Validation of daily increments in the otoliths of Atlanto-Iberian sardine larvae (*Sardina pilchardus* Walbaum 1792) reared at three different temperatures

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Project VITAL **FCT - PTDC/MAR/111304/2009**

http://projectvital.fc.ul.pt/

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- Main pelagic fish resource of purse-seine fisheries of Portugal and Spain
  - Both recruitment strength and total abundance have been declining in the last decade. (ICES, 2013)
- Recruitment success is highly dependent of egg and larval survival
  - Larval survival is thought to be largely size-selective

Smaller larvae are more vulnerable

Starvation Predation

Very important to properly quantify growth in field caught larvae!

Introduction | Objectives | Methods | Results | Discussion

- Larvae otolith microstructure analysis assumes increment rate as 1 per day → Larvae age, Growth history

BUT → Temperature, food availability, salinity, photoperiod and oxygen

AGE ESTIMATION ERRORS

Validation is essential

- To determine the influence of temperature in the deposition rate in the otoliths of sardine larvae reared in controlled laboratory conditions with no food limitation at 3 different temperatures (13, 15 and 17ºC).

- ✓ Sardine adults captured in the Portuguese coast and maintained at Oceanário de Lisboa.
- ✓ Spawning was induced by temperature and photoperiod manipulation and salinity was kept constant at 35.
- ✓ Fertilized eggs collected → 30L tanks where larvae hatch and grow under different temperatures (13, 15 and 17ºC) with no food limitation (*Gymnodinum* sp, rotifers *Brachionus* sp., calanoid copepod *Acartia grani* naupli, copepodite and adults).

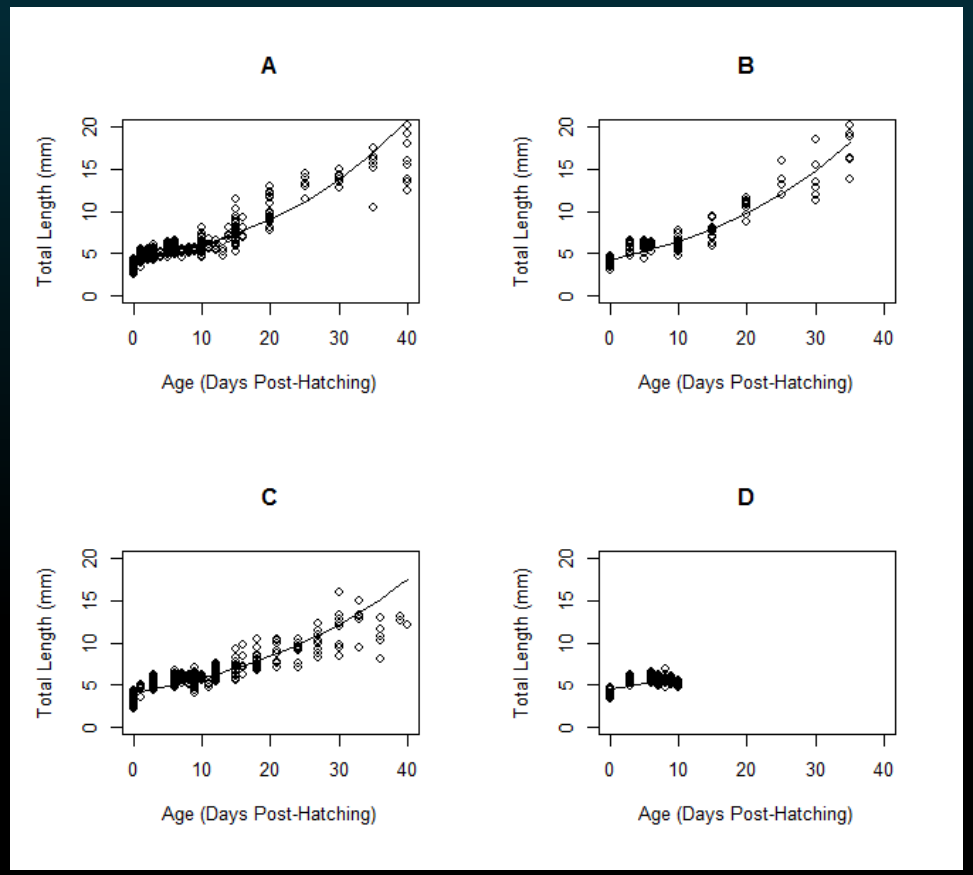
- ✓ Otolith extraction and analysis under an inverted light microscope with Optvar magnification system

