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High-resolution carbon and oxygen isotope record in modern brachiopod *Pictothyris picta* collected off Okinoshima, Japan

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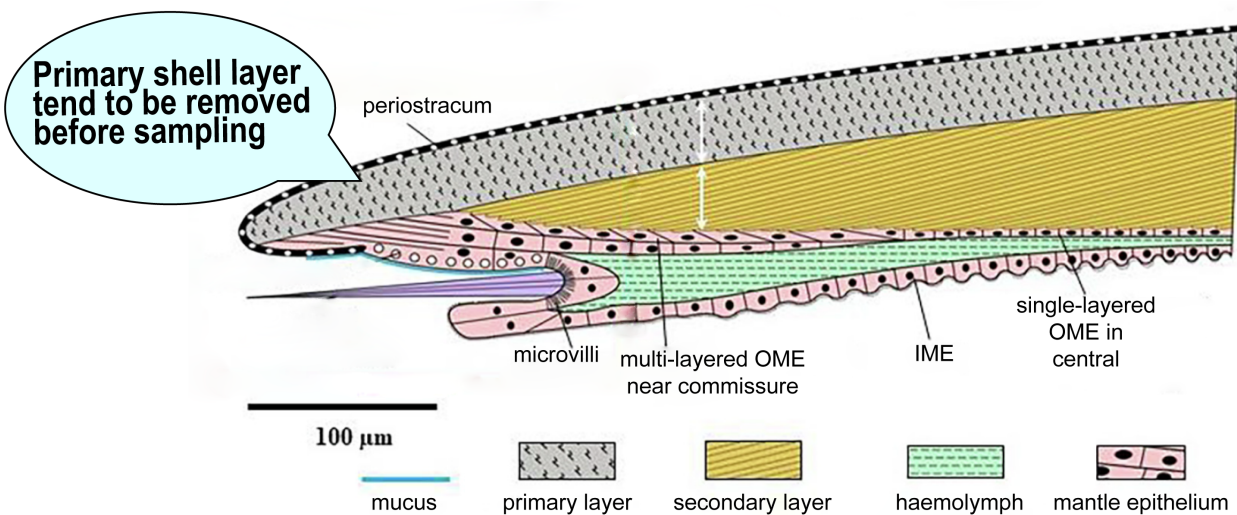
Why do we focus on fossil brachiopods?

Preferential use of fossil brachiopods is due to...

- ✓ common and extensive occurrences throughout the Phanerozoic, **especially the Paleozoic** (Williams et al., 1996; Powell, 2009).
- ✓ the relatively **low susceptibility to diagenetic alteration** on account of their mineralogy (low-magnesium calcite) and dense microstructure (Brand and Veizer, 1980; Morse and Mackenzie, 1990).
- ✓ the assumed precipitation of their shells in oxygen isotopic equilibrium with ambient seawater (Lowenstam, 1961).

However, recent studies revealed that carbon and oxygen isotope composition of brachiopods shell are also influenced by biological and physiological fractionation effects.

Recent knowledge on isotopic composition of modern brachiopods shells



Primary layer vs. Secondary layer

Partly modified from Simonet-Roda et al (2019b)

	Primary layer	Secondary layer
Previous studies	Poorly studied	Several studies
Isotopic composition	Significantly lower isotopic composition compared to those of equilibrium calcite	Large within-shell variation (partly isotopic equilibrium/disequilibrium with ambient seawater)
Fluid in contact with OME	Seawater (through mucus) [Ca ²⁺] > [CO ₃ ²⁻] (hundredfold)	Haemolymph (Simonet-Roda et al., 2019a)
Thickness of calcification site	No data	nano scale (Simonet-Roda et al., 2019a)

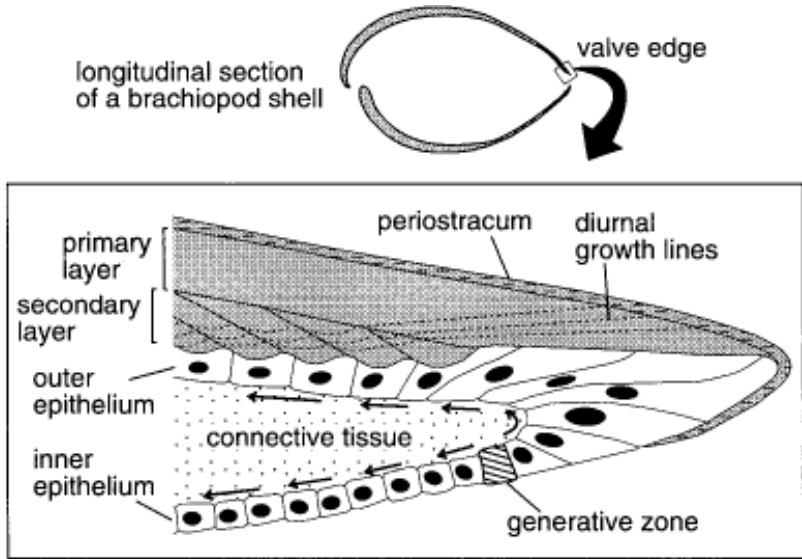
The **primary layer (PL)** is unsuitable for paleoenvironmental reconstructions due to significant low isotopic composition. (Carpenter and Lohmann, 1995; Brand et al., 2003; Parkinson et al., 2005)

The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values from the uppermost secondary layer (SL) show within-shell, intraspecific, and interspecific variations even even when the shells were secreted under the same environmental conditions (Yamamoto et al., 2010a, b, 2011, 2013; Takayanagi et al., 2012, 2013, 2015).



In order to achieve more reliable paleoenvironmental reconstructions using $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values from fossil brachiopods, it is necessary to improve our understanding of how and to what extent biological isotope fractionation effects occur during their shell formation.

New calcification model of brachiopods



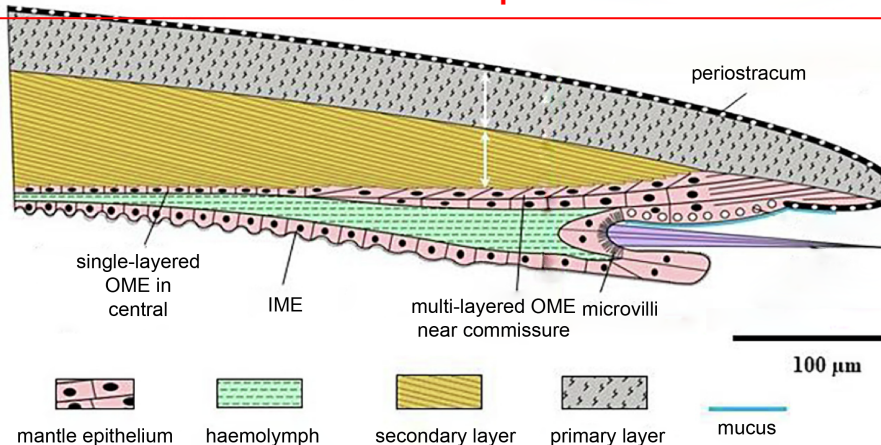
Modified from Williams (1971) and Hiller (1988).

The **'conveyor belt'** model assumes a one-to-one relationship of the cells and fibers (= a single epithelial cell contributes to the formation of only one fiber).

