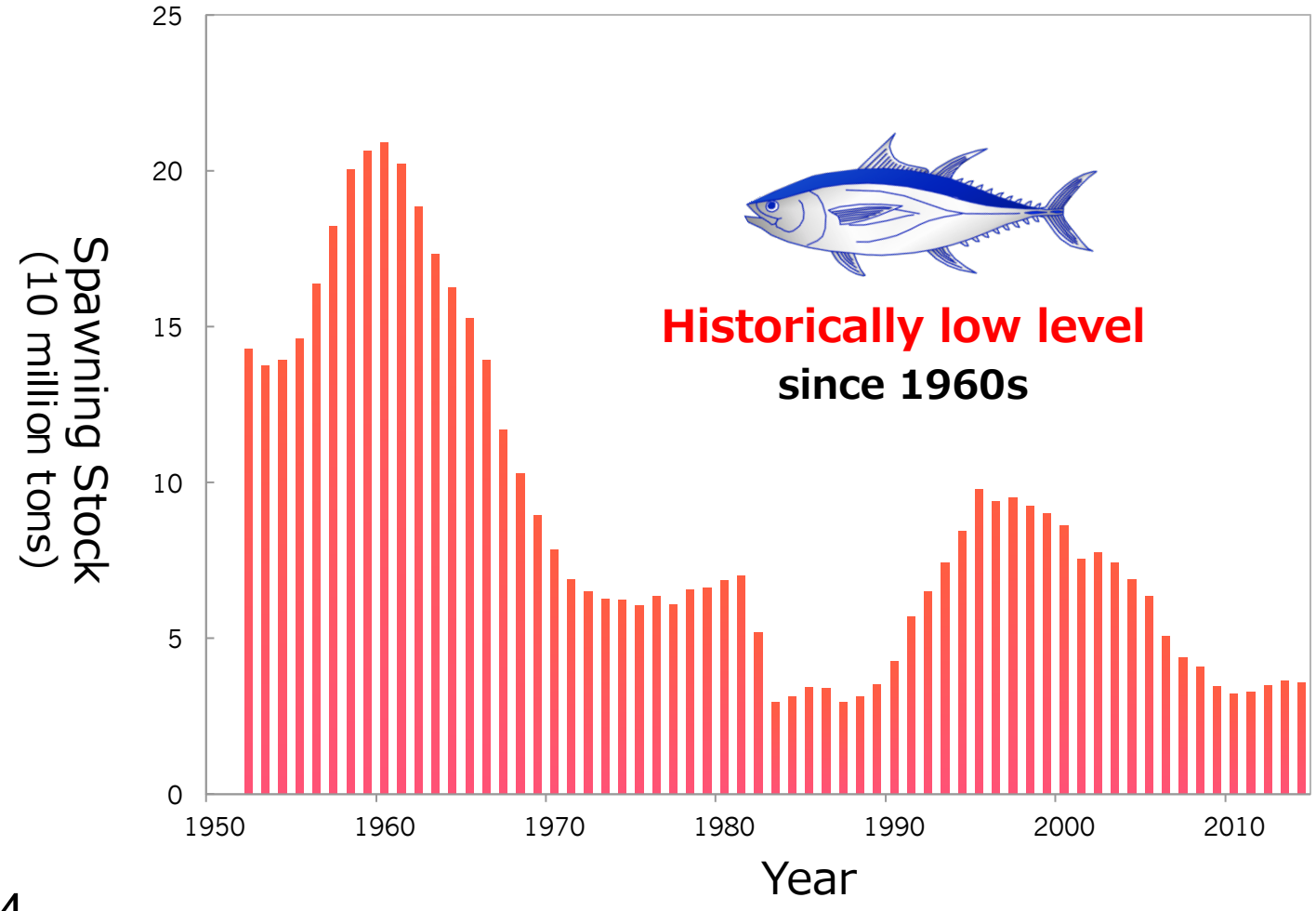
PICES 2017 Annual Meeting Workshop 3
September 22, 2017

Development of methodology for analyses of larval ambient water temperature of Pacific bluefin tuna using SIMS

Yulina Hane[1], Shingo Kimura[1], Yusuke Yokoyama[1], Yosuke Miyairi[1], and Takayuki Ushikubo[2]
[1]The University of Tokyo, Kashiwa, Japan
[2]Kochi Institute for Core Sample Research, JAMSTEC, Nankoku, Japan