  - Growth increments (GI) counted
  - With help of Leica Application Suite software:
    - Total diameter (OD)
    - Increment width

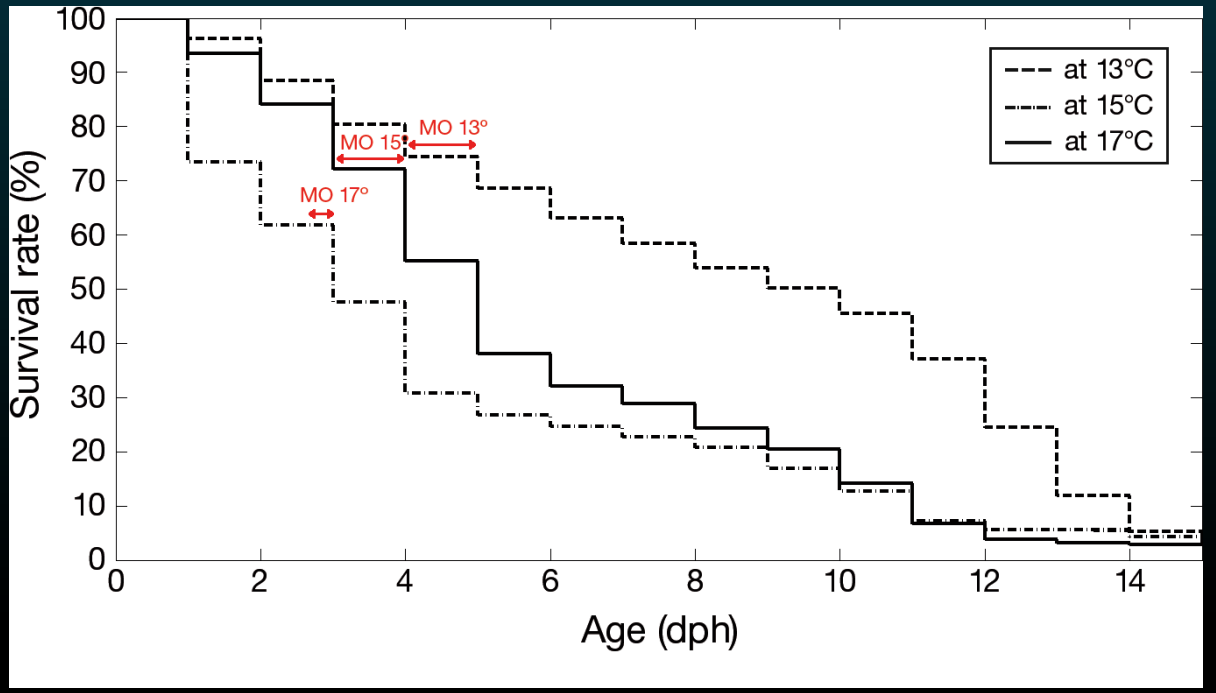
- ✓ Validation:

  - Some of the larger otoliths fixed in Depex resin were polished and etched with EDTA 4% (5-7 minutes)
  - Scanning Electron Microscopy (SEM):
    - Increment count
    - Increment width

