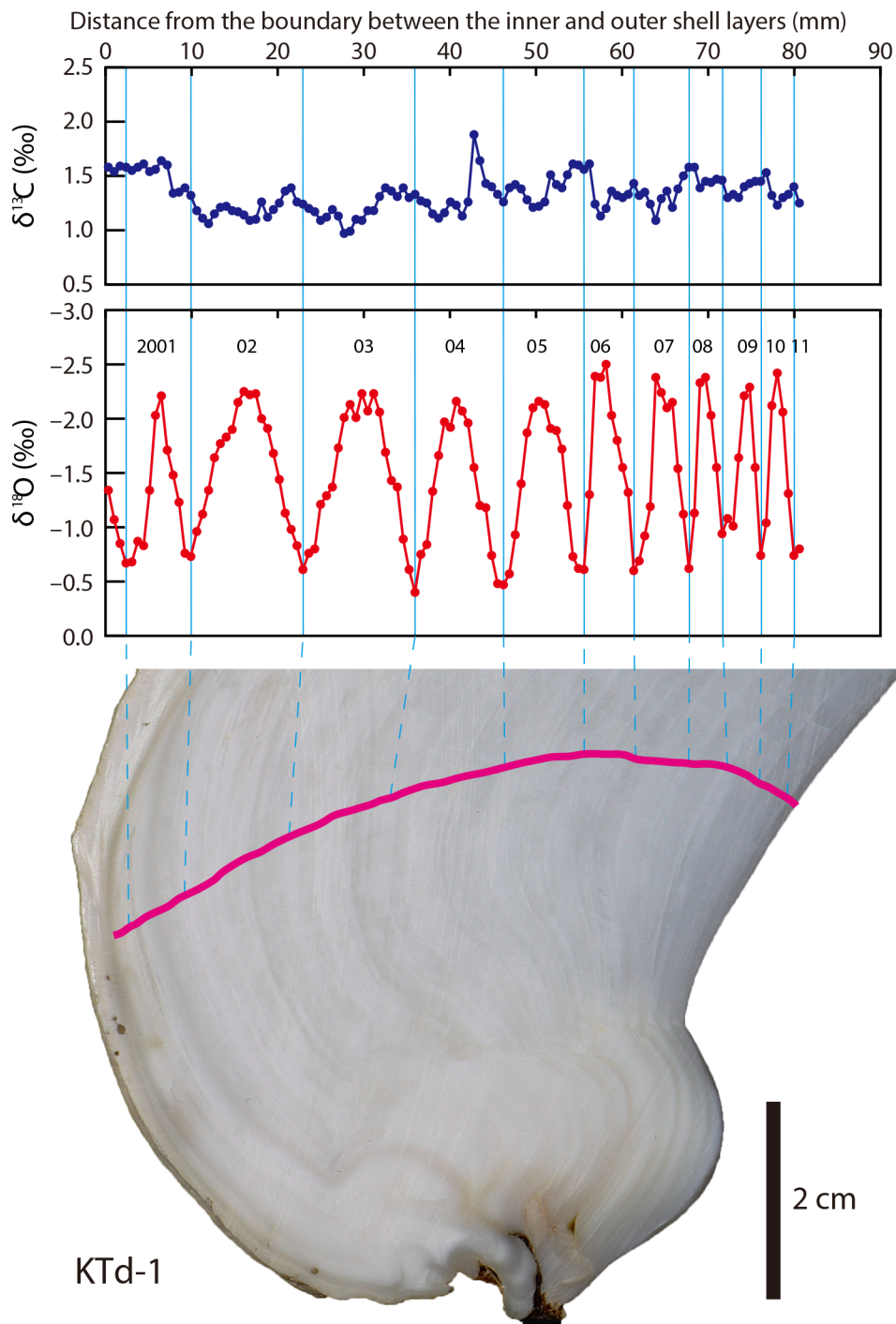


Fig 3. $\delta^{13}\text{C}_{\text{shell}}$ and $\delta^{18}\text{O}_{\text{shell}}$ profiles of a *Tridacna derasa* shell (KTd-1). Pink line indicates the sampling transect.

doi:10.1371/journal.pone.0157659.g003

squamosa, and *T. maxima* are widely distributed in the Indian and Pacific Oceans, with the latter two extending their distribution into the Red Sea, whereas *T. squamosa*, *T. rosewateri*, and *T. mbalavuana* have restricted distributions (Red Sea, Mauritius, and Fiji to Tonga, respectively) [44]. Shell length of *T. derasa* reaches 50–60 cm. They live byssally anchored at a