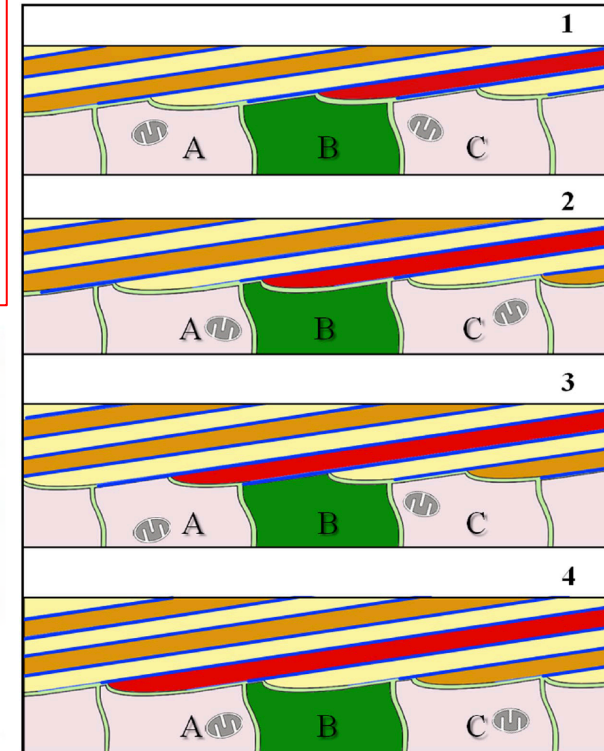
The **new ion transport mechanisms** proposed by Simonet Roda et al. (2019a) demonstrated that epithelial cells and fibers are in a one-to-many relationship and the cells can secrete not only calcium carbonate but also an extracellular biopolymer at different portions.



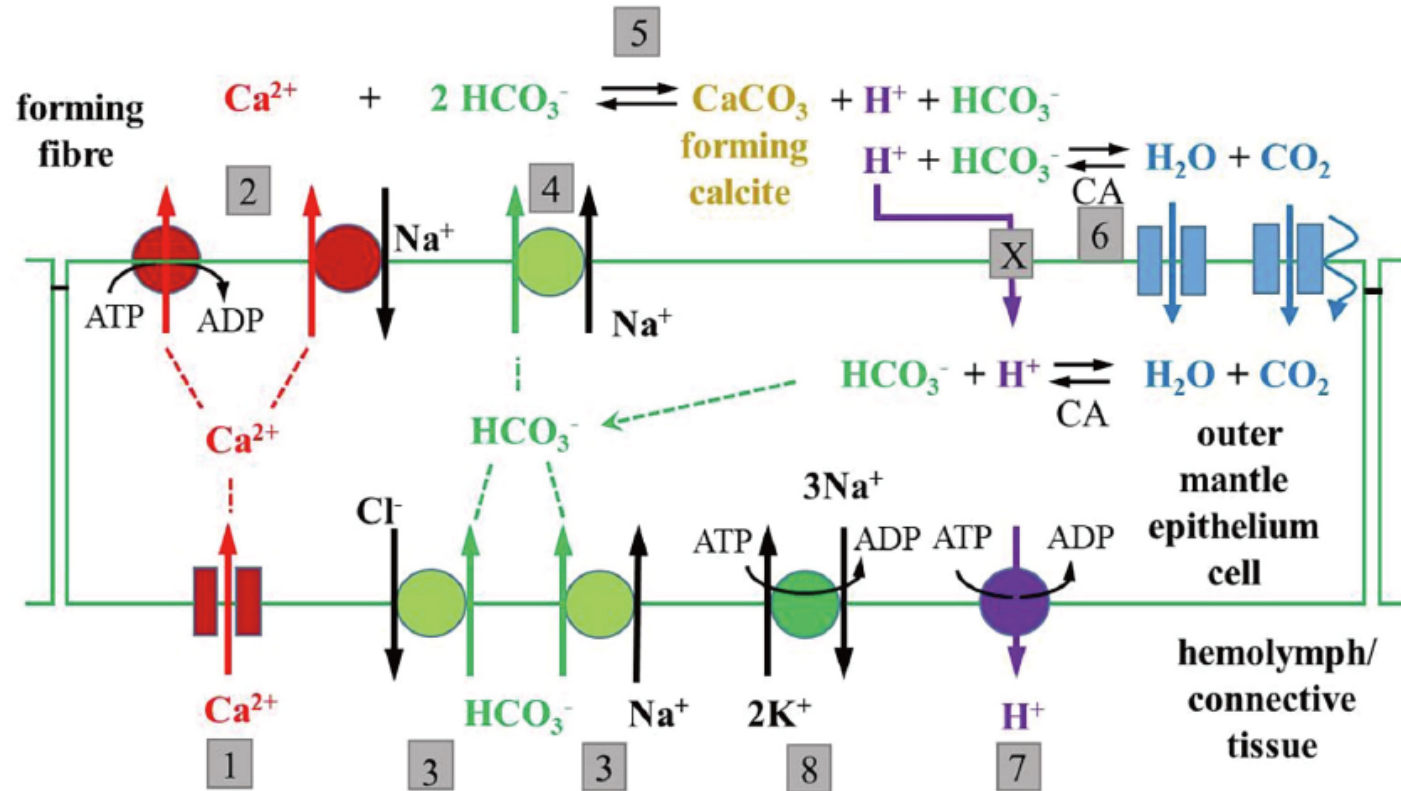
Brachiopod shells are likely to be formed under strong biological regulations, and calcite of different shell portions would be precipitated from calcifying fluids with different chemical compositions.



Simonet-Roda et al. (2019a, b)



New calcification model of brachiopods



We need to re-examine causes and processes of biological isotope fractionation effects by following the latest calcification models of brachiopods proposed by Simonet Roda et al. (2019a) .

Brachiopod's ion transport model from Simonet Roda et al. (2019b)

Aims of this study

Comparing $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of different shell layers and portions formed under the same environmental and ecological conditions (i.e., within-shell variability) will be essential for assessing the contribution of biological fractionation.

However

- Limited studies have been done on a direct comparison of high-resolution $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ variations between the PL and SL within a single shell (e.g., Carpenter and Lohmann, 1995).
- There are no detailed studies dealing with sexual differences in isotope composition within a single species.

Therefore

This study aims to illustrate high-resolution within-shell variations in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values from the PL and the SL of modern brachiopod "*Pictothyris picta*" in order to specify possible causes and to estimate extent of the biological isotope fractionation effects based on the new calcification models proposed by Simonet Roda et al. (2019a, b).

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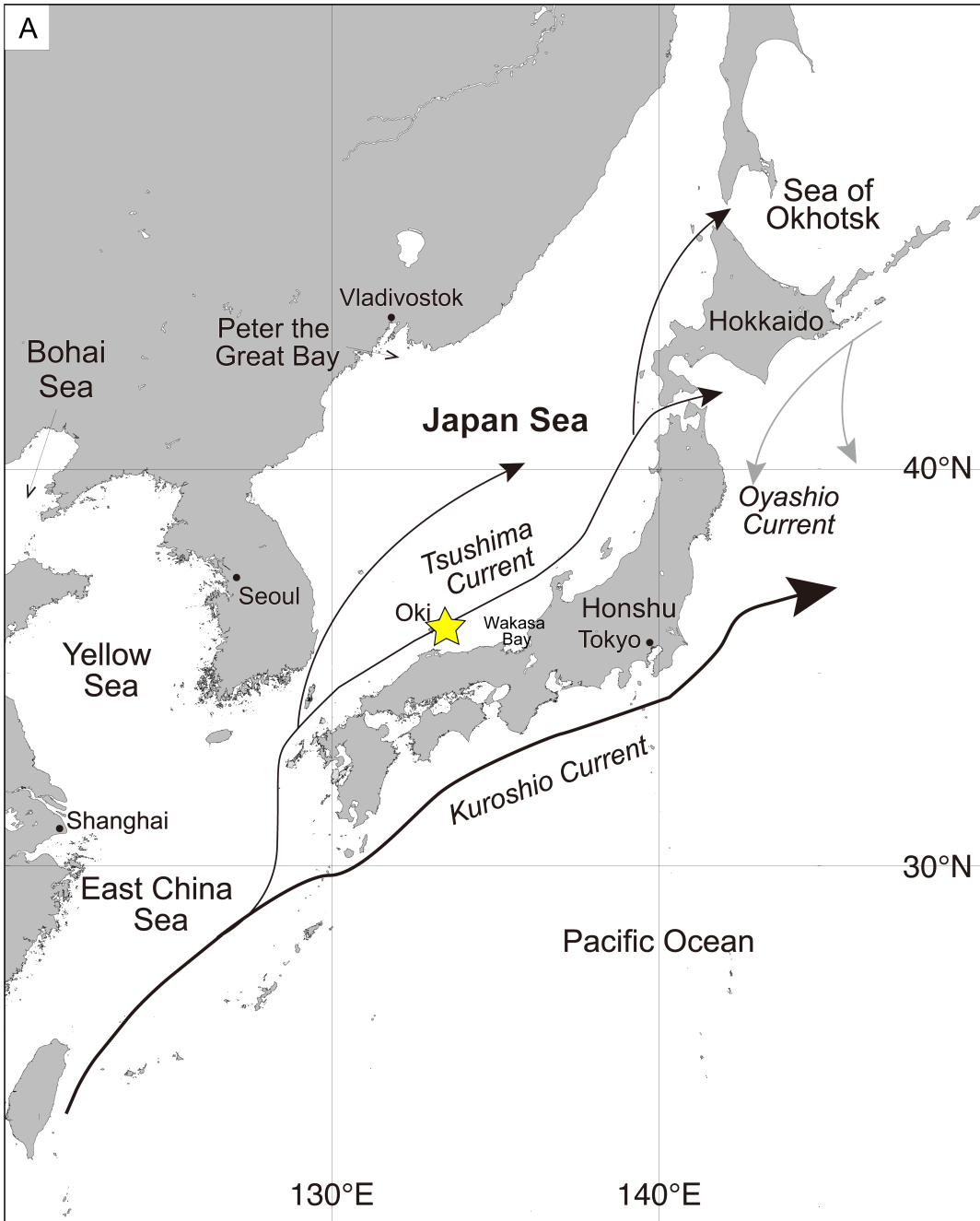
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Study area and materials