Stock of Pacific bluefin tuna

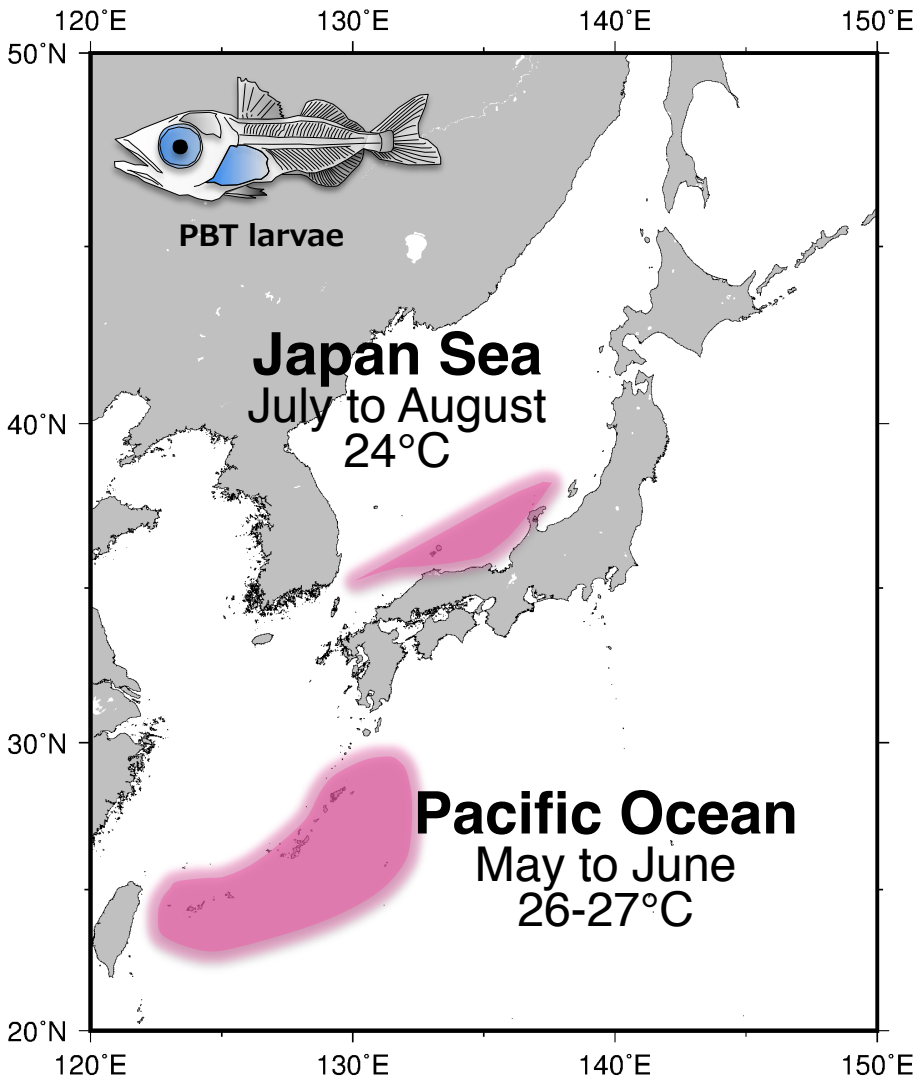


2014

Listed as **endangered species** on IUCN's red list

Need **for Sustainable stock management**

Spawning grounds of Pacific bluefin tuna

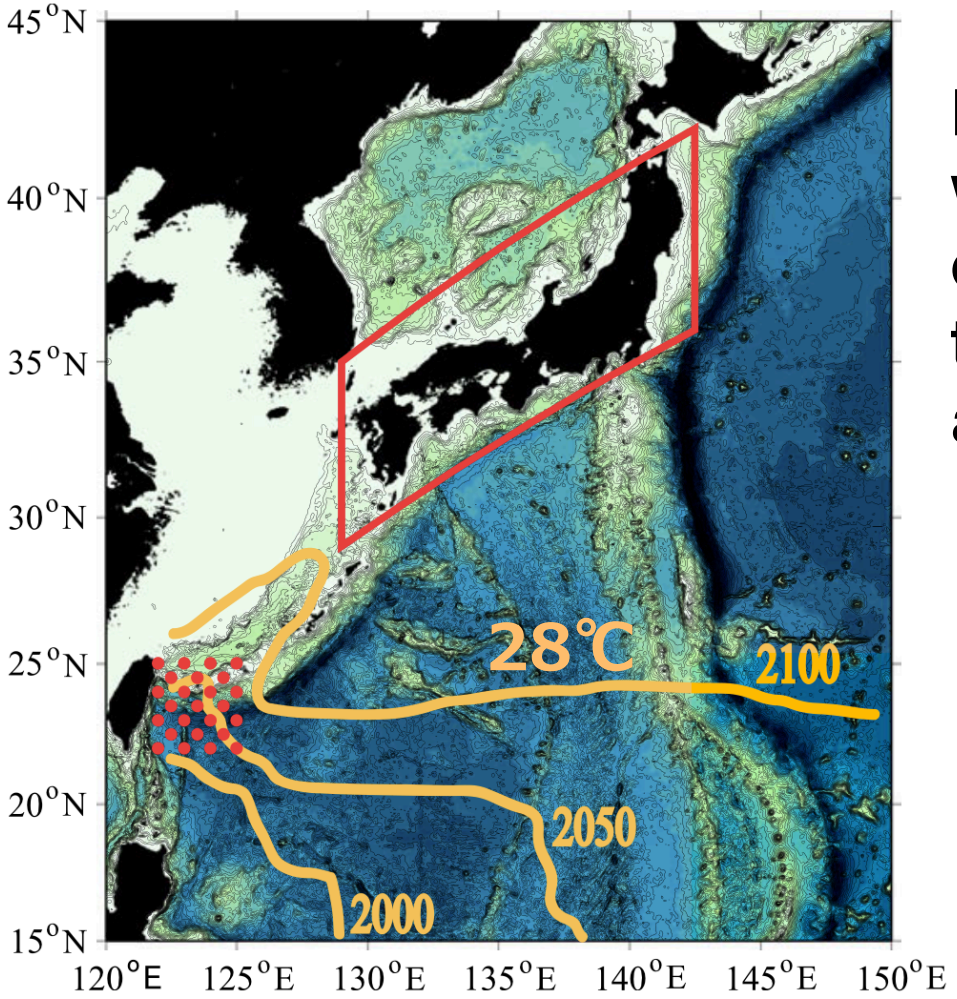


- Spawning grounds and seasons are **limited**

- **Vulnerable** to oceanic fluctuations

The effect of long-term oceanic fluctuations on PBT spawning grounds

(Kimura et al. 2010)



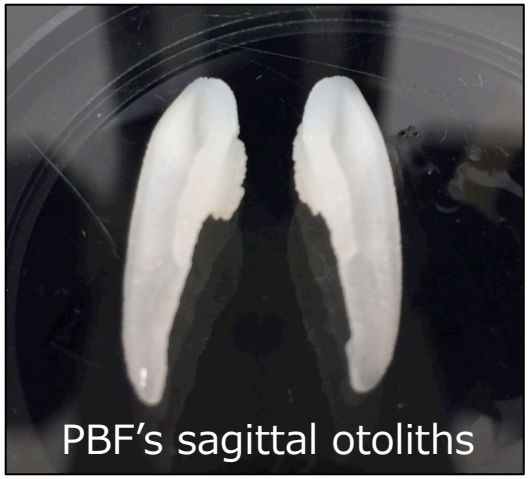
Most of spawning grounds will be out of the range of optimal spawning temperature for egg hatching and larval survival.

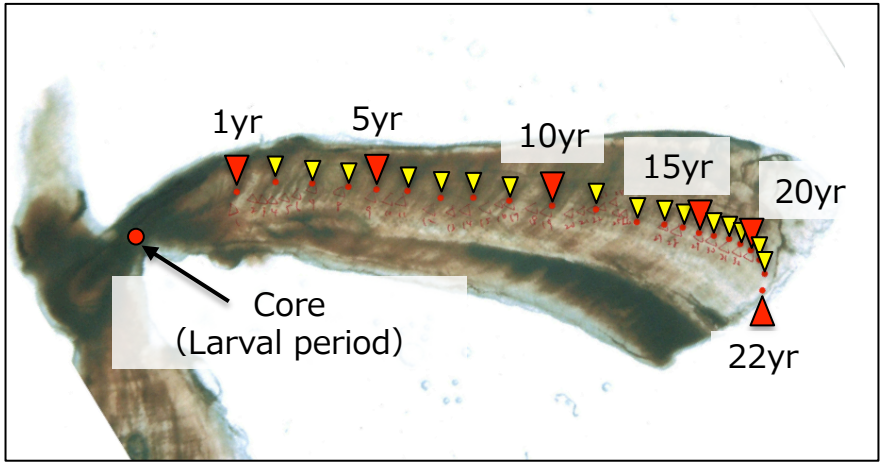
↓

Potential northward shift

in PBF spawning grounds

Otolith:
Effective indicator of environmental history





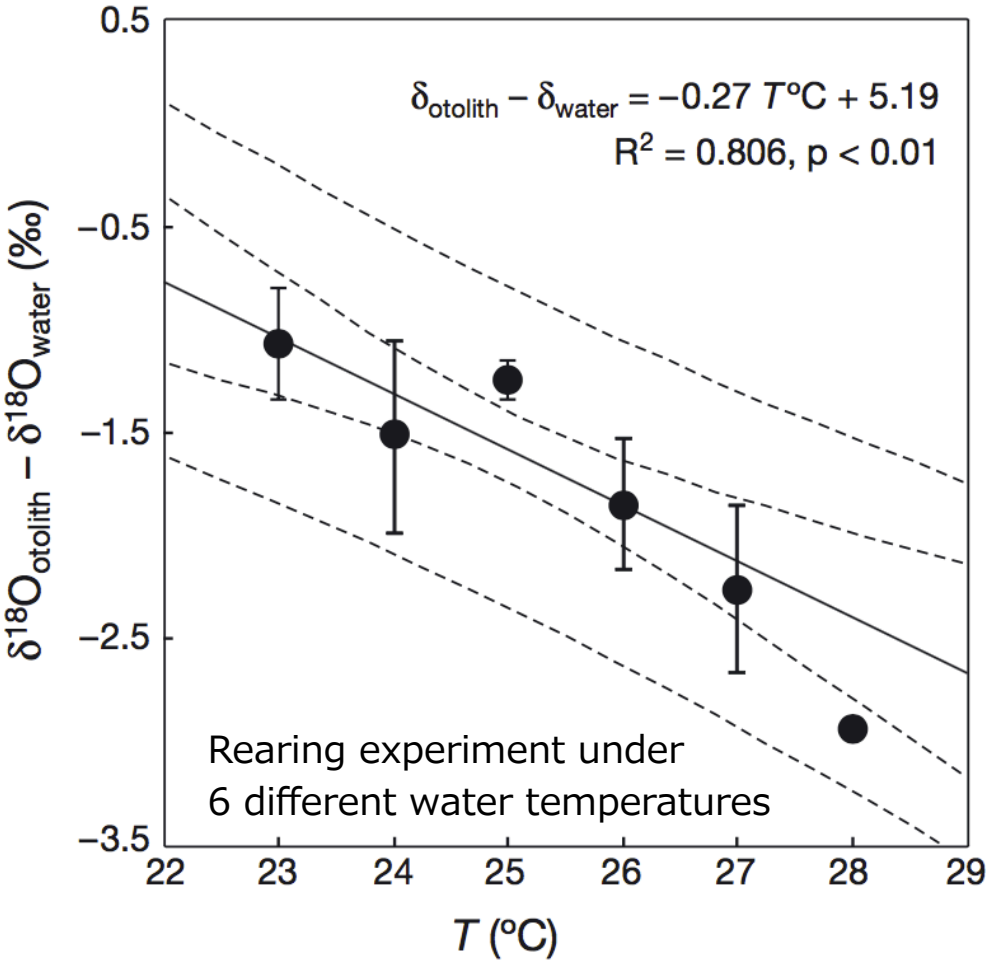
- **Rock-like structure** made of calcium carbonate
- **Metabolically inert**
- **Trace elements and isotopes** kept after death of fish