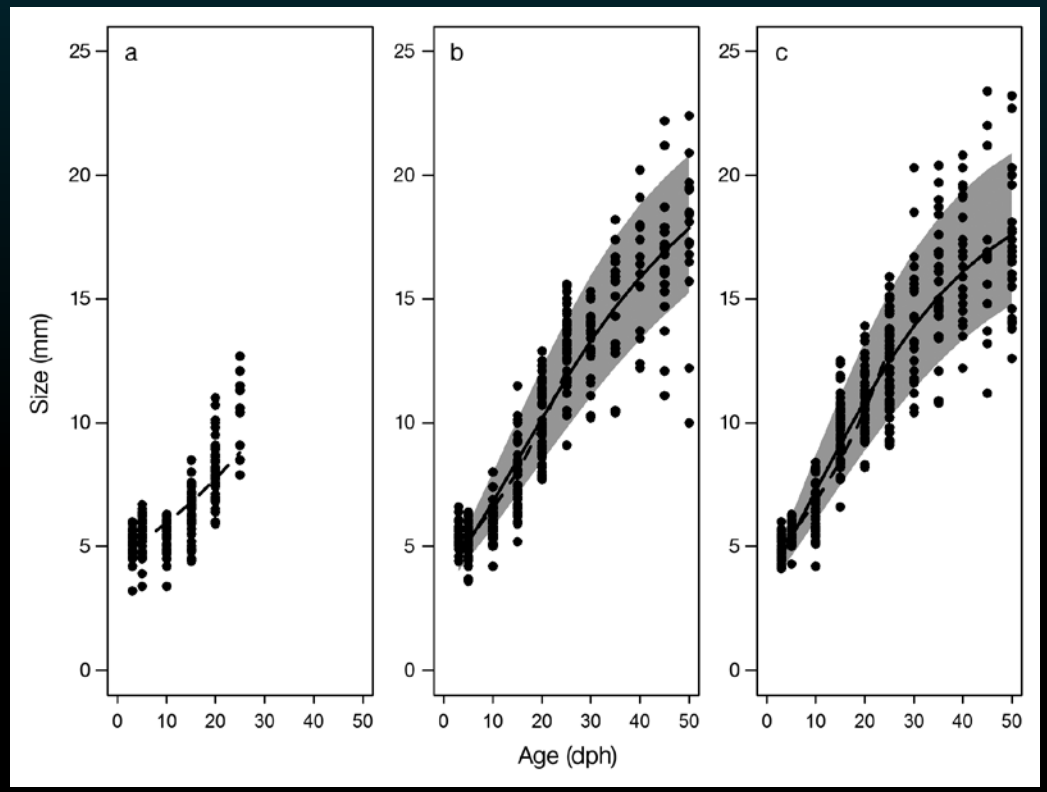
- Food availability influence on growth rates