Brachiopod samples

- Living *Pictothyris picta* (three specimens)
- Sex was determined by their reproductive organs

Sampling site

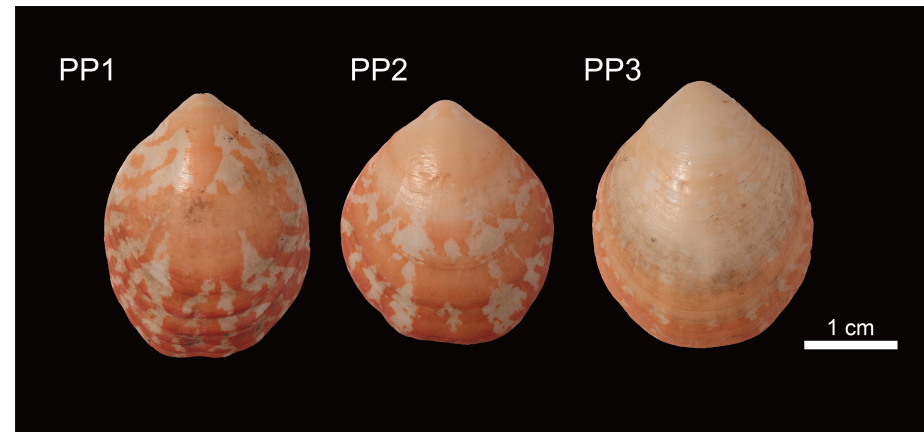
- Off Okinoshima in Japan Sea (water depth: 61 m)

Sampling date

- 28th July 2017

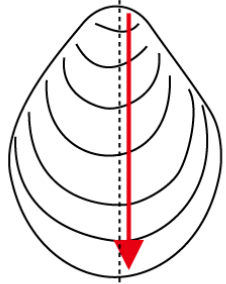
Oceanographic data

- CTD observations were conducted off Wakasa Bay by the R/V *Seifu-Maru*, Maizuru Marine Meteorological Observatory from 2000 to 2009.

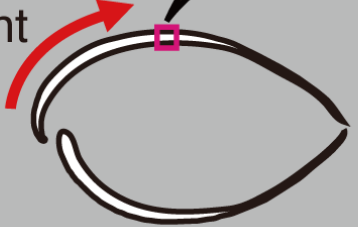


Sampling method

Ontogenetic series
(Ont)



Ont

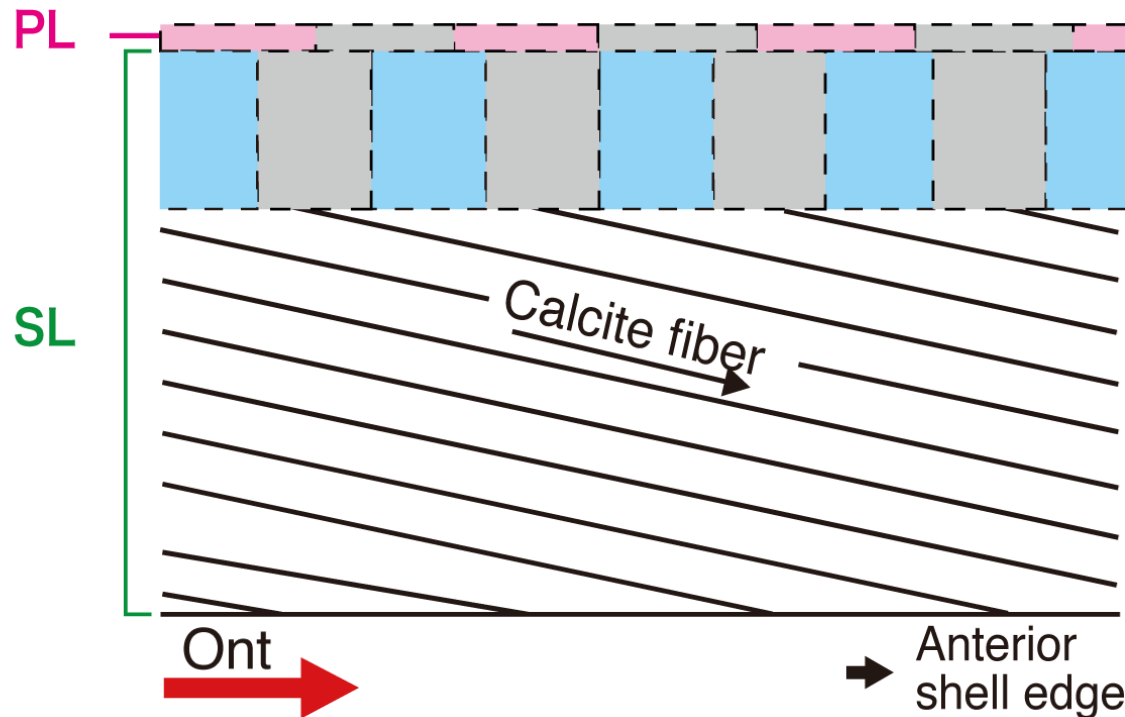


Longitudinal section

Carbon and oxygen isotope analyses

PL-Ont SL-Ont

Trace element analysis
(Do not use in this study)



High-resolution sampling at intervals of 0.10-0.20 mm and 0.09-0.17 mm from the primary layer (PL-Ont) and the uppermost secondary layer (SL-Ont), respectively

Number of samples

	PP1	PP2	PP3	Total
PL-Ont	98	125	104	327
SL-Ont	114	141	113	368

Stable carbon and oxygen isotope analyses of shell calcite

ThermoFisher DeltaV Advantage mass spectrometer, coupled with a ThermoQuest Kiel-III automated carbonate device, at Institute of Geology and Paleontology, Graduate School of Science, Tohoku University.



external precision

$$\delta^{13}\text{C} = \pm 0.03 (\text{‰}) (1\sigma) \quad \delta^{18}\text{O} = \pm 0.04(\text{‰}) (1\sigma)$$

based on replicate measurements of the laboratory reference material (JcT-1; $n = 58$)

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Carbon and oxygen isotope variations

Amplitude of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ variations
PL-Ont > SL-Ont