$\delta^{18}O$: Effective proxy for **estimating water temperature**

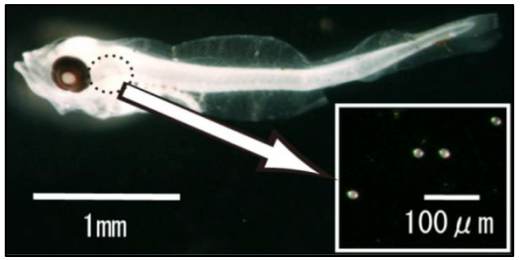
Kitagawa et al. (2013)

Otolith $\delta^{18}O$ of PBF larvae and ambient water temperature

(Kitagawa et al., 2013)



Rearing experiment



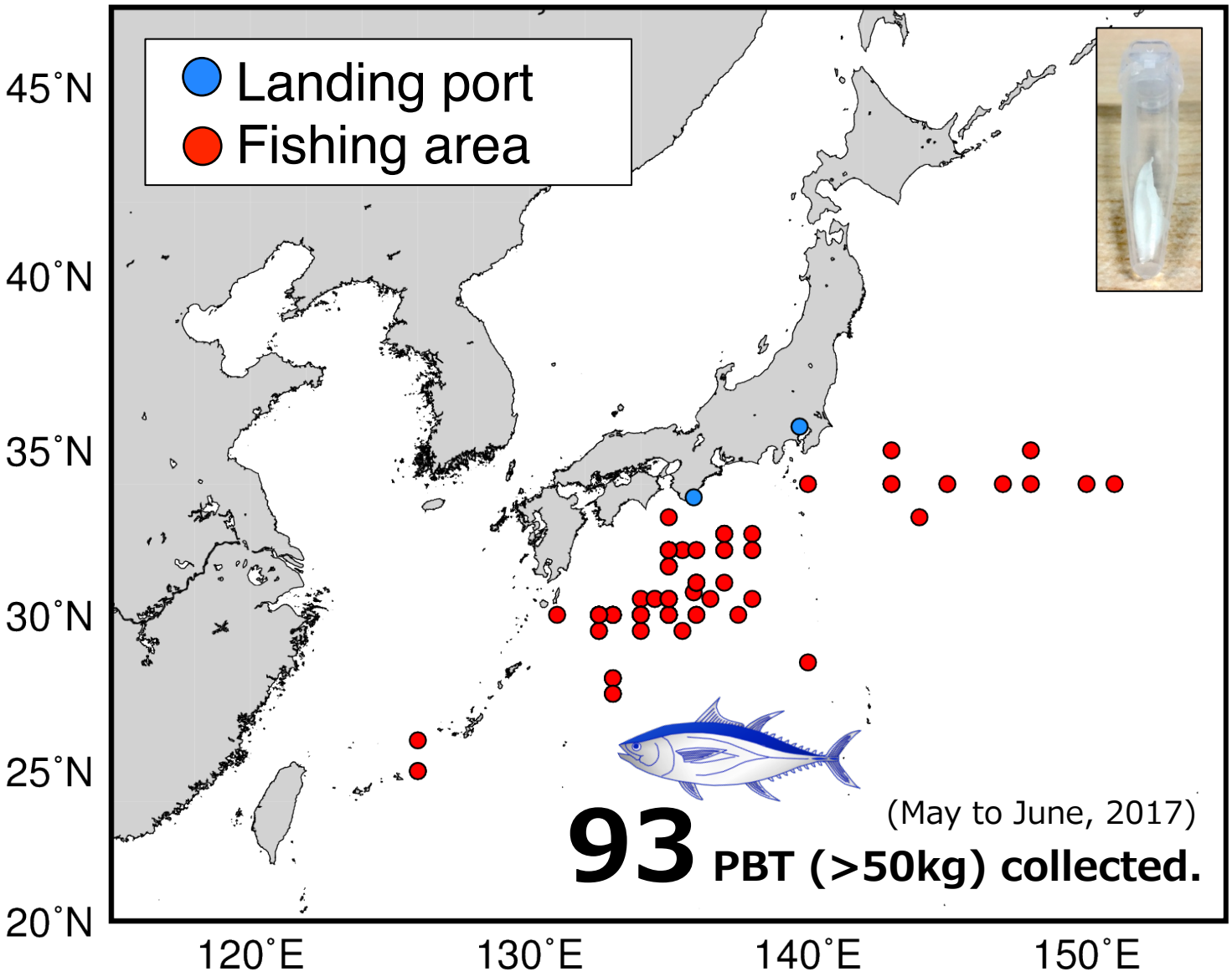
Negative correlation between otolith $\delta^{18}O$ and water temperature

Otolith core $\delta^{18}O$ of mature fish → Spawning temperature

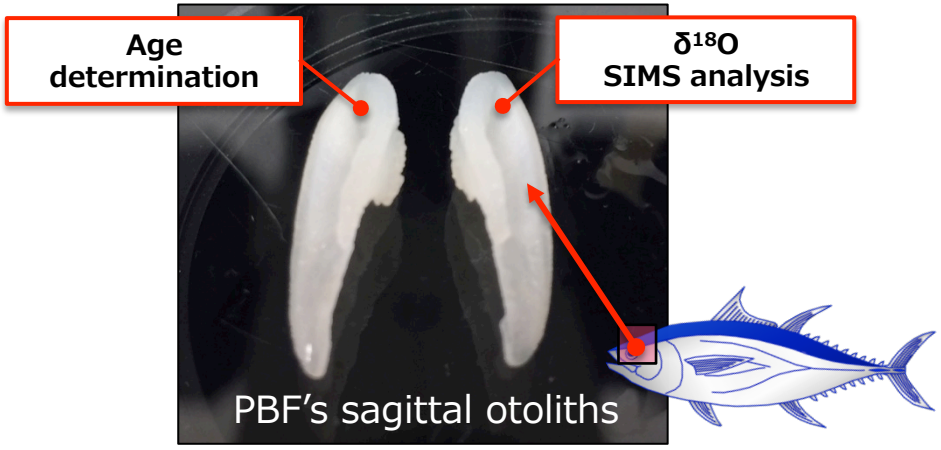
Objective

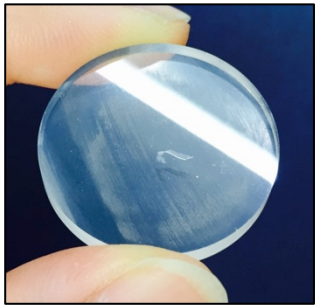
1. **Estimate spawning temperature and spawning grounds** using otolith core $\delta^{18}O$
2. Investigate changes in **spawning grounds related to long-term oceanic climate change**