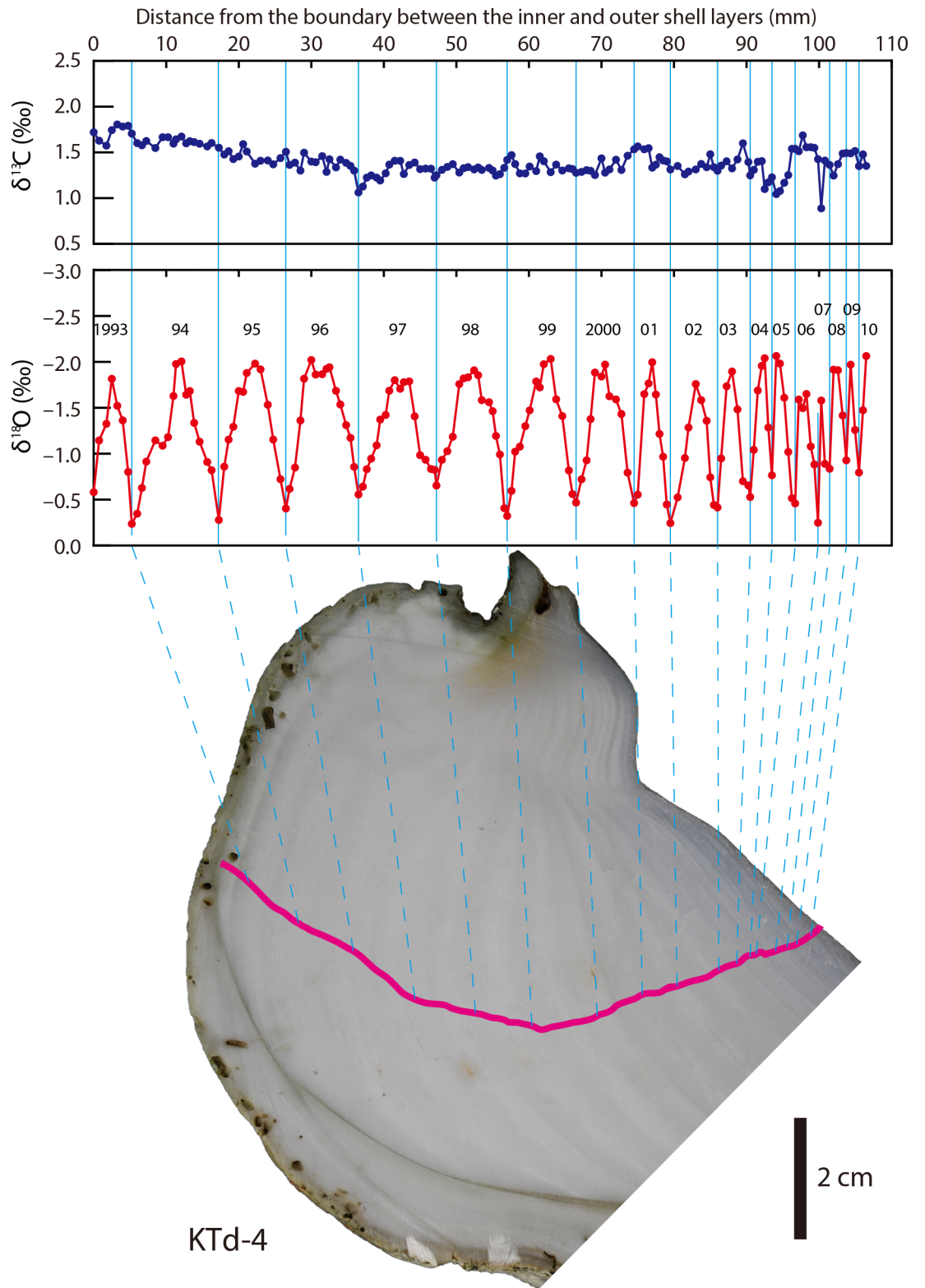


Fig 4. $\delta^{13}\text{C}_{\text{shell}}$ and $\delta^{18}\text{O}_{\text{shell}}$ profiles of a *Tridacna derasa* shell (KTd-4). Pink line indicates the sampling transect.

doi:10.1371/journal.pone.0157659.g004

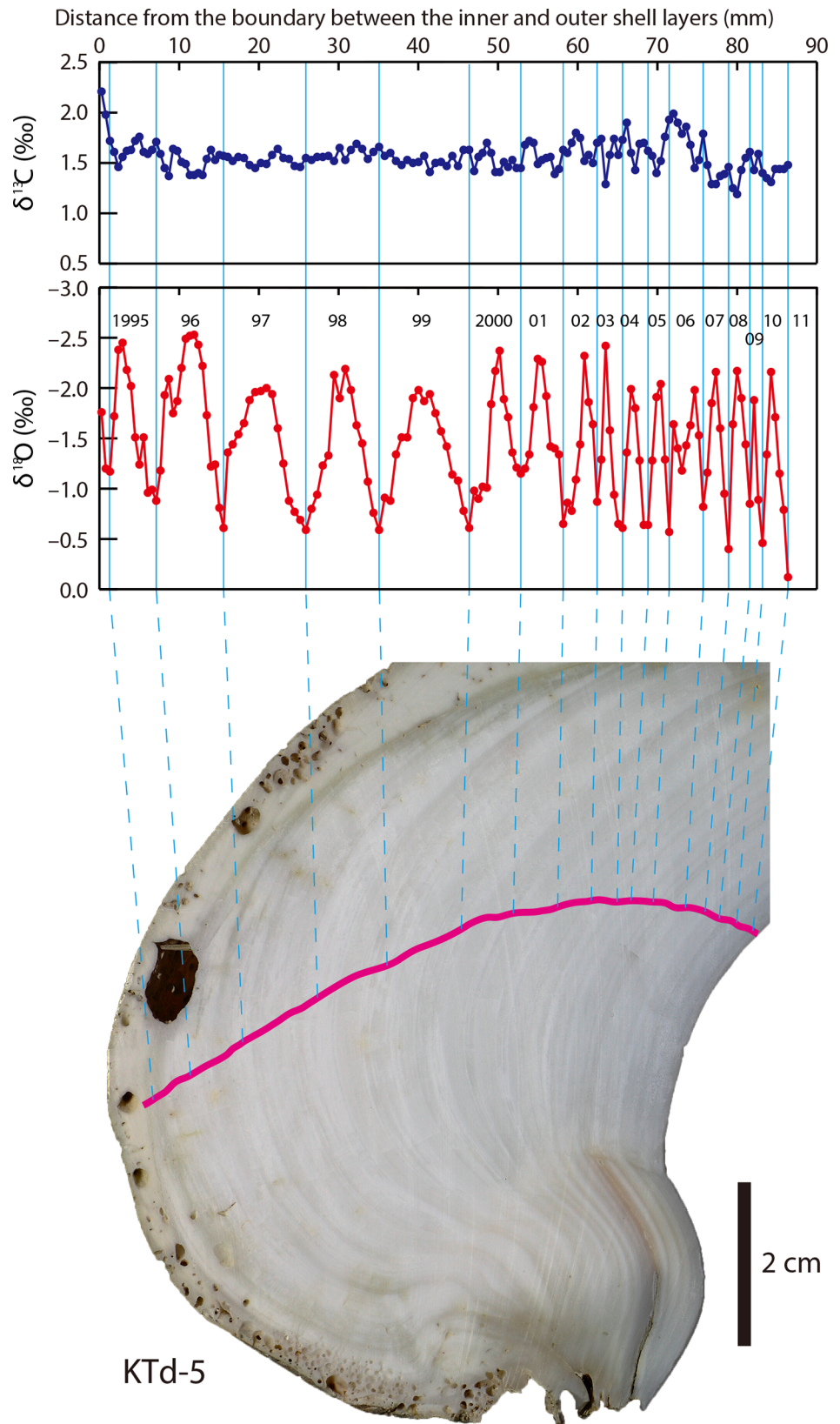


Fig 5. $\delta^{13}\text{C}_{\text{shell}}$ and $\delta^{18}\text{O}_{\text{shell}}$ profiles of a *Tridacna derasa* shell (KTd-5). Pink line indicates the sampling transect.