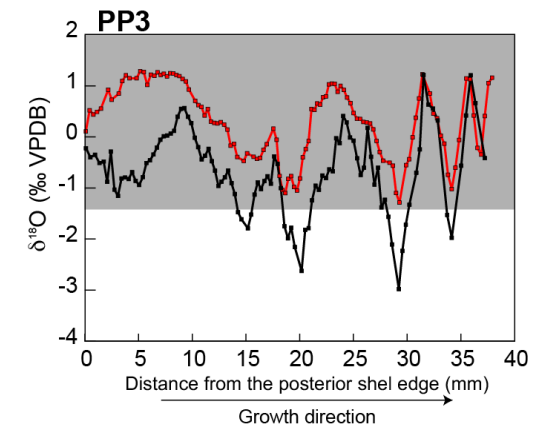
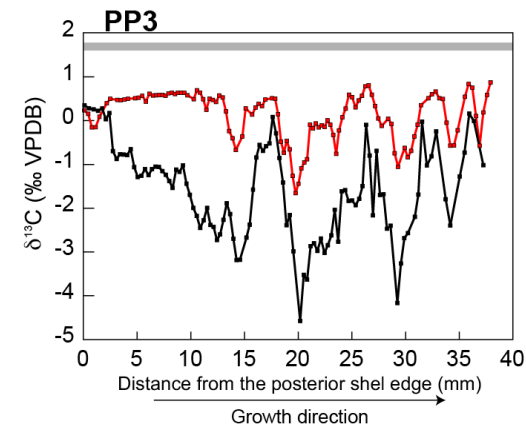
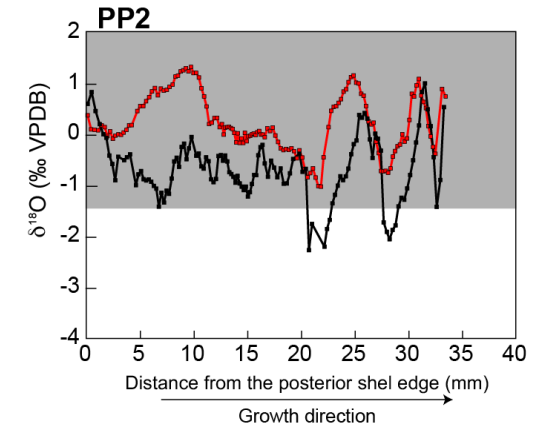
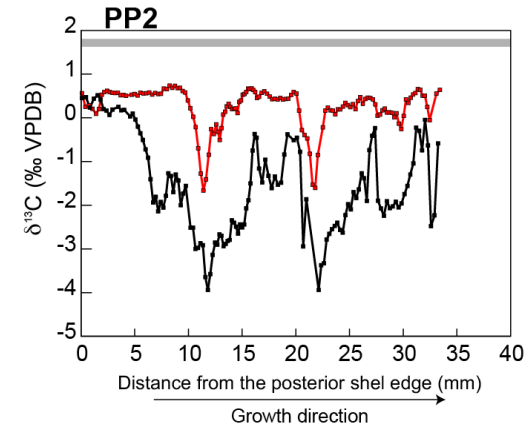
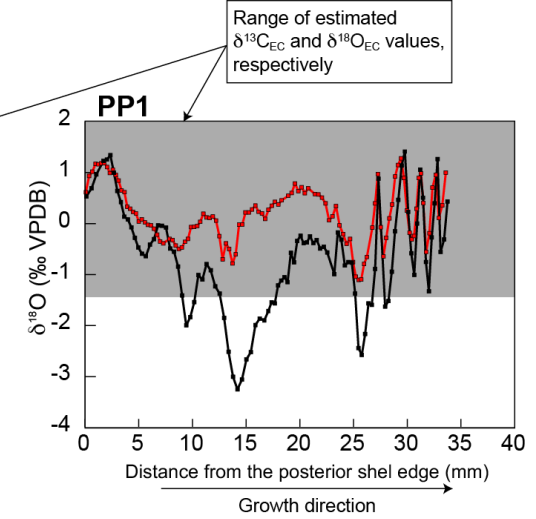
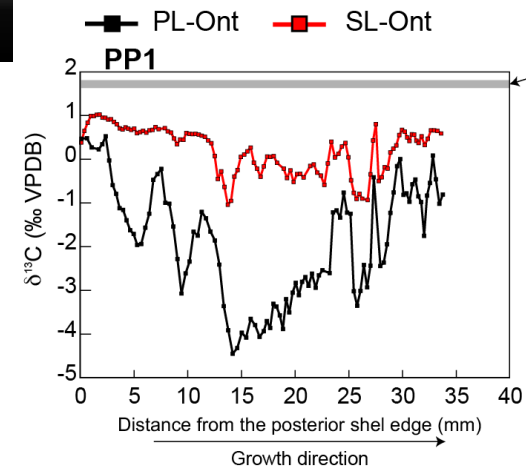
Trends of results