Materials and methods: Sample collection



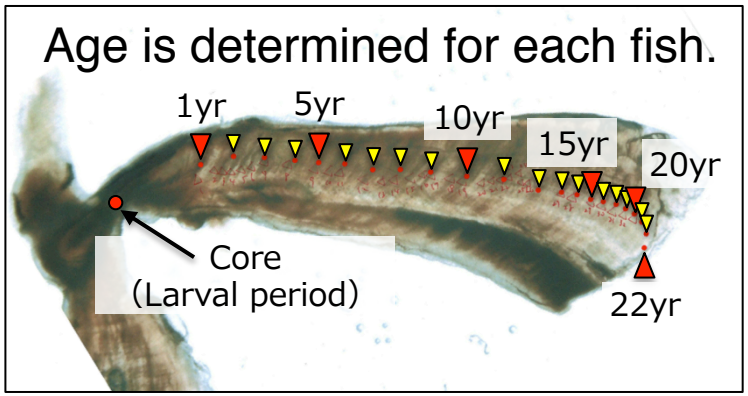
1. Otolith preparation



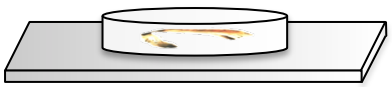


A thin section with **a mirror-finished surface** is prepared.

Sample preparation protocol needed!



2. $\delta^{18}O$ analysis by SIMS



Measure $\delta^{18}O$ by SIMS technique.

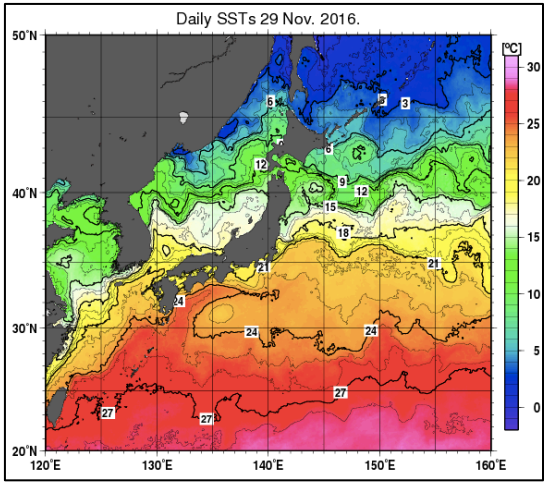
$$\delta_{otolith} - \delta_{water} = -0.27\,T\,^{\circ}C + 5.19$$
$$(R^2 = 0.806,\ p < 0.01)$$

(Kitagawa *et al*., 2013)

Estimate spawning temperature.

3. Spawning grounds estimation

(SST衛星画像, 気象庁)

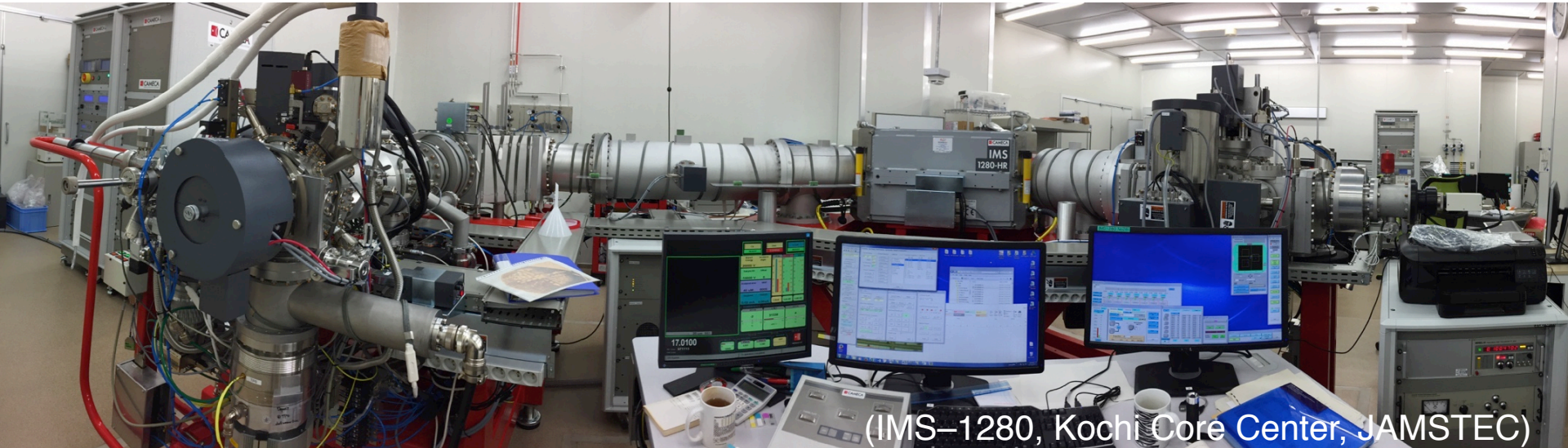


Spawning ground is estimated using SST satellite images and in-situ data.

Materials and methods

SIMS: Secondary Ion Mass Spectrometry

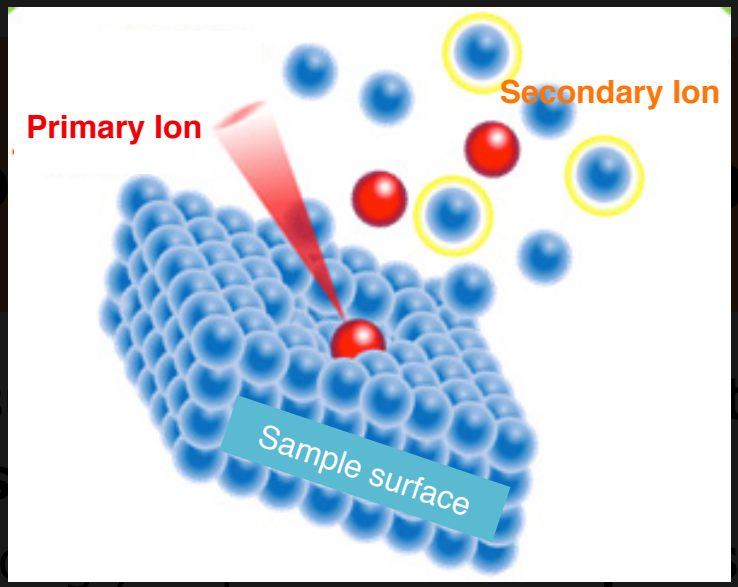
- High sensitive surface analysis of **sub-μm to 10μm**
- **Isotope ratios** and trace element analysis
- Data quality strongly depends on **sample surface condition**



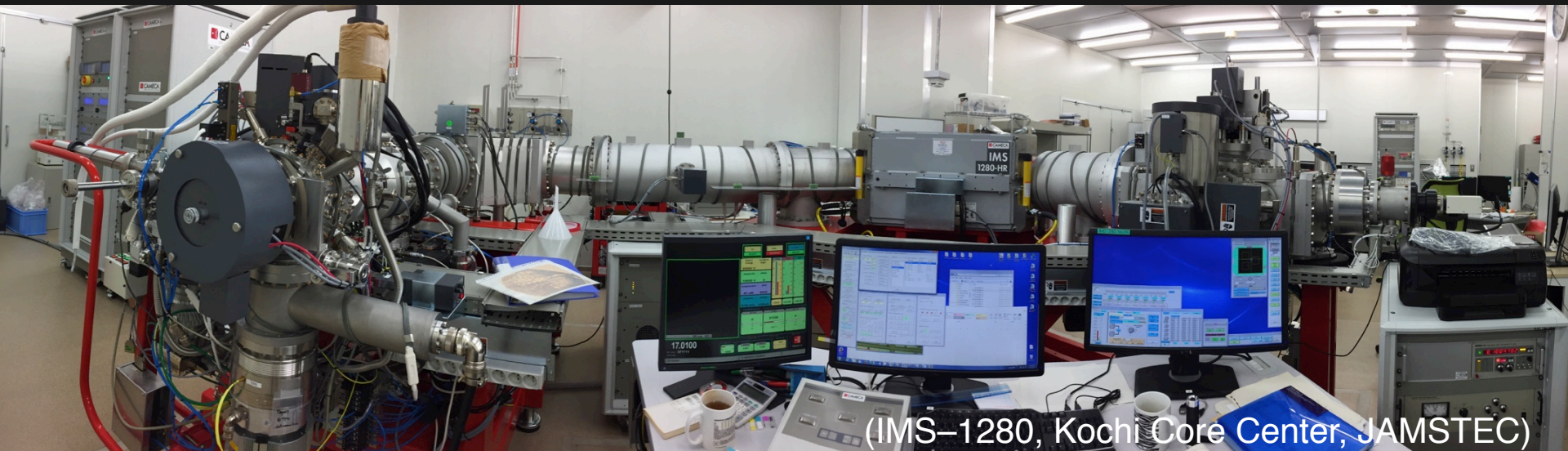
(IMS–1280, Kochi Core Center, JAMSTEC)