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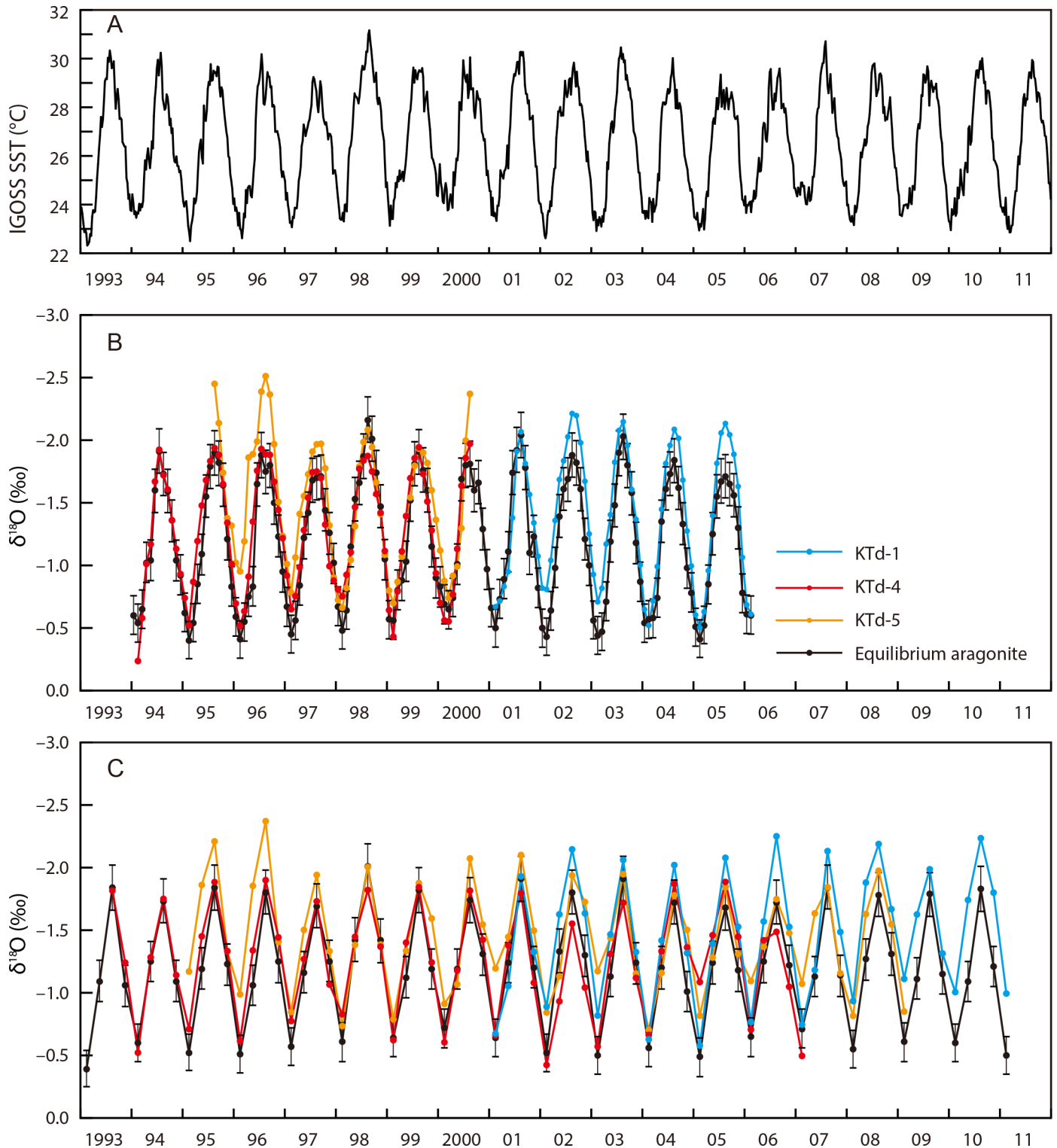


Fig 6. Comparison of time series of sea surface temperature and $\delta^{18}\text{O}_{\text{shell}}$ values for *Tridacna derasa* shells. (A) IGOSST SST data around Ishigakiji-jima (1° resolution gridded data, centered at $24^\circ 30' \text{N}$ and $124^\circ 30' \text{E}$) during the period 1993–2011. (B) Monthly resolved $\delta^{18}\text{O}_{\text{shell}}$ profiles of the KTd-1, KTd-4, and KTd-5 shells and equilibrium aragonite. (C) Seasonally resolved $\delta^{18}\text{O}_{\text{shell}}$ profiles of the KTd-1, KTd-4, and KTd-5 shells and equilibrium aragonite. Each error bars indicates ± 1 standard error.

doi:10.1371/journal.pone.0157659.g006

Table 1. Climate in Ishigaki-jima.

	Atmospheric temperature (°C) ¹	Sea surface temperature (°C) ²	Rainfall (mm) ¹
Annual mean	24.3	26.4	2106.8
Mean monthly maximum	29.5 (July)	30.6 (August)	261.6 (August)
Mean monthly minimum	18.6 (January)	23.1 (February)	126.3 (December)

¹ Data for 1981–2010 are from the Japan Meteorological Agency

² Integrated Global Ocean Services System (IGOSS) data (1° resolution gridded data, centered at 24°30'N and 124°30'E) for 1994–2011.

doi:10.1371/journal.pone.0157659.t001

juvenile stage, but when approaching maturity their byssus glands atrophy, and the adult clams take up unattached existence on coral rubble or hard packed sand [45]. Phylogenetic analyses indicated that the divergent time was estimated to be ~10 Ma between *T. derasa* and *T. gigas-T. mbalavuana* [44].

Materials

Three *T. derasa* shells, designated KTd-1, KTd-4, and KTd-5 (Fig 2), were collected in 1990 (KTd-4 and KTd-5) and 1998 (KTd-1) at the stage of fertilized egg and grown at a depth of 2 m in a large culture pond at the Yaeyama Branch of the Okinawa Prefectural Fisheries Research and Extension Center located at Kabira, Ishigaki-jima, Ryukyu Islands, southwestern Japan (24°28'N, 124°09'E; Fig 1B). They were collected on 7 May 2011 (KTd-4 and KTd-5) and 12 May 2011 (KTd-1). The water temperature and salinity in the pond were roughly equivalent to those of the outer ocean because the seawater was always pumped up from the outer ocean into the pond. The shell height and length of KTd-1 were 25.8 cm and 40.4 cm, respectively, those of KTd-4 were 26.6 cm and 41.7 cm, respectively, and those of KTd-5 were 25.3 cm and 38.3 cm, respectively. After the soft tissue was removed, a ~1 cm thick slab was cut vertically from each shell along the maximum growth axis (Fig 2). The inner and outer shell layers were clearly discernible on each slab. Carbonate samples for isotope analysis were manually obtained along a roughly median line on the inner shell layer at ~0.5–0.7 mm intervals using drill bits with diameters of 0.6 mm and 1.0 mm (Figs 3–5). The numbers of carbonate samples obtained from KTd-1, KTd-4, and KTd-5 were 121, 163, and 149, respectively.