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

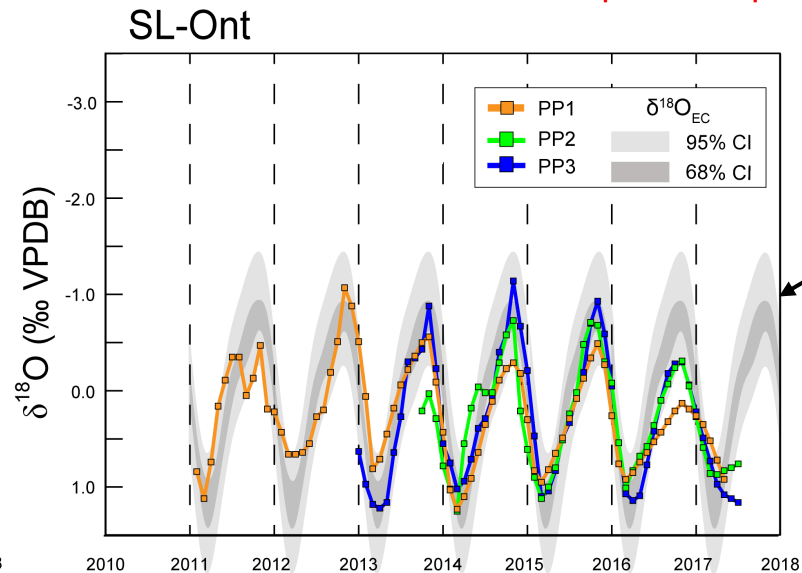
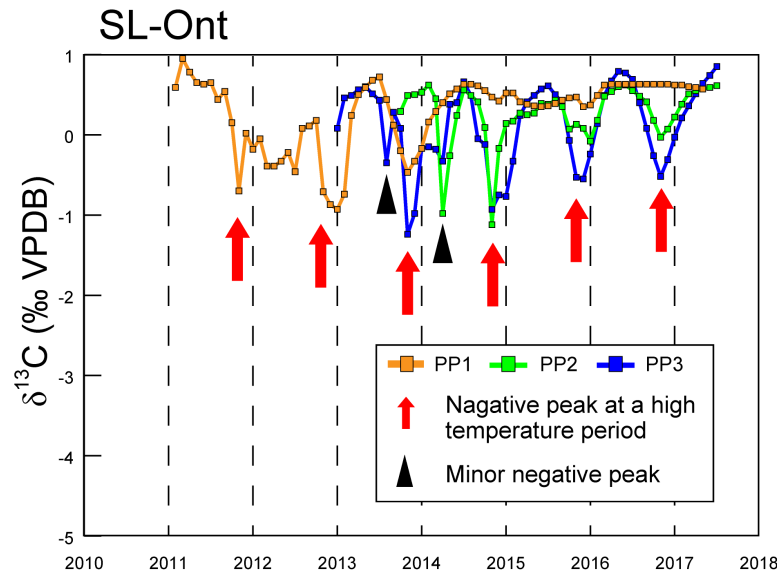
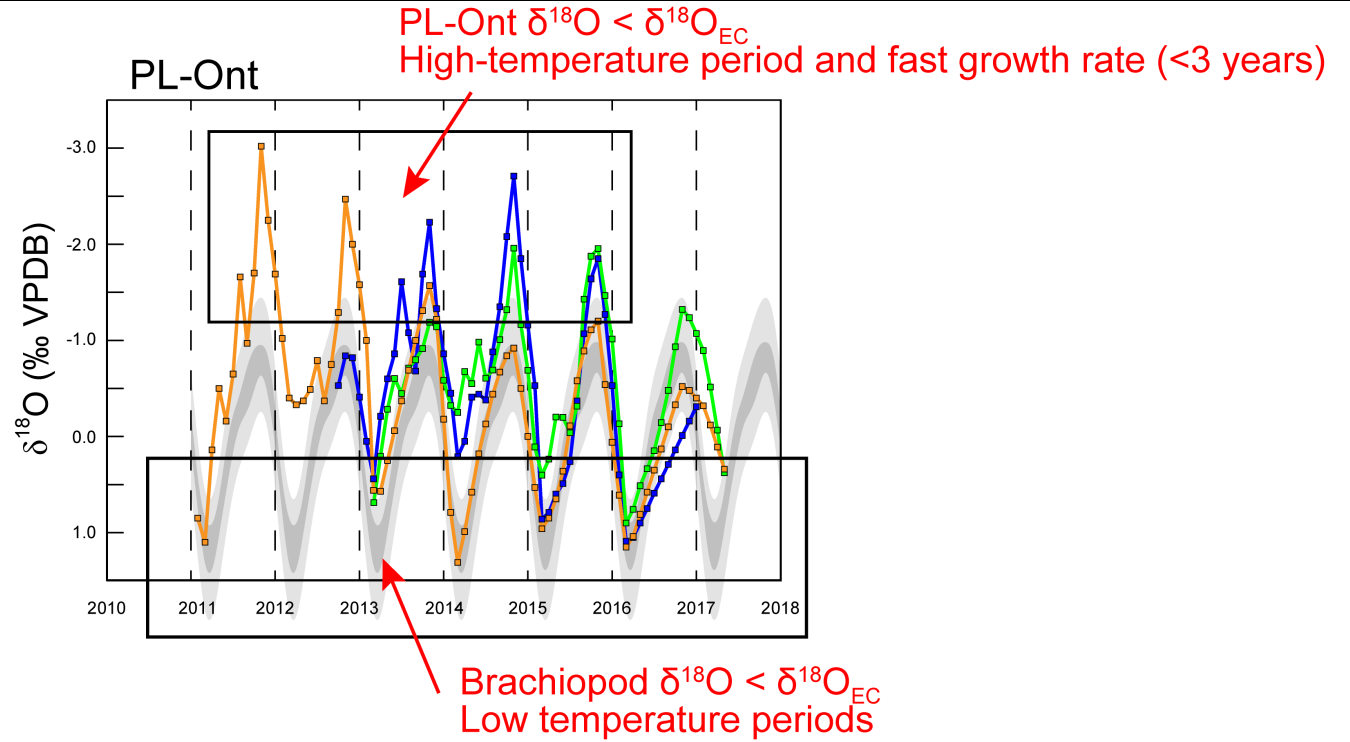
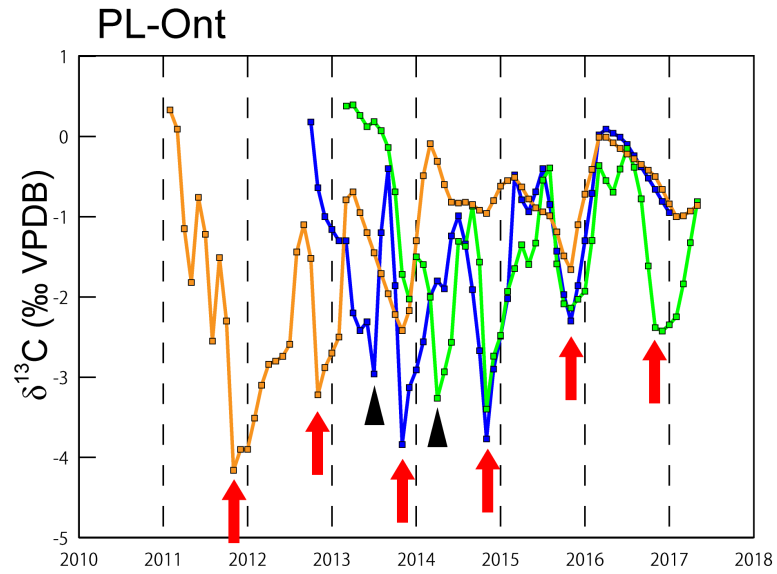
- ✓ PL-Ont $\delta^{13}\text{C}$: 1.2-6.3‰
< $\delta^{13}\text{C}$ values of equilibrium calcite
- ✓ The SL-Ont $\delta^{13}\text{C}$ values
Relatively narrow range
Associated with one or two minor negative spikes

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

- ✓ Both layers
Significant seasonal variation.
- ✓ The SL-Ont
Fall in the range of $\delta^{18}\text{O}$ values of equilibrium calcite

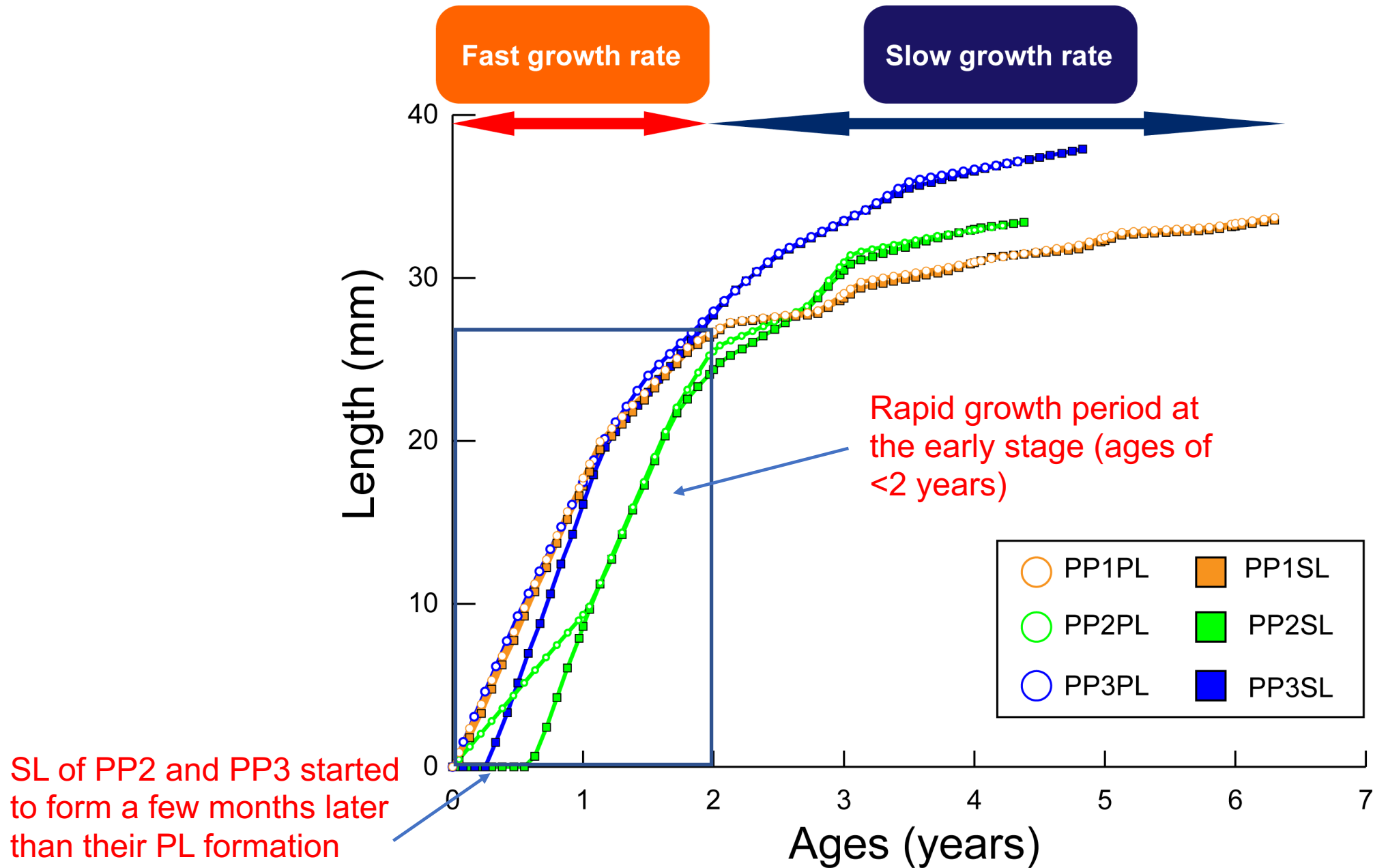


Time series $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ variations in the primary and secondary shell layers