- Temperature influence on survival rates

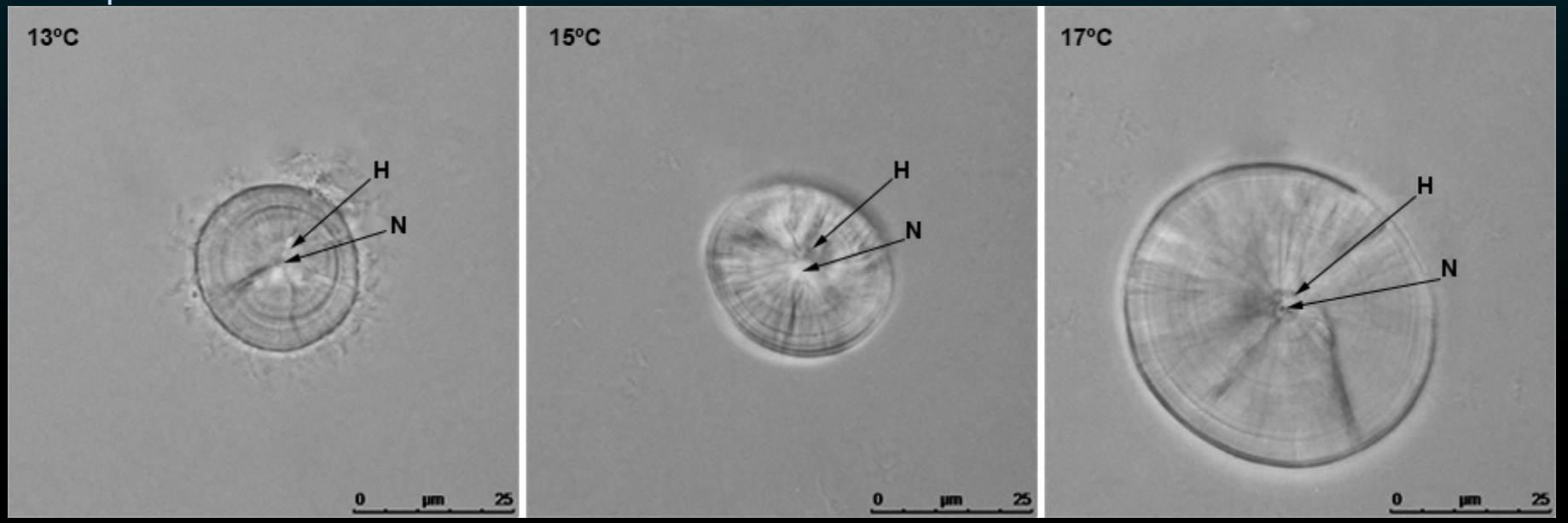


- Larvae under 15 and 17ºC survived the entire experimental period while the ones under 13ºC only survived until 25 dph.
- Different temperatures → different growth rates (17ºC significantly higher).

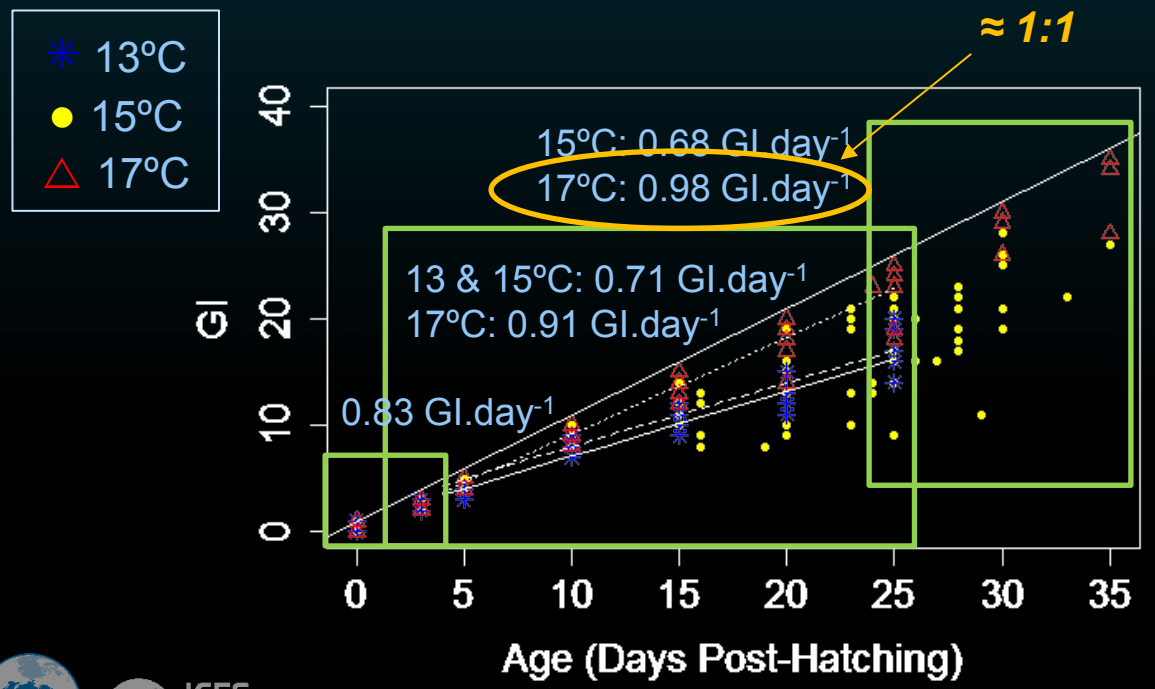


- At hatching, otolith with a diameter of 10.56 µm (± 1.07 SD) showing first increment.
- Nucleus diameter with no significant differences (mean 4.42 µm ± 0.75 SD).
- At the same age, otoliths are always smaller for larvae at 13ºC and wider for larvae at 17ºC.