Isotope analysis

Stable isotope analyses of the shell aragonite were performed with a Thermo Scientific Delta V Advantage mass spectrometer, coupled with a ThermoQuest Kiel III Carbonate Device, at the Graduate School of Science, Tohoku University, Sendai, Japan. The $\delta^{13}\text{C}_{\text{shell}}$ and $\delta^{18}\text{O}_{\text{shell}}$ values were calibrated for the NBS-19 international standard relative to VPDB. The external precision (1σ) for the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ analyses, based on replicate measurements of the laboratory reference sample (Jct-1; [46]), were $\pm 0.02\text{‰}$ and $\pm 0.04\text{‰}$ ($n = 112$), respectively. The correlations between the $\delta^{13}\text{C}_{\text{shell}}$ and $\delta^{18}\text{O}_{\text{shell}}$ values were evaluated with a reduced major axis regression technique [47], the significance of which was examined statistically with a two-sided Pearson test and a 95% confidence limit.

The distance domain $\delta^{18}\text{O}_{\text{shell}}$ profiles clearly showing seasonal cycles were converted to time series for better interpretation and comparison with those of aragonite precipitated in oxygen isotope equilibrium with ambient seawater (equilibrium aragonite), because it is well known that the aragonitic shells of tridacnids are generally precipitated in oxygen isotope equilibrium with ambient seawater [30, 32]. The temporal resolution of the $\delta^{18}\text{O}_{\text{shell}}$ values, represented by the number of isotope data per year, varied from ~19 days to ~4 months per sample.

Therefore, we converted the distance domain $\delta^{18}\text{O}_{\text{shell}}$ profiles to time series with peak-to-peak matching (i.e., annual maximum and minimum values in a year) with the equilibrium aragonite $\delta^{18}\text{O}$ profiles using AnalySeries software [48]. The time for the data point of annual maximum and minimum $\delta^{18}\text{O}_{\text{shell}}$ values were assigned to February and August, respectively. The monthly and seasonally resolved shell $\delta^{18}\text{O}$ profiles were then calculated on the assumption of constant growth between each shell $\delta^{18}\text{O}$ peak. The $\delta^{13}\text{C}_{\text{shell}}$ time series were generated simultaneously.

Results

$\delta^{13}\text{C}_{\text{shell}}$ and $\delta^{18}\text{O}_{\text{shell}}$ values

The $\delta^{13}\text{C}_{\text{shell}}$ profiles of KTD-1, KTD-4, and KTD-5 showed no distinct cycles (Figs 3–5). The $\delta^{13}\text{C}_{\text{shell}}$ values for KTD-1, KTD-4, and KTD-5 ranged from 0.97‰ to 1.88‰ (average = 1.33‰, σ (standard deviation) = 0.17‰), from 0.89‰ to 1.81‰ (average = 1.40‰, σ = 0.15‰), and from 1.19‰ to 2.21‰ (average = 1.56‰, σ = 0.15‰), respectively (S1 Table).

The $\delta^{18}\text{O}_{\text{shell}}$ profiles are characterized by a series of regular cycles of varying amplitudes and frequencies. The numbers of cycles in the profiles of KTD-1, KTD-4, and KTD-5 are 10, 17, and 16, respectively (Figs 3–5). The $\delta^{18}\text{O}_{\text{shell}}$ values for KTD-1, KTD-4, and KTD-5 range from -2.50‰ to -0.40‰ (average = -1.47‰, σ = 0.59‰), from -2.06‰ to -0.24‰ (average = -1.26‰, σ = 0.51‰), and from -2.53‰ to -0.12‰ (average = -1.46‰, σ = 0.54‰), respectively (S1 Table).

$\delta^{13}\text{C}_{\text{EA}}$ and $\delta^{18}\text{O}_{\text{EA}}$ values

We estimated the approximate ranges of the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of equilibrium aragonite ($\delta^{13}\text{C}_{\text{EA}}$ and $\delta^{18}\text{O}_{\text{EA}}$, respectively) using previously published $\delta^{13}\text{C}$ values of dissolved inorganic carbon ($\delta^{13}\text{C}_{\text{DIC}}$) and $\delta^{18}\text{O}_{\text{sw}}$, respectively, of seawater samples collected around Okinawa-jima [23] and Ishigaki-jima [41]. The $\delta^{13}\text{C}_{\text{DIC}}$ values at Kabira, where *T. derasa* grew, were assumed to be 1.1‰–1.6‰. Because the pH of the surface seawater at Ishigaki-jima ranged from 7.9 to 8.0, it was assumed that the $\delta^{13}\text{C}_{\text{HCO}_3^-}$ values for this seawater were ~0.2‰ greater than the $\delta^{13}\text{C}_{\text{DIC}}$ values [49–51]. Therefore, the $\delta^{13}\text{C}_{\text{EA}}$ values calculated using the $\delta^{13}\text{C}_{\text{DIC}}$ values (1.1‰–1.6‰) and the aragonite HCO_3^- -enrichment factor ($2.7 \pm 0.6\%$; [50]) should range from 3.4‰ to 5.1‰ at the *T. derasa* growth site.

The $\delta^{18}\text{O}_{\text{EA}}$ time series were calculated using the IGOSS SST data [42] and the monthly average $\delta^{18}\text{O}_{\text{sw}}$ values at Ishigaki Port, ~14 km south of the *T. derasa* growth site, during the period from December 1997 to May 2004, measured by Abe et al. (2009) [41] and based on the following equation [52]:

$$10^3 \ln \alpha_{\text{aragonite-water}} = (18.45 \pm 0.4) * 10^3 / T(K) - (32.45 \pm 1.5)$$

The $\delta^{18}\text{O}_{\text{sw}}$ values [41] varied between 0.09‰ and 0.29‰, and showed clear seasonal cycles, except for abrupt short-term drops (down to -0.30‰) during or just after heavy rainfall. The monthly average $\delta^{18}\text{O}_{\text{EA}}$ values were calculated, and range from -0.31‰ to -2.16‰ (annual mean of -1.18‰), which demonstrated distinct seasonal cycles (Fig 6). The annual maximum and minimum $\delta^{18}\text{O}_{\text{EA}}$ values were recorded in August and February, respectively.

Discussion

$\delta^{13}\text{C}_{\text{shell}}$ values

Because *T. derasa* is a zooxanthellae (symbiont)-bearing giant clam, its metabolic activity is expected to be closely related to organic carbon production by zooxanthellan photosynthesis,

which is considered to show seasonal cycles corresponding to those of solar radiation. However, the $\delta^{13}\text{C}_{\text{shell}}$ values show no seasonal cycles (Figs 3–5). This is true for the inner shell layers of not only *T. derasa* but also other tridacnids [35, 38, 53–55]. These indicate that such a link is unlikely because only minor amounts of metabolic carbon are incorporated into the bivalve shells as shown in non-zooxanthellate bivalves [56, 57].