The entire $\delta^{18}\text{O}$ values of the SL fall within the range of $\delta^{18}\text{O}_{\text{EC}}$ values and small intraspecific variations.

Growth curves of the PL and SL based on the PL-Ont and SL-Ont $\delta^{18}\text{O}$ values



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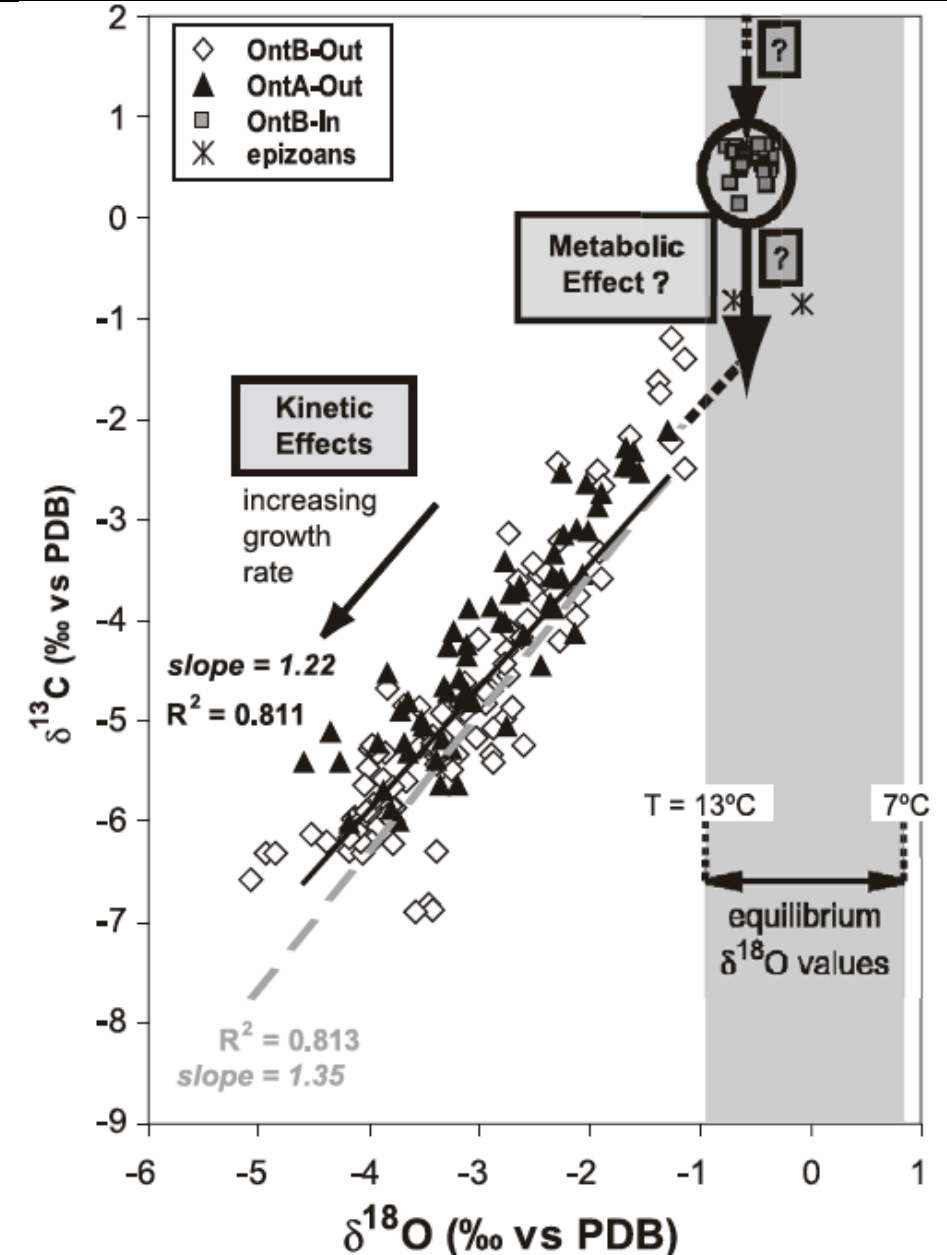
Biological effects on biogenic carbonates

Kinetic isotope effect (KIE)

- Mostly result from kinetic discrimination against the heavy isotopes ^{18}O and ^{13}C during CO_2 hydration and hydroxylation in extrapallial fluids
- Relate to shell growth rates
- Show significant positive correlations in cross-plots of $\delta^{13}\text{C}$ versus $\delta^{18}\text{O}$ values.

Metabolic effect

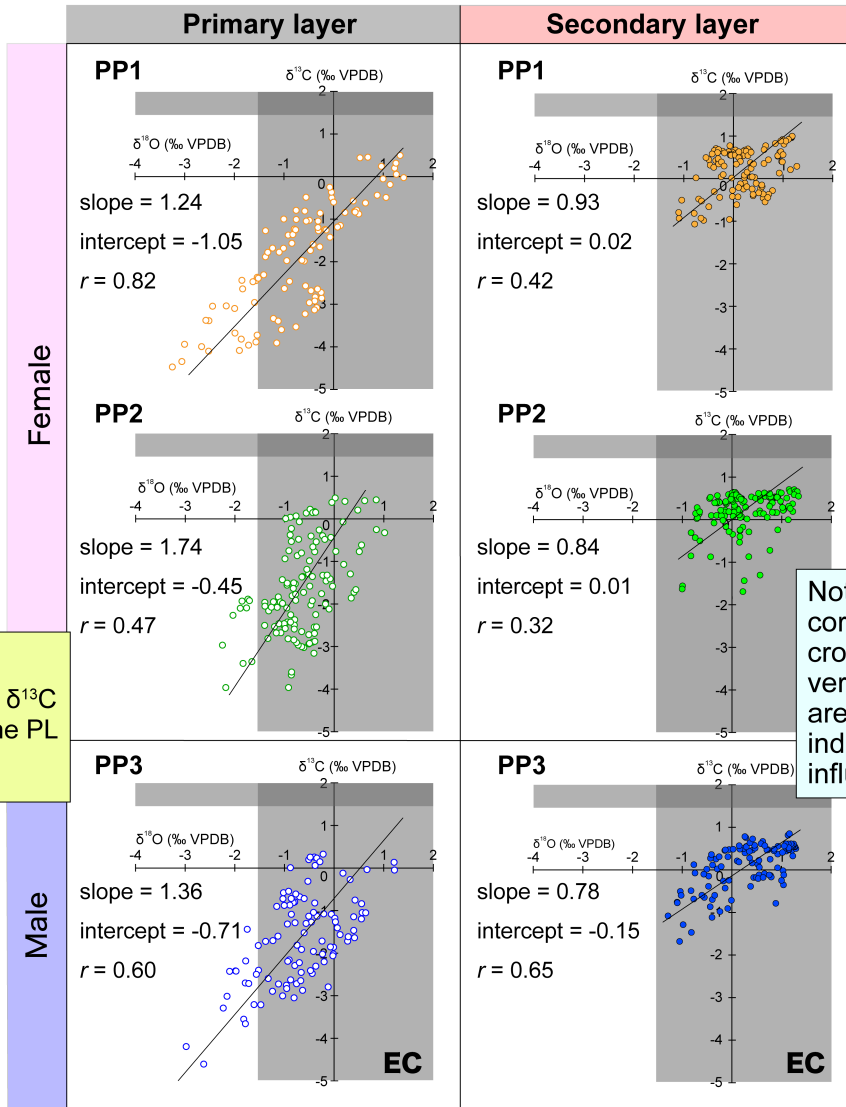
- Caused by contribution of respiration-derived CO_2 (depleted in ^{13}C) during shell formation.
- Affect only $\delta^{13}\text{C}$ composition (McConnaughey, 1989a).