25 dph



- GI increased with age for all temperatures
  - ✓ 0-3 dph - no differences between temperatures
  - ✓ 3-25 dph - 13ºC and 15ºC similar while 17ºC with significant higher increment counts-at-age
  - ✓ 25-35 dph – 15ºC and 17ºC significantly different



Slope Confidence Interval
(95% confidence bounds)

TºC	0-3 dph	3-25 dph	25-35 dph
13	0.55 – 0.88	0.55 – 0.67	-
15	0.45 – 0.82	0.45 – 0.74	0.49 – 1.58
17	0.56 – 0.80	0.85 – 0.97	0.91 – 1.27

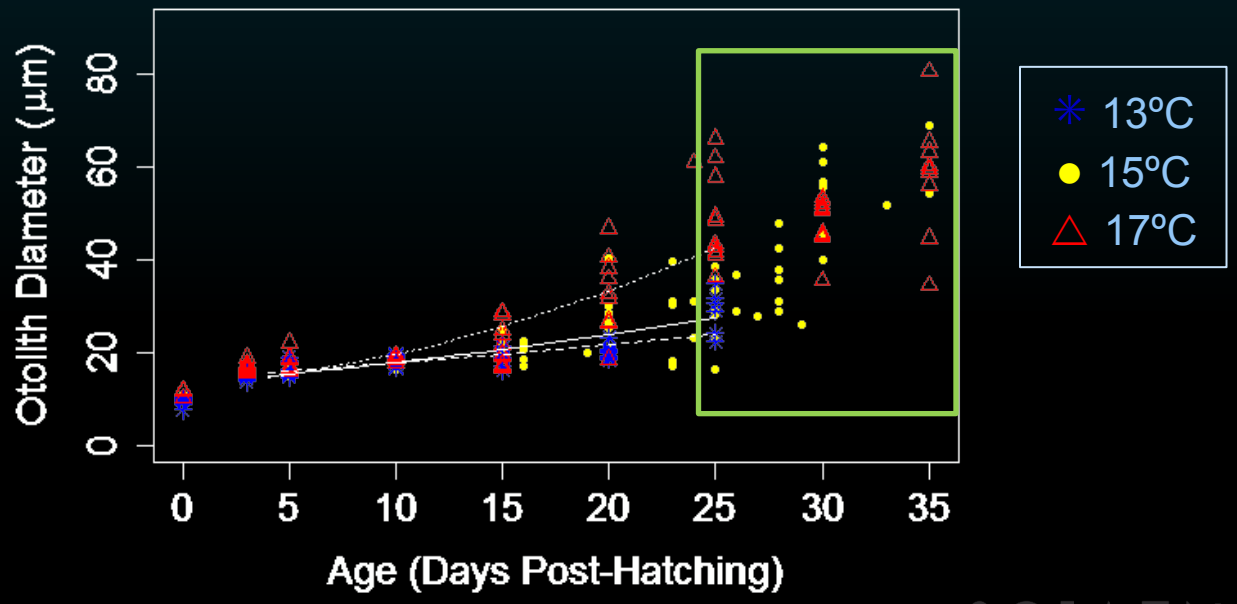
F=0.42, $p<0.0001$

- GI increased with age for all temperatures
  - ✓ 0-3 dph - no differences between temperatures
  - ✓ 3-25 dph - 13ºC and 15ºC similar while 17ºC with significant higher increment counts-at-age
  - ✓ 25-35 dph – 15ºC and 17ºC significantly different
- OD increased with age (13ºC < 17ºC) except for larvae of 25-35 dph at 17ºC → individual variablity