Materials and methods

SIMS: Seco ectrometry



- High sensitive s to 10μm
- **Isotope ratios**
- Data quality str urface condition

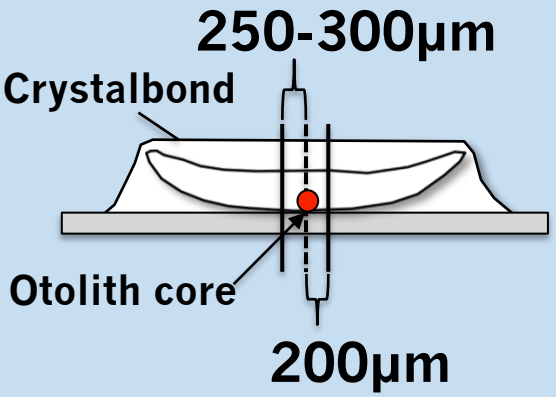


(IMS–1280, Kochi Core Center, JAMSTEC)

Sample preparation protocol for SIMS analysis

1. Cutting and embedding

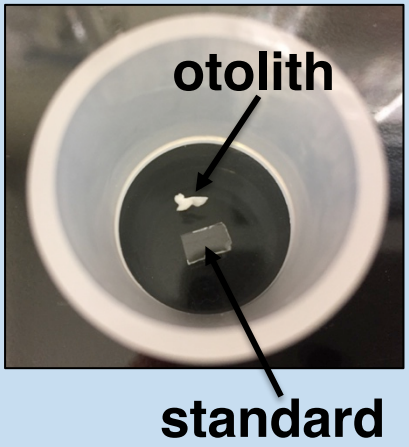
1. Draw lines



2. Cut a thin section

(ISOMET5000, Struers)

3. Embed the otolith



- **Draw lines** on each side of the otolith core
- **Cut a thin section** with an automated slow speed saw
- **Embed otoliths** in epoxy resin with standard materials

Sample preparation protocol for SIMS analysis

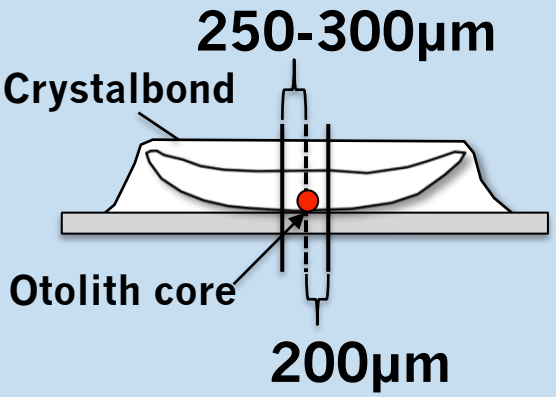
(Inverted microscope, Olympus GX51)

- You can clearly **view otolith core** under the inverted microscope

Sample preparation protocol for SIMS analysis

1. Cutting and embedding

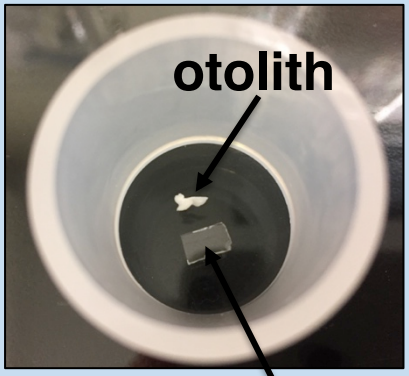
1. Draw lines



2. Cut a thin section

(ISOMET5000, Struers)

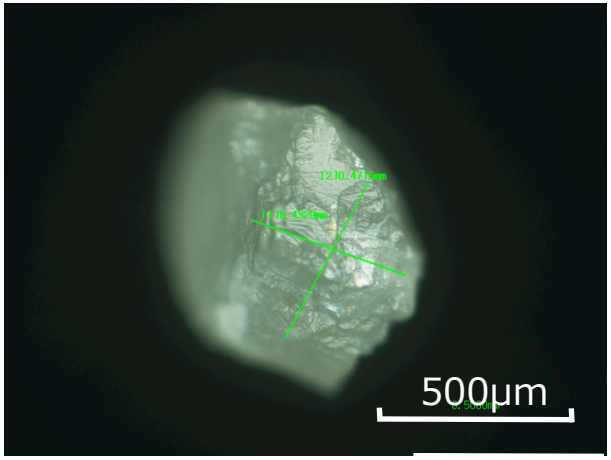
3. Embed the otolith

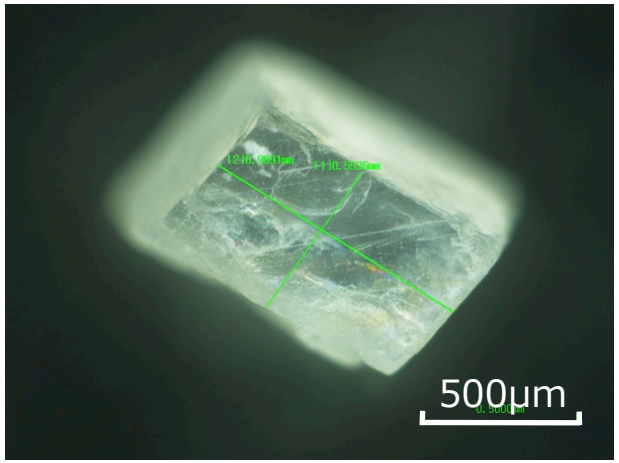


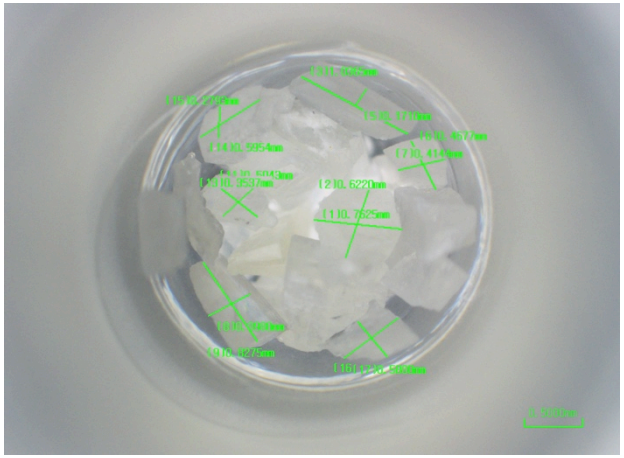
- **Draw lines** on each side of the otolith core
- **Cut a thin section** with an automated slow speed saw
- **Embed otoliths** in epoxy resin with standard materials