The $\delta^{13}\text{C}_{\text{shell}}$ values show no statistically significant ontogenetic trend. Previous studies have shown the same results in the inner shell layers of other tridacnids [32, 35, 38, 55]. In contrast, distinct ontogenetic decreases in $\delta^{13}\text{C}$ values have been detected in some bivalves [58–60]. Based on the relationship between metabolic rate and body size, Lorrain et al. (2004) [60] attributed this ontogenetic decrease to the increased incorporation of respiratory CO_2 during growth. As mollusks grow, more metabolic (= respiration-derived) CO_2 becomes available to them, whereas the amount required for shell formation decreases, resulting in the incorporation of more metabolic carbon (^{12}C -enriched) into their shells. However, ontogenetic increases in $\delta^{13}\text{C}$ values are known from non-zooxanthellate and zooxanthellate (tridacnid) bivalve shells, indicating that the model proposed by Lorrain et al. (2004) [60] may not be a general model for all bivalves [19, 53].

The spawning period *Mytilus edulis* is reflected by more negative $\delta^{13}\text{C}_{\text{shell}}$ values, although the $\delta^{13}\text{C}_{\text{DIC}}$ is generally becoming more positive, which is explained by higher metabolic rates just after spawning, as energy lost during spawning is restored [61]. Vander Putten et al. (2000) [62] also reported these patterns in $\delta^{13}\text{C}_{\text{shell}}$ values in *M. edulis* as being a result of increased respiration associated with periods of higher food availability. However, no such negative peaks have been found in the $\delta^{13}\text{C}$ profiles of *T. derasa* or other tridacnid shells [32, 35, 38, 55].

It was shown that decadal variability of $\delta^{13}\text{C}_{\text{DIC}}$ values relating to phytoplankton productivity and large-scale ocean dynamics are possible causes of ontogenetic trends of $\delta^{13}\text{C}$ values from long-lived bivalve shells [63]. However, the three $\delta^{13}\text{C}_{\text{shell}}$ profiles of *T. derasa* are not long enough to discuss such relationships.

The relatively constant $\delta^{13}\text{C}_{\text{shell}}$ values, characterized by the absence of seasonal cycles, ontogenetic decreases, and abrupt short-term drops in $\delta^{13}\text{C}_{\text{shell}}$ that are attributable to reproductive activity, suggests that the calcification site of this species is not affected by CO_2 uptake resulting from photosynthesis or the incorporation of CO_2 from respiration. This is common in other tridacnid species [32, 35, 38, 55]. The $\delta^{13}\text{C}_{\text{shell}}$ and $\delta^{18}\text{O}_{\text{shell}}$ values show weak or no significant correlations (KTd-1, $r = 0.37$, $p < 0.05$; KTd-4, $r = 0.09$, $p < 0.05$; KTd-5, $r = 0.32$, $p < 0.05$; Fig 7), suggesting that there is no kinetic effect [64] or a very weak one on isotope fractionation during the precipitation of the carbonate-forming *T. derasa* shells.

The $\delta^{13}\text{C}_{\text{shell}}$ values are 1.0‰–2.2‰ lower than the lowest $\delta^{13}\text{C}_{\text{EA}}$ values (3.4‰). At present, we have no explanation why the $\delta^{13}\text{C}_{\text{shell}}$ values are so low relative to the $\delta^{13}\text{C}_{\text{EA}}$ values. Possible causative factors include the pH at the calcification site, as pH is known to affect the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of skeletal carbonates [51, 56, 65].

$\delta^{18}\text{O}$ values

The $\delta^{18}\text{O}_{\text{shell}}$ profiles are characterized by a series of regular seasonal cycles of varying amplitudes and frequencies. The amplitude of the cycles in the $\delta^{18}\text{O}_{\text{shell}}$ profiles ranges from 1.03‰ to 1.90‰ for KTd-1, from 1.19‰ to 1.73‰ for KTd-4, and from 1.16‰ to 2.04‰ for KTd-5. Because the seasonal amplitude of the $\delta^{18}\text{O}_{\text{sw}}$ values is $< 0.2\text{‰}$ (excluding the extremely low outliers recorded during or just after short-term heavy rainfall), the contribution of $\delta^{18}\text{O}_{\text{sw}}$ to the $\delta^{18}\text{O}_{\text{shell}}$ variations is less than $< 19\%$. Therefore, the cycles of the $\delta^{18}\text{O}_{\text{shell}}$ profiles correspond predominantly to seasonal changes in seawater temperature. $\delta^{18}\text{O}_{\text{shell}}$ profiles characterized by distinct seasonal cycles have previously been reported for the inner shell layers of tridacnids [30, 33–35, 37, 53, 54, 66].