(Auclair et al., 2003)

Kinetic isotope effect (KIE) on the PL and SL of *P. picta*

Cross plots $\delta^{13}\text{C}$ versus $\delta^{18}\text{O}$ values

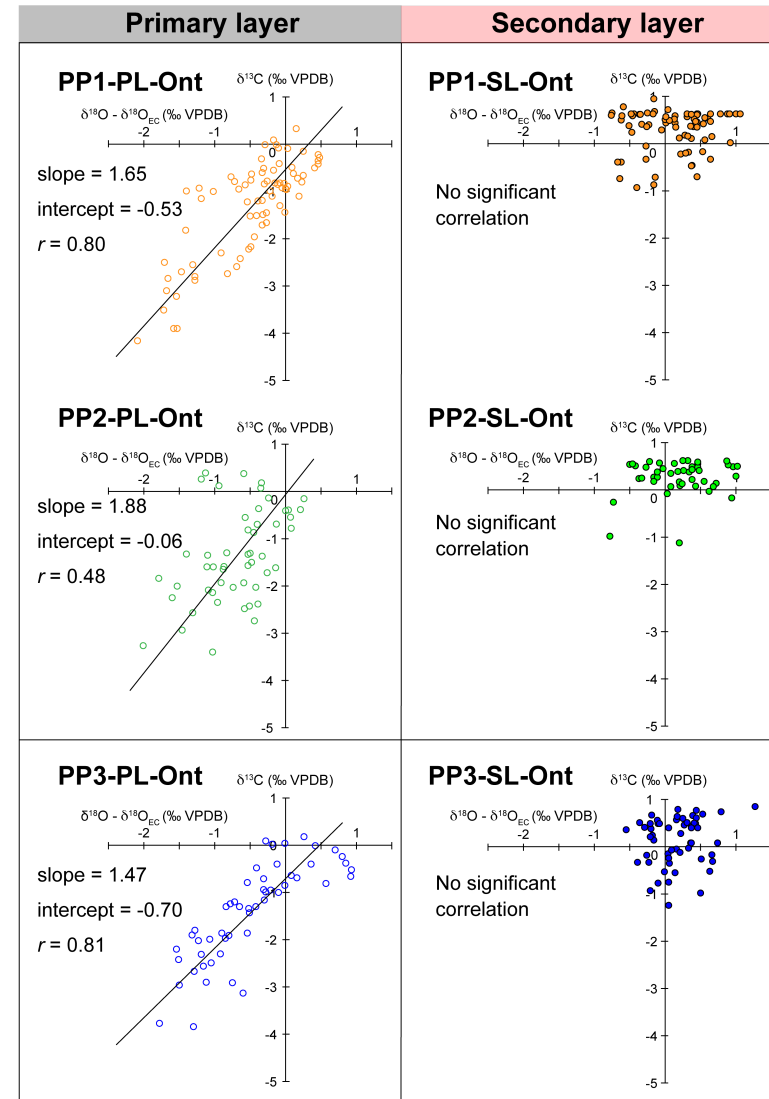


Note that positive correlations in cross-plots of $\delta^{13}\text{C}$ versus $\delta^{18}\text{O}$ values are not always an indicator of the influence of KIE.

No sexual differences in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ of the PL and SL

Positive correlations between $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of the SL reflecting seasonal variations in environmental factors (e.g., seawater temperature and $\delta^{18}\text{O}_{\text{sw}}$).

Cross plots of $\delta^{13}\text{C}$ versus $\delta^{18}\text{O} - \delta^{18}\text{O}_{\text{EC}}$
Remove factors of seasonal variations in $\delta^{18}\text{O}_{\text{EC}}$



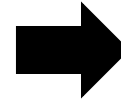
Significant positive correlations even after removing seasonal variations in $\delta^{18}\text{O}_{\text{EC}}$.

Primary layer (PL)
Significant positive correlation = stronger KIE

Secondary layer (SL)
No correlation = negligible KIE

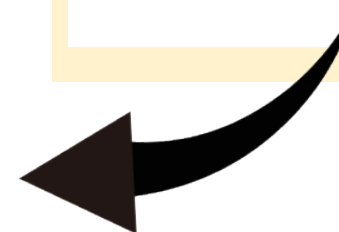
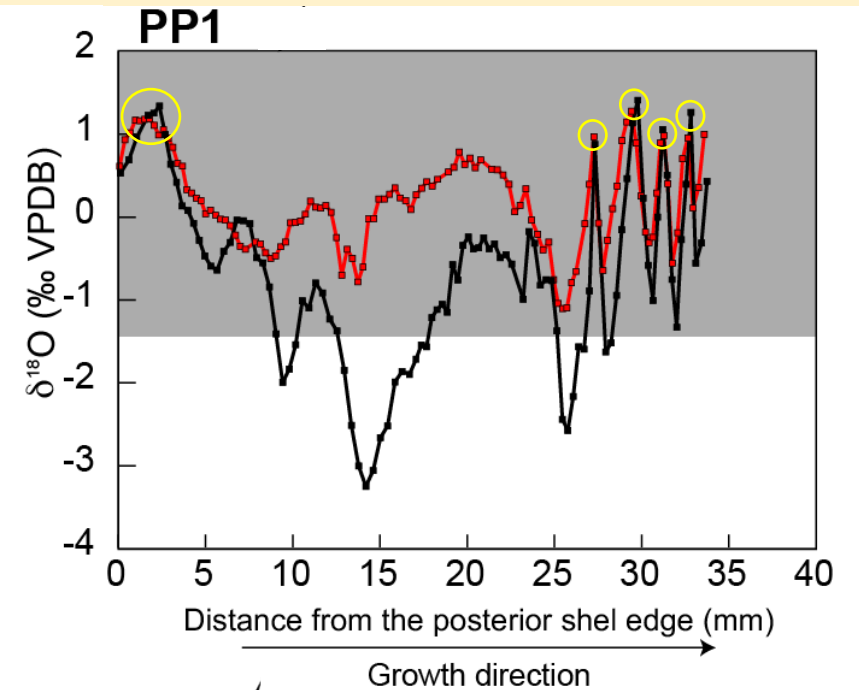
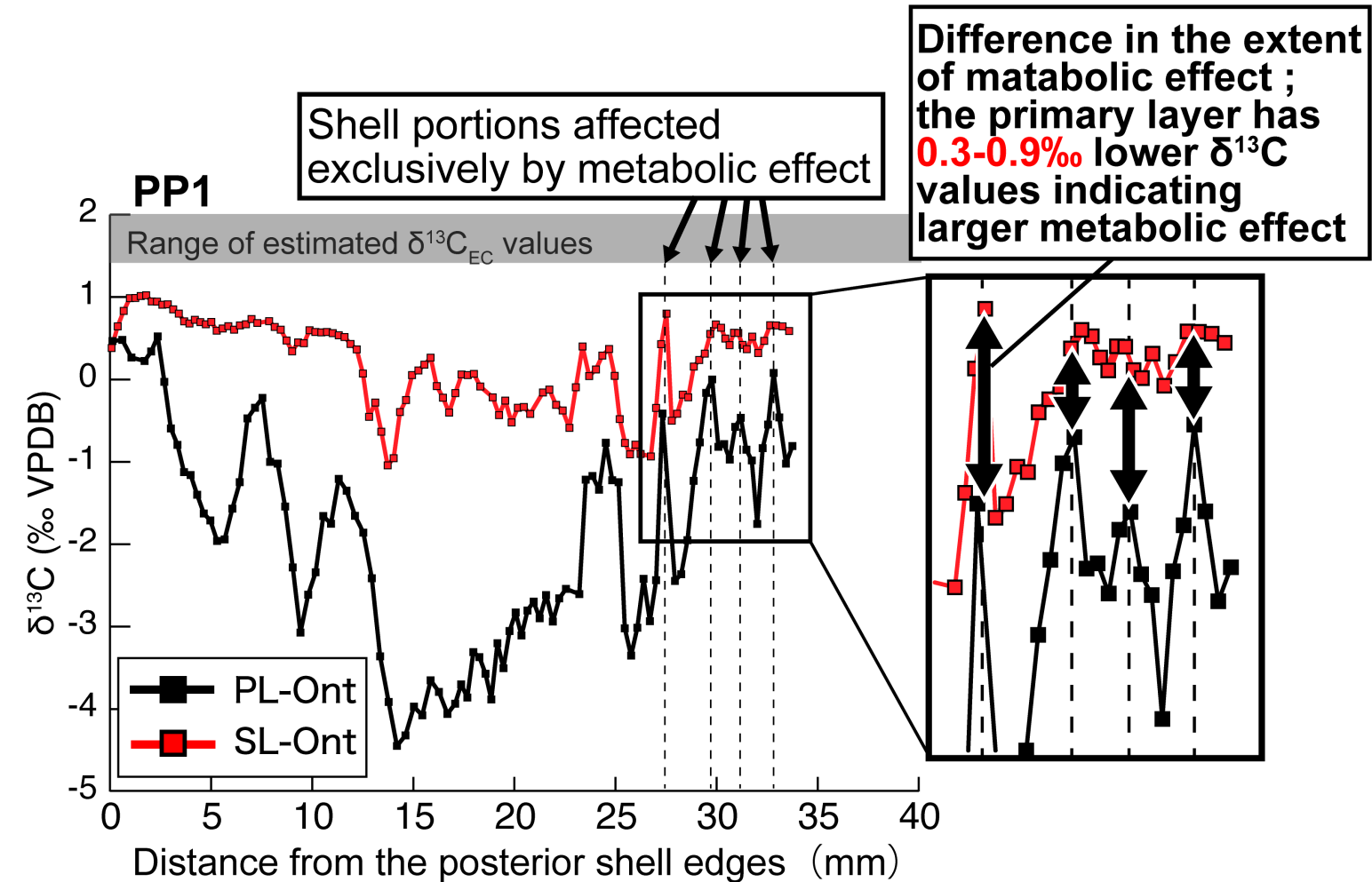
Metabolic effect on the PL and SL of *P. picta*