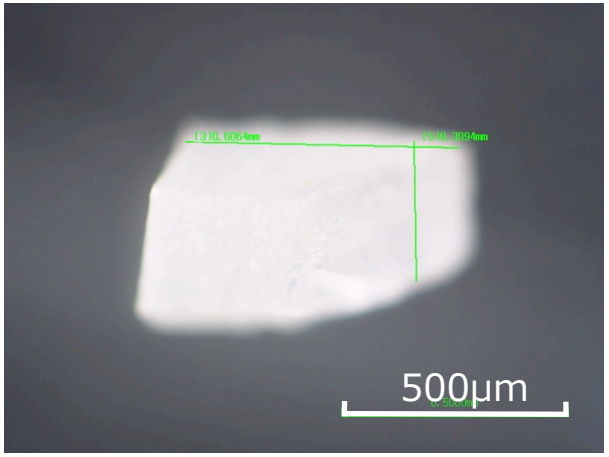
Sample preparation protocol for SIMS analysis

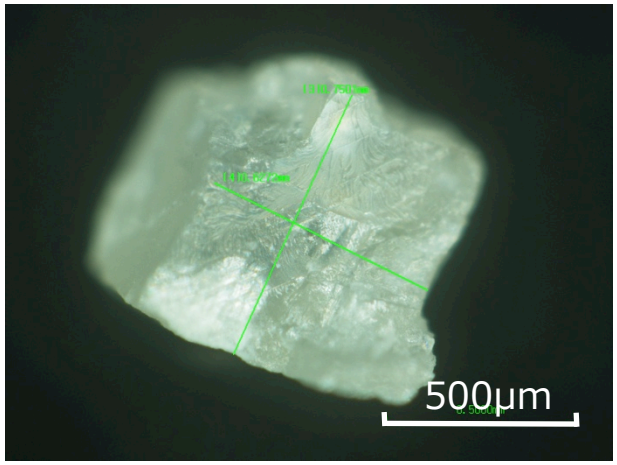
2. Preparation of standard materials



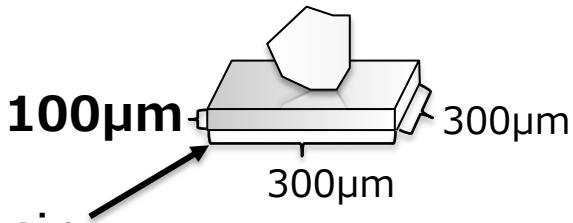








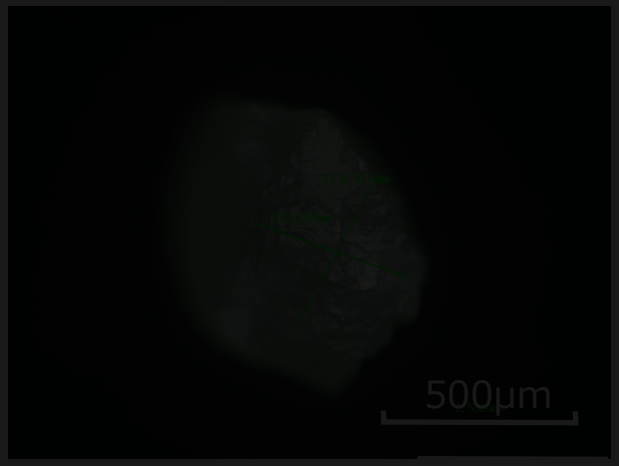
- Different sizes of standard materials

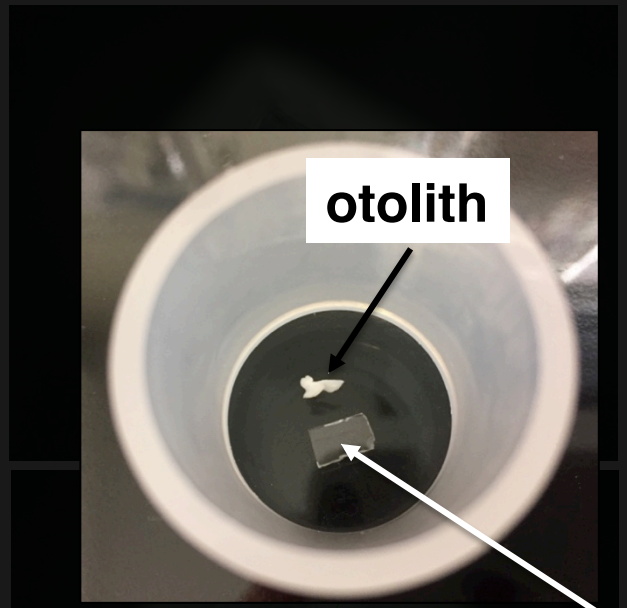


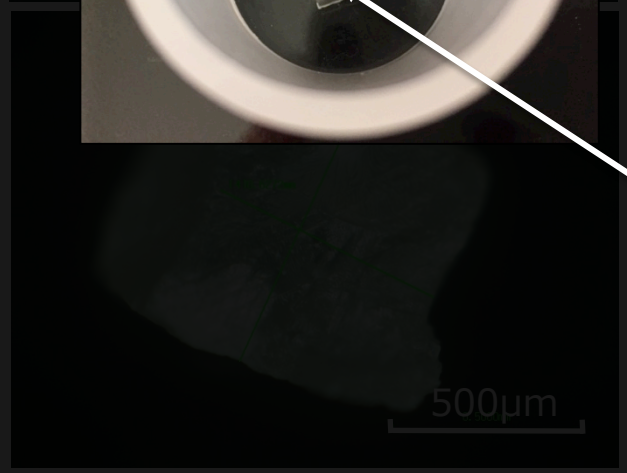
Stand made of epoxy resin

Sample preparation protocol for SIMS analysis

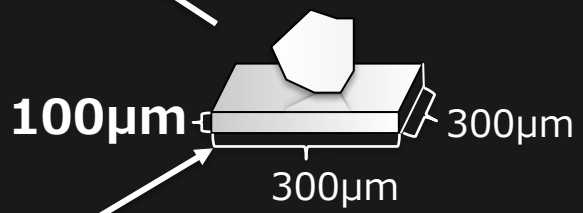
2. Preparation of standard materials







- Different sizes of standard materials (300 – 700μm)



Stand made of epoxy resin

Sample preparation protocol for SIMS analysis

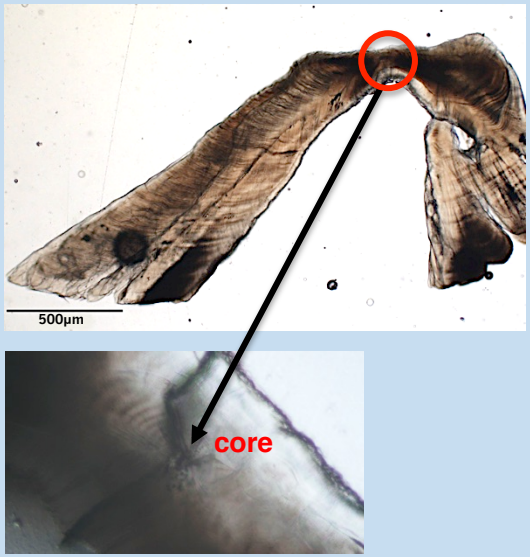
3. Grinding and polishing

1. Grinding

(Discoplan, Struers)