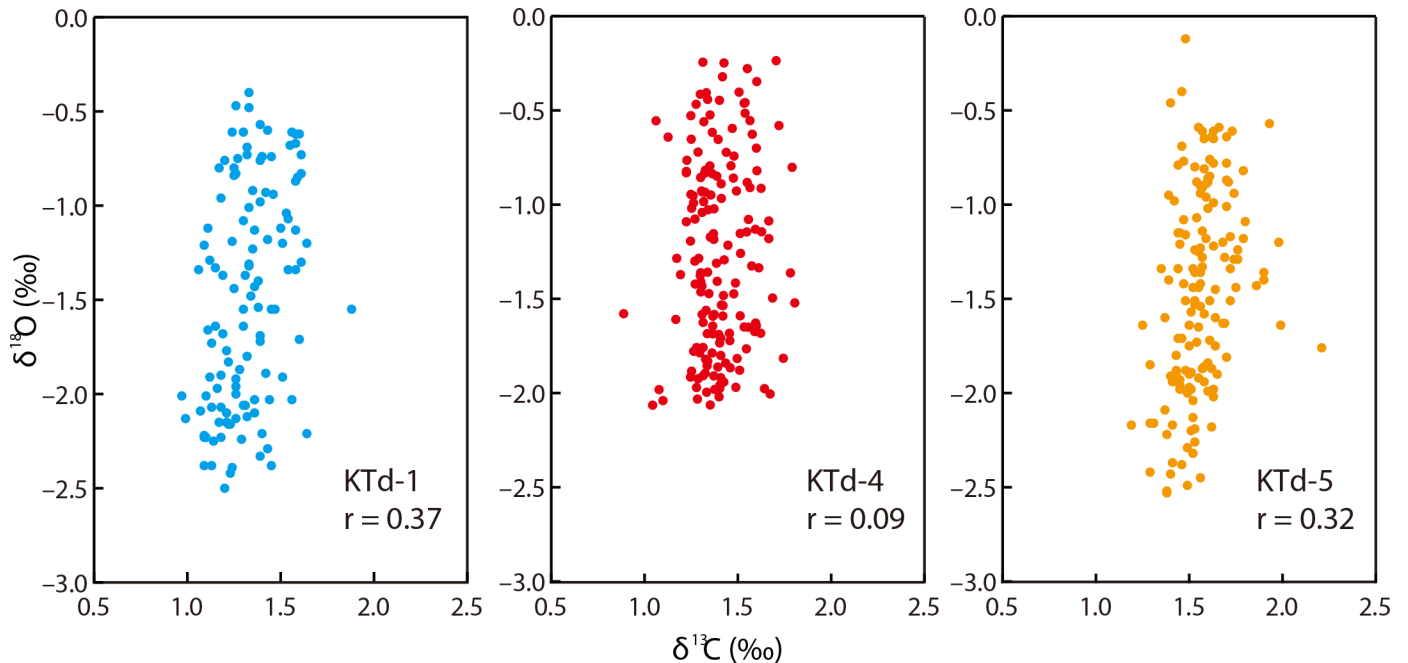


Fig 7. Cross-plots of $\delta^{13}\text{C}_{\text{shell}}$ versus $\delta^{18}\text{O}_{\text{shell}}$ for the studied *Tridacna derasa* shells. The $\delta^{13}\text{C}_{\text{shell}}$ and $\delta^{18}\text{O}_{\text{shell}}$ values show weak or no significant correlations.

doi:10.1371/journal.pone.0157659.g007

The aragonitic shells of tridacnids are known to be precipitated in oxygen isotope equilibrium with ambient seawater [30, 32]. However, the $\Delta\delta^{18}\text{O}$ values, defined as the $\delta^{18}\text{O}_{\text{shell}}$ values minus the $\delta^{18}\text{O}_{\text{EA}}$ values, are not negligible, ranging from -0.25‰ to 0.00‰ (KTd-1; $n = 11$), from -0.12‰ to 0.15‰ (KTd-4; $n = 14$), and from -0.38‰ to 0.00‰ (KTd-5; $n = 11$), if we calculate them using the $\delta^{18}\text{O}_{\text{shell}}$ and $\delta^{18}\text{O}_{\text{EA}}$ values for the coolest and warmest months in the monthly resolved data from the shell portion in which 12 or more samples for isotope analysis were collected (Fig 6B). The differences are larger if calculated from the summer and winter values using seasonally resolved data (KTd-1, -0.35‰ to 0.00‰ , $n = 21$; KTd-4, -0.45‰ to 0.07‰ , $n = 28$; KTd-5, -0.52‰ to 0.00‰ , $n = 29$) (Fig 6C). These differences generally cause the reconstructed seawater temperatures to be overestimated. The largest monthly and seasonally resolved $\Delta\delta^{18}\text{O}$ values correspond to differences of 1.7°C and 2.3°C , respectively. However, the monthly time series for $\delta^{18}\text{O}_{\text{shell}}$ agrees well with that for $\delta^{18}\text{O}_{\text{EA}}$, except for the period 1995–1997 and the warmest months in 2000 for the KTd-5 profile (Fig 6C). The small differences ($<0.25\text{‰}$) between $\delta^{18}\text{O}_{\text{shell}}$ and $\delta^{18}\text{O}_{\text{EA}}$ for 1997–2006 allow the accurate reconstruction of seawater temperatures, with an error of $<1.1^\circ\text{C}$. Cross-plots of $\delta^{18}\text{O}_{\text{shell}}$ versus $\delta^{18}\text{O}_{\text{EA}}$ indicate that these values are not completely identical, but correlate positively, with the slopes and intercepts of the regression lines ranging from 0.95 to 1.06 and from -0.23 to -0.12 , respectively, and the cross-correlation coefficients ranging from 0.73 to 0.91 ($p < 0.05$) for all monthly resolved data (Fig 8). If annual maximum and minimum $\delta^{18}\text{O}_{\text{shell}}$ values ($= \delta^{18}\text{O}_{\text{shell}}$ values for the coolest and warmest months, respectively) are used, the slopes and intercepts of the regression lines range from 0.99 to 1.05 and from -0.22 to -0.03 , respectively, and the cross-correlation coefficients are 0.91–0.97 ($p < 0.05$) (Fig 8). Taking into account the statistical errors ($\delta^{18}\text{O}_{\text{EA}}$ estimation and sampling errors), these results suggest that the seawater temperatures reconstructed from the $\delta^{18}\text{O}_{\text{shell}}$ values are largely the same as the actual temperatures.