The PL and SL $\delta^{13}\text{C}$ influenced by KIE and metabolic effects.

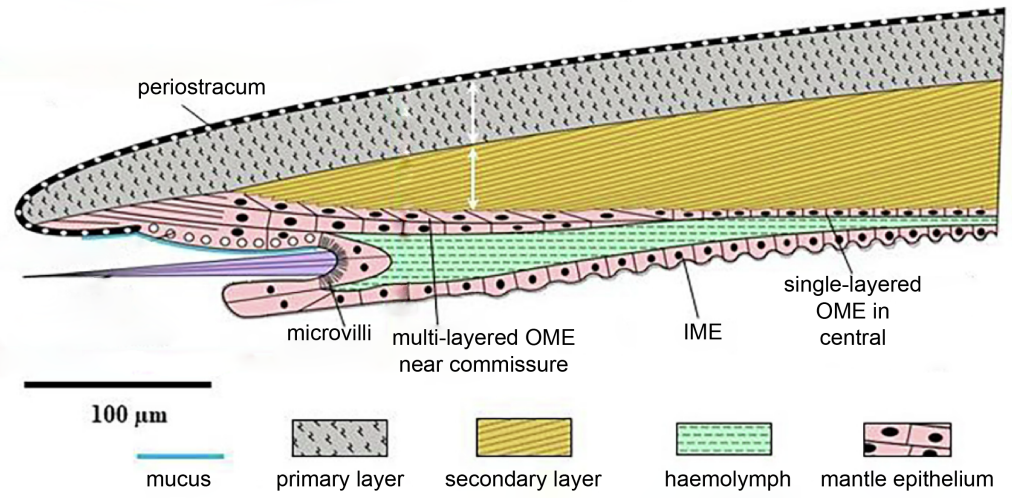


The PL and SL partly show same $\delta^{18}\text{O}$ values and are in isotopic equilibrium with ambient seawater, indicating negligible KIE influences on these portions (circled in yellow).

→ The KIE and metabolic effect on $\delta^{13}\text{C}$ can be evaluated separately.



Summary of biological effects on *P. picta* shells



Internal structure of brachiopod shells
Partial reorganization of Simonet-Roda et al. (2019b)

Different extent of biological effects between the PL and SL of *P. picta* can be accounted for the new calcification model proposed by Simonet-Roda et al. (2019a, b).

Summary of biological effects (i.e., kinetic and metabolic effects) influenced on the PL and SL.

	Primary layer	Secondary layer
Previous studies	Poorly studied	Several studies
Isotopic composition	Significantly lower isotopic composition compared to those of equilibrium calcite	Large within-shell variation (partly isotopic equilibrium/disequilibrium with ambient seawater)
Fluid in contact with OME	Seawater (thorough mucus) [Ca ²⁺] > [CO ₃ ²⁻] (hundredfold)	Haemolymph (Simonet-Roda et al., 2019a)
Thickness of calcification site	No data	nano scale (Simonet-Roda et al., 2019a)

	PL-Ont	SL-Ont	Possible cause
Kinetic isotope effect	Strong	Negligibly small	<ul style="list-style-type: none"> Crystal system Thickness of calcification site Existence of ACC
Metabolic effect	Affected (PL > SL)	Affected	Different ion transport processes through outer mantle epithelium (OME)

DIC Residence Time = [DIC] · h · R⁻¹

h = Thickness of the calcifying space
R = Calcification rate

Reaction shorter than this time cause kinetic isotope effects

e.g. coral
[DIC] = 2 × 10³ mol/m³
h = 10⁻⁶m
R = 10⁻⁷mol/m²/s

→ **ca. 5.5h**
(McConnaughey, 2003)

Modern brachiopods have three orders of magnitude smaller extrapallial spaces than those of other calcifying organisms, suggesting a shorter residence time of DIC.

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We conducted high-resolution within-shell variations in $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values from the primary and uppermost secondary shell layers (PL and SL, respectively) of three modern *Pictothyris picta* collected at the water depth of 61 m off Okinoshima, southeastern Japan Sea. Objectives of this study is 1) to evaluate differences in extent of biological effects (such as kinetic and metabolic effects) between the PL and the SL, and 2) to specify possible causes based on the **new** calcification models. The results of this study can be summarized as follows:

- There are very small isotopic differences between male and female brachiopod shells, indicating negligible impacts of physiological factors (e.g., reproduction) on calcifying fluid.
- The $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values of the PL are mostly lower than those of the SL.
- The SL of *P. picta* shells were mostly precipitated in oxygen isotopic equilibrium with surrounding seawater, indicating the minimal kinetic isotope effect.
- The extent of the metabolic effect on the PL is $\sim 0.3\text{--}1.1\text{‰}$ larger than that in the SL.
- Comparison of the $\delta^{18}\text{O}$ profiles between the PL and the SL indicate that the SL formation may start later than that of the PL formation at the earliest stage of the shell formation.