2. 3-steps polishing

3. Expose the core



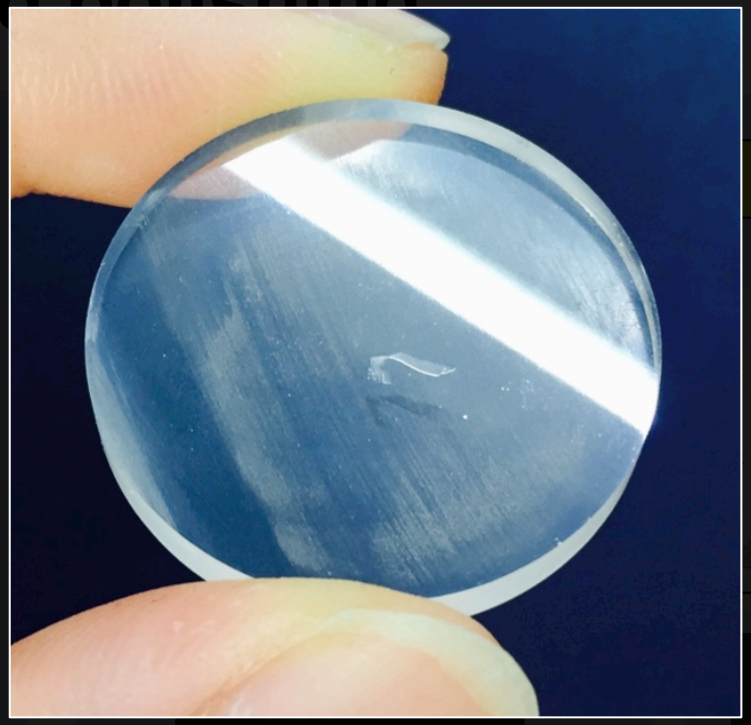
- Grind otoliths with precision grinding machine to near final thickness
- **Polish in 3 steps** with a composite disc using diamond spray

Sample preparation protocol for SIMS analysis

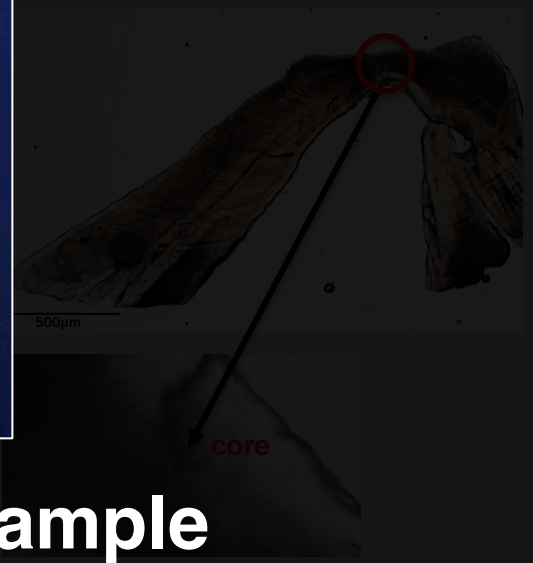
3. Grinding and polishing

1. Grinding

(Discoplan, Struers)



3. Expose the core



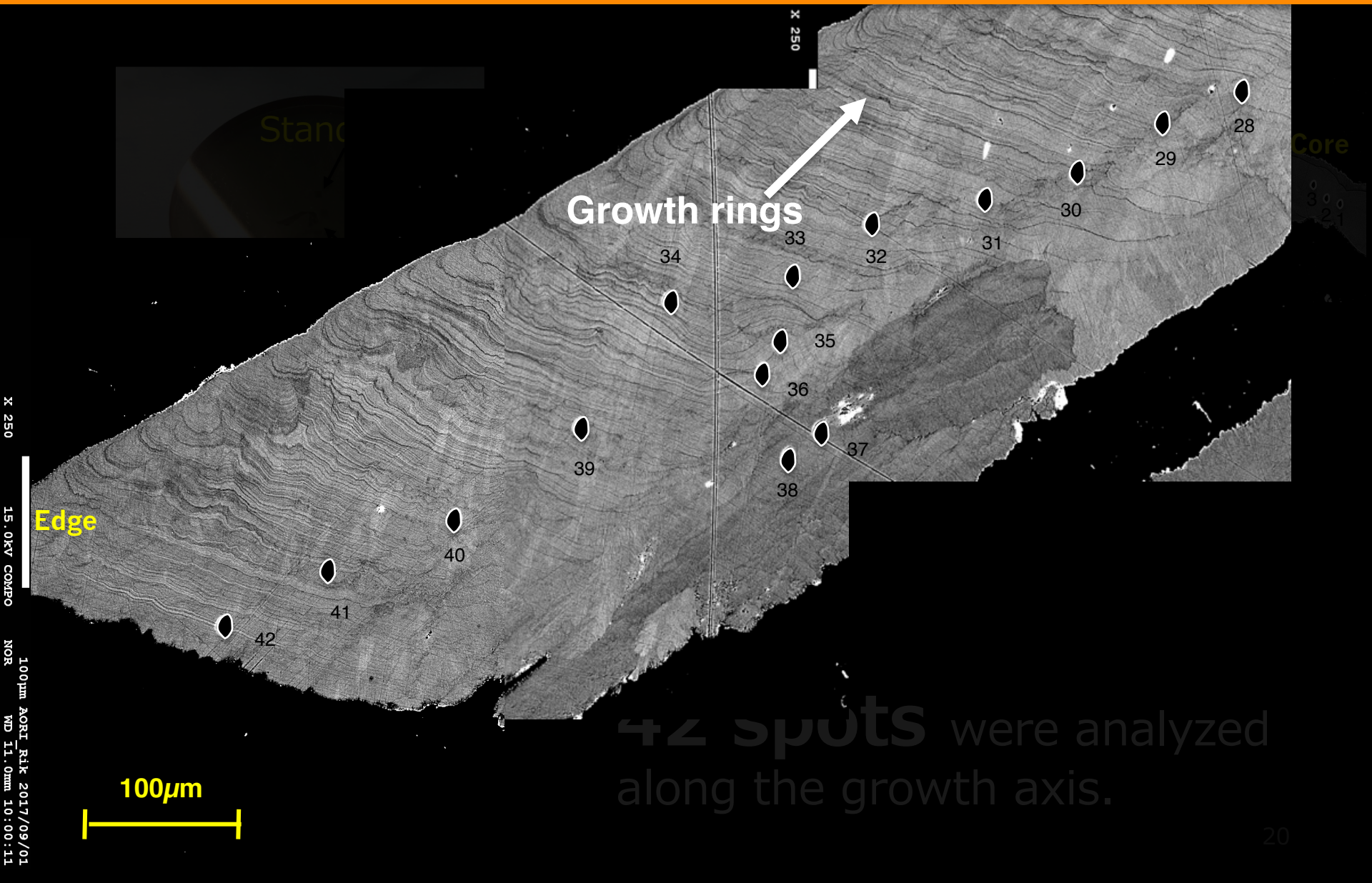
Mirror-finished surface sample

- Grind otoliths with precision grinding machine to near final thickness
- **Polish in 3 steps** with a composite disc using diamond spray