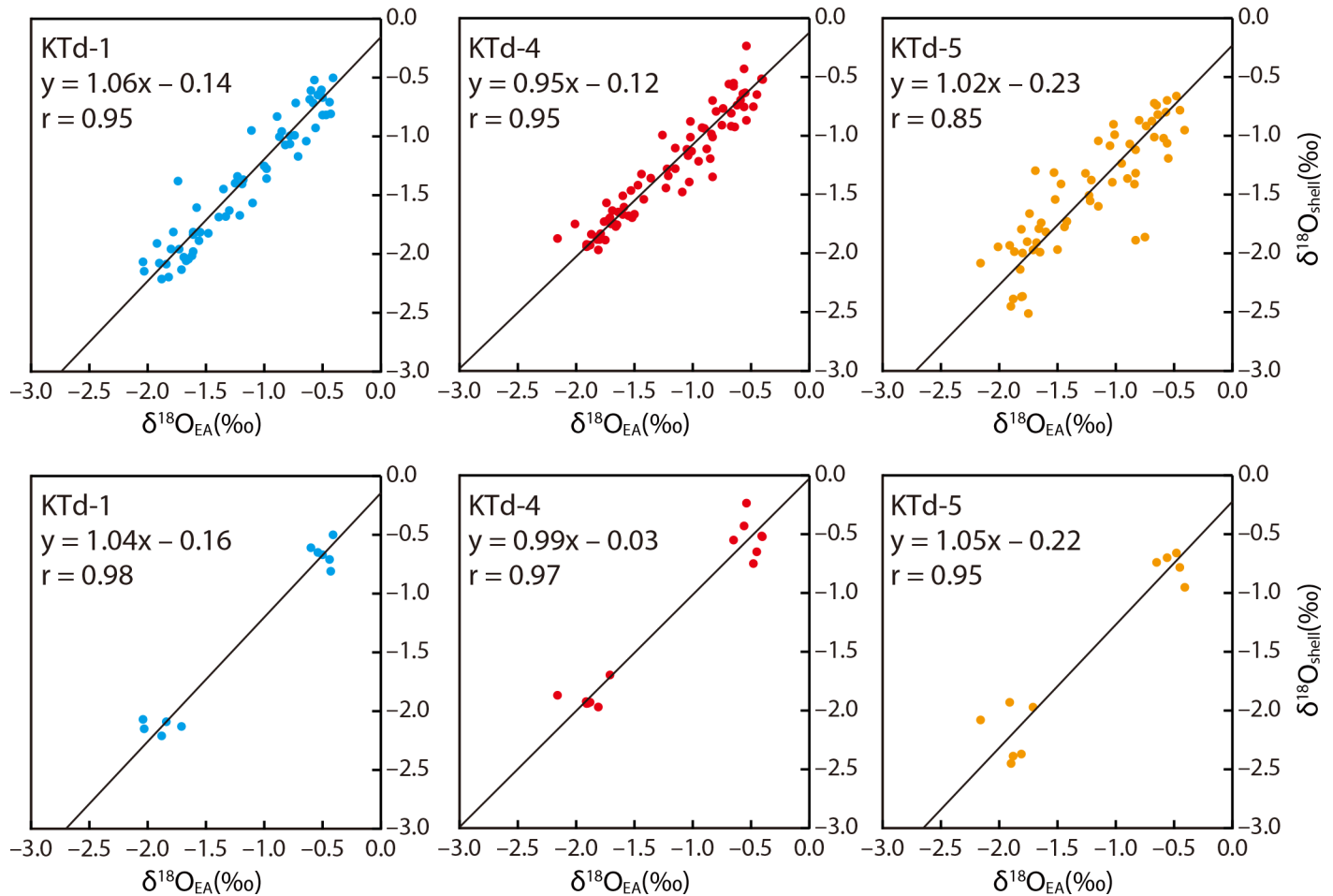


Fig 8. Cross-plots of monthly resolved $\delta^{18}\text{O}_{\text{shell}}$ versus $\delta^{18}\text{O}_{\text{EA}}$ for the studied *Tridacna derasa* shells. All monthly resolved data are shown in the upper row. Annual maximum and minimum $\delta^{18}\text{O}_{\text{shell}}$ values ($= \delta^{18}\text{O}_{\text{shell}}$ values for the coolest and warmest months, respectively) are indicated in the lower row. Black line is the regression line.

doi:10.1371/journal.pone.0157659.g008

The intraspecific variability in the $\delta^{18}\text{O}_{\text{shell}}$ values was relatively small in the period between 1997 and 2006 but relatively large in the periods between 1995 and 1997 (0.51‰ on monthly data and 0.46‰ on seasonal data between KTd-4 and KTd-5) and between 2006 and 2009 (0.76‰ between KTd-1 and KTd-4/5) (Fig 6). These differences may be due, at least in part, to different sampling resolutions and/or biological offsets in the juvenile and senescent stages of shell growth, which have been reported in several studies (brachiopods [23, 24, 67, 68], mollusks [69]). Therefore, the high (more than monthly) temporal resolution of the $\delta^{18}\text{O}_{\text{shell}}$ values in the adult stage of shell growth are most suitable for paleoenvironmental reconstructions using *T. derasa*.

The shells of *T. derasa* (and other tridacnids) have potential advantages over the skeletal carbonates of other organisms, such as brachiopods and corals, because they are precipitated very close to oxygen isotope equilibrium with ambient seawater at least along the axis of maximum growth, which allows the $\delta^{18}\text{O}_{\text{shell}}$ values from any part of the inner shell layer to be converted directly to seawater temperature if the contribution of $\delta^{18}\text{O}_{\text{sw}}$ is negligible. The secondary shell layer of brachiopod shells is believed to be precipitated in carbon and oxygen isotope equilibrium with ambient seawater. However, recent investigations have shown that

the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of modern brachiopod shells may be partly or wholly outside the range of those values for equilibrium calcite, which is attributed, at least in part, to kinetic and metabolic isotope fractionation effects [22–24, 67, 68, 70–73] or unidentified chemical conditions at the calcification sites [23]. It is well known that the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of coral skeletons deviate significantly from those of equilibrium aragonite because of the effects of kinetic and metabolic isotope fractionation [64]. Our study shows that the $\delta^{18}\text{O}_{\text{shell}}$ values for the adult growth stages of *T. derasa* shells, with little intraspecific variability, are in good agreement with the $\delta^{18}\text{O}_{\text{EA}}$ values. This relationship can be used to generate high-resolution $\delta^{18}\text{O}_{\text{shell}}$ time series of seawater temperatures and $\delta^{18}\text{O}_{\text{sw}}$ in coral reef environments. It should also be noted that the use of multiple shell samples provides a more reliable reconstruction of seawater temperatures, with an error of $<1.1^\circ\text{C}$.

Growth curves and growth lines

Seasonal variations in $\delta^{18}\text{O}_{\text{shell}}$ allowed the construction of growth curves for the tridacnid shells studied. A comparison of the $\delta^{18}\text{O}_{\text{shell}}$ and $\delta^{18}\text{O}_{\text{EA}}$ profiles indicated life spans of 10, 17, and 16 years for KTd-1, KTd-4, and KTd-5, respectively (Fig 6). The annual rate of shell thickening defined as a distance between annual $\delta^{18}\text{O}_{\text{shell}}$ maxima (= seawater temperature minima), which was measured perpendicular to growth lines/bands, was 5.0–15.5 mm/year during the juvenile to adult stages, and decreased to 1.0–7.2 mm/year during the senescent stage. The growth curves representing shell thickening (Fig 9) show similar shapes to those of many other organisms characterized by growth rates that are initially high and later low (tridacnids [32, 33, 74], bivalves [75], brachiopods [72]).

Clear growth banding, composed of alternating wide light-grey bands and narrow white bands, is discernible on cross-sections of the inner shell layer (Figs 3–5). Different terms have been used to describe the growth bands, depending partly on differences in the instruments

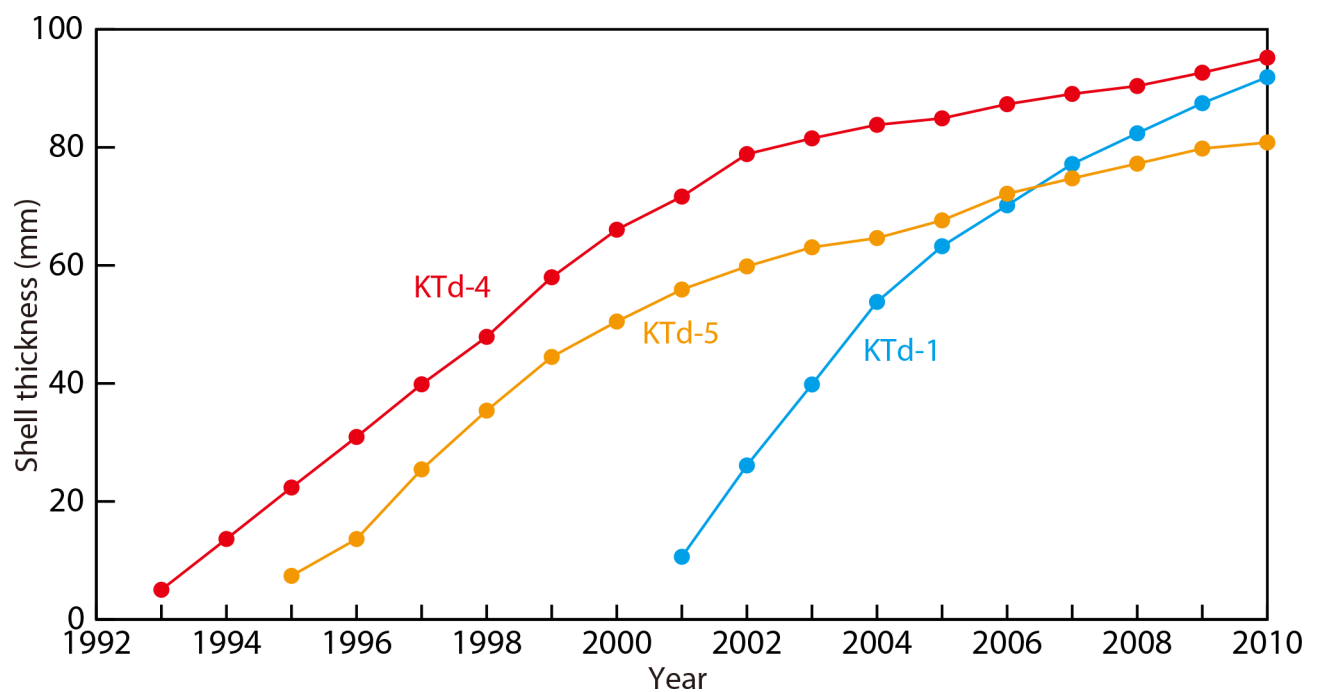


Fig 9. Annual rate of shell thickening of *Tridacna derasa* estimated from seasonal variations in $\delta^{18}\text{O}_{\text{shell}}$ values. Each annual increment represents a distance between annual $\delta^{18}\text{O}_{\text{shell}}$ maxima, which was measured perpendicular to growth lines/bands.

doi:10.1371/journal.pone.0157659.g009

used to observe them, such as transmitted [32, 36, 74] or reflected illumination [2, 55], which has led to confusion. In this study, we describe the growth bands based on observations made under reflected light. The annual $\delta^{18}\text{O}_{\text{shell}}$ maxima (= seawater temperature minima) correlated with the white growth bands. This indicates that the white bands were predominantly formed during winter, which is consistent with previous findings [2, 34, 36, 74].

Conclusions

We investigated the intraspecific variations in the $\delta^{13}\text{C}_{\text{shell}}$ and $\delta^{18}\text{O}_{\text{shell}}$ values of samples from the inner shell layers, taken from cross-sections, of three modern *T. derasa* shells from Ishigaki-jima, Ryukyu Islands, southwestern Japan. The results can be summarized as follows.

The $\delta^{13}\text{C}_{\text{shell}}$ profiles of the samples fall into a relatively narrow range and show no seasonal cycles, ontogenetic decreases, or abrupt short-term drops that might be related to reproductive activity. These observations suggest that the calcification site of *T. derasa* is unlikely to be affected by CO_2 uptake or influx caused by photosynthesis or respiration, respectively. The $\delta^{13}\text{C}_{\text{shell}}$ values and the $\delta^{18}\text{O}_{\text{shell}}$ values show no or very weak positive correlations, indicating no or little kinetic isotope fractionation during the carbonate precipitation of the *T. derasa* shells.

The $\delta^{18}\text{O}_{\text{shell}}$ profiles are characterized by distinct cycles corresponding to seasonal changes in seawater temperature. The $\delta^{18}\text{O}_{\text{shell}}$ values are usually greater than the $\delta^{18}\text{O}_{\text{EA}}$ values by up to 0.38‰ and 0.52‰ when calculated from monthly and seasonally resolved data, respectively. These differences lead to seawater temperature to be overestimated by 1.7°C and 2.3°C, respectively. However, these differences are small (<0.25‰) in the parts of the shell that formed in the adult stage, which allows the reconstruction of accurate seawater temperatures with an error of <1.1°C. Therefore, the high-temporal-resolution $\delta^{18}\text{O}_{\text{shell}}$ data from the adult stage are most suitable for paleoenvironmental reconstructions using *T. derasa*. However, the maximum intraspecific differences in the monthly and seasonally resolved $\delta^{18}\text{O}_{\text{shell}}$ values are as large as 0.51‰ and 0.76‰, corresponding to differences in water temperature of 2.2°C and 3.3°C, respectively. This result suggests that multiple $\delta^{18}\text{O}_{\text{shell}}$ records should be used to reconstruct seawater temperatures, because although the intraspecific variations in the $\delta^{18}\text{O}$ values are not negligible, especially during the juvenile and senescent stages. If multiple data are collected, the reconstructed seawater temperatures are expected to be very close to the actual temperatures.

Cross-sections of the inner shell layer are characterized by growth banding, composed of alternating wide light-grey bands and narrow white bands. The $\delta^{18}\text{O}_{\text{shell}}$ data indicate that the narrow white bands and the wide light-grey bands were formed in winter and the other seasons, respectively (Figs 3–5).

Supporting Information

S1 Table. Stable carbon and oxygen isotope ratios of the KTd-1, KTd-4, and KTd-5 shells. (XLSX)

Acknowledgments

We are grateful to K. Inoue (Okinawa Prefectural Fisheries Research and Extension Center) for providing the *T. derasa* shells and environmental records. The manuscript was significantly improved by the comments and suggestions from G. J. Vermeij, B. Bijma, and two anonymous reviewers.

Author Contributions

Conceived and designed the experiments: HT RA YI. Performed the experiments: JY HT AI. Analyzed the data: JY HT RA YI. Contributed reagents/materials/analysis tools: YI. Wrote the paper: JY HT RA YI.

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