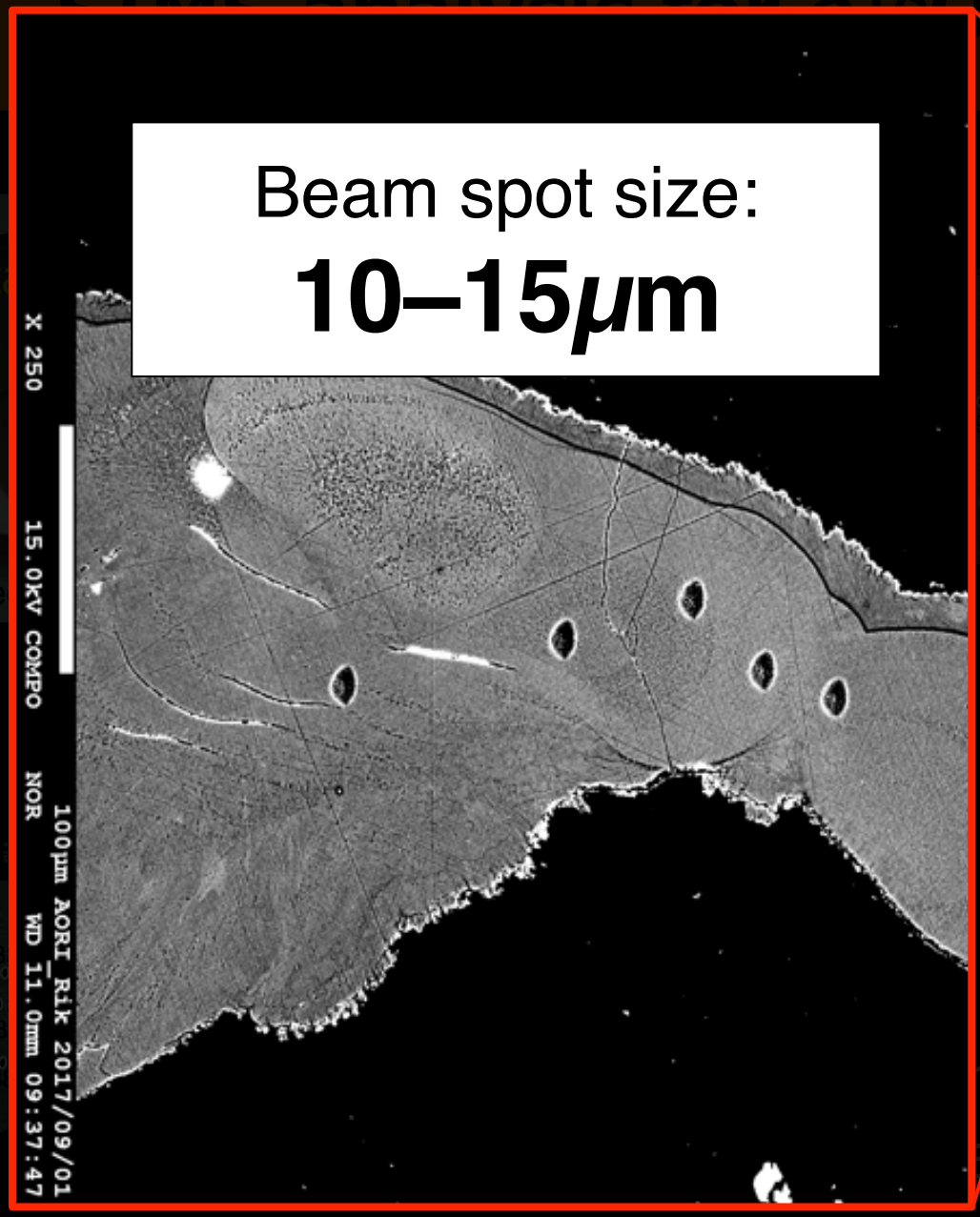
$\delta^{18}O$ SIMS analysis

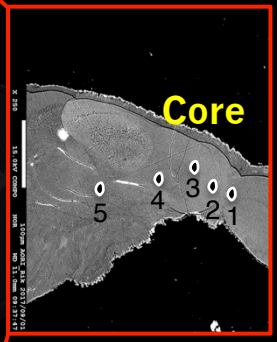
42 spots were analyzed along the growth axis.

SIMS analysis for $\delta^{18}O$



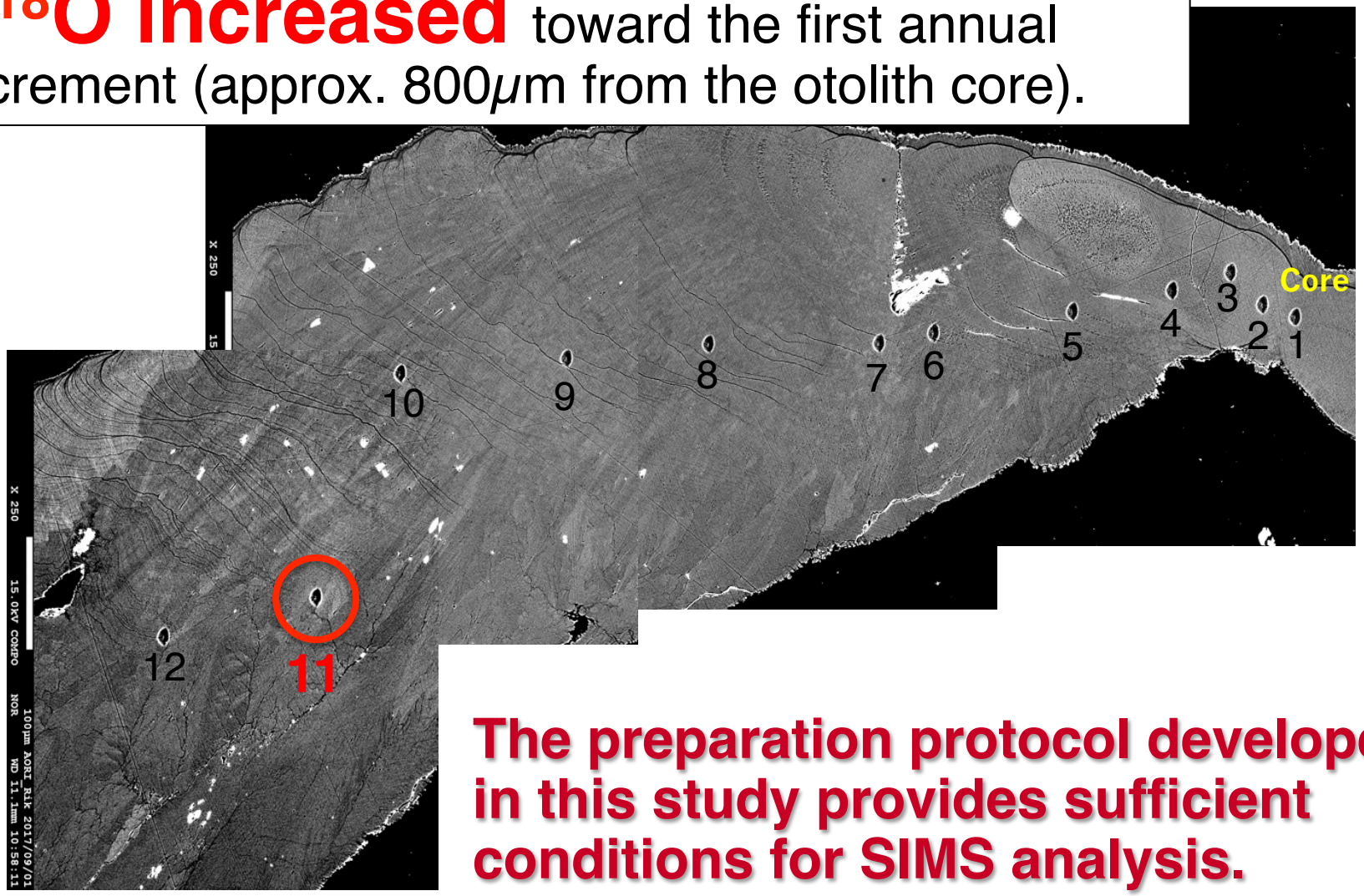
42 spots were analyzed along the growth axis.


Beam spot size:
10–15μm
X 250
15.0kV COMPO
NOR
100μm AORI_Rik 2017/09/01
WD 11.0mm 09:37:47


Core
5
4
3
2
1

SIMS analysis for $\delta^{18}O$

$\delta^{18}O$ increased toward the first annual increment (approx. 800μm from the otolith core).



The preparation protocol developed in this study provides sufficient conditions for SIMS analysis.

Future Plan

- **The sample preparation protocol developed in this study assures data quality** for SIMS analysis, as increasing trends in $\delta^{18}O$ values was measured toward the first annual increment of PBT's otolith.

- For more efficient analysis, **we need to further develop a method to embed multiple otolith cores in one resin disk.**