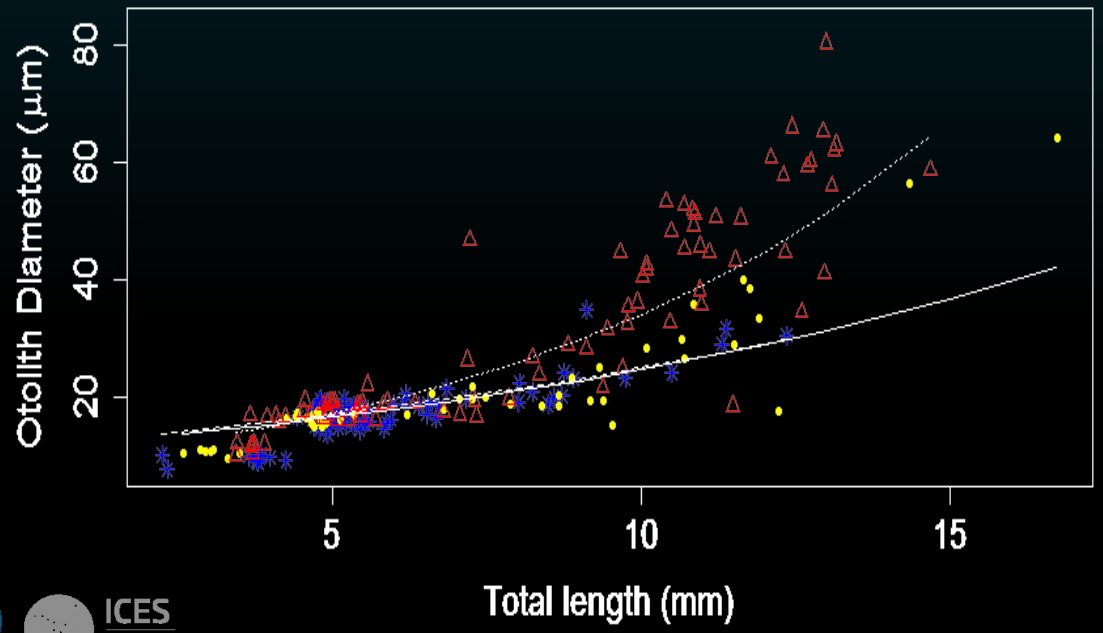
Slope Confidence Interval
(95% confidence bounds)

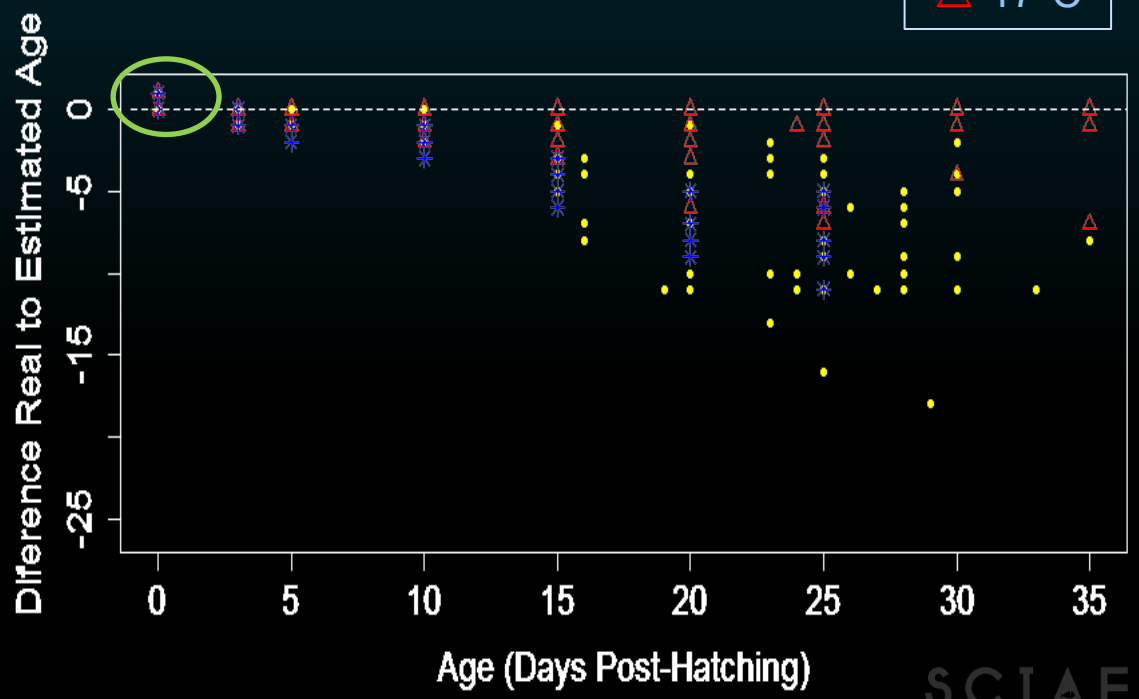
TºC	0-3 dph	3-25 dph	25-35 dph
13	1.63 – 2.14	0.015 – 0.026	-
15	1.54 – 1.91	0.019 – 0.038	0.052 – 0.119
17	1.52 – 2.14	0.043 – 0.060	-0.003 – 0.031

p<0.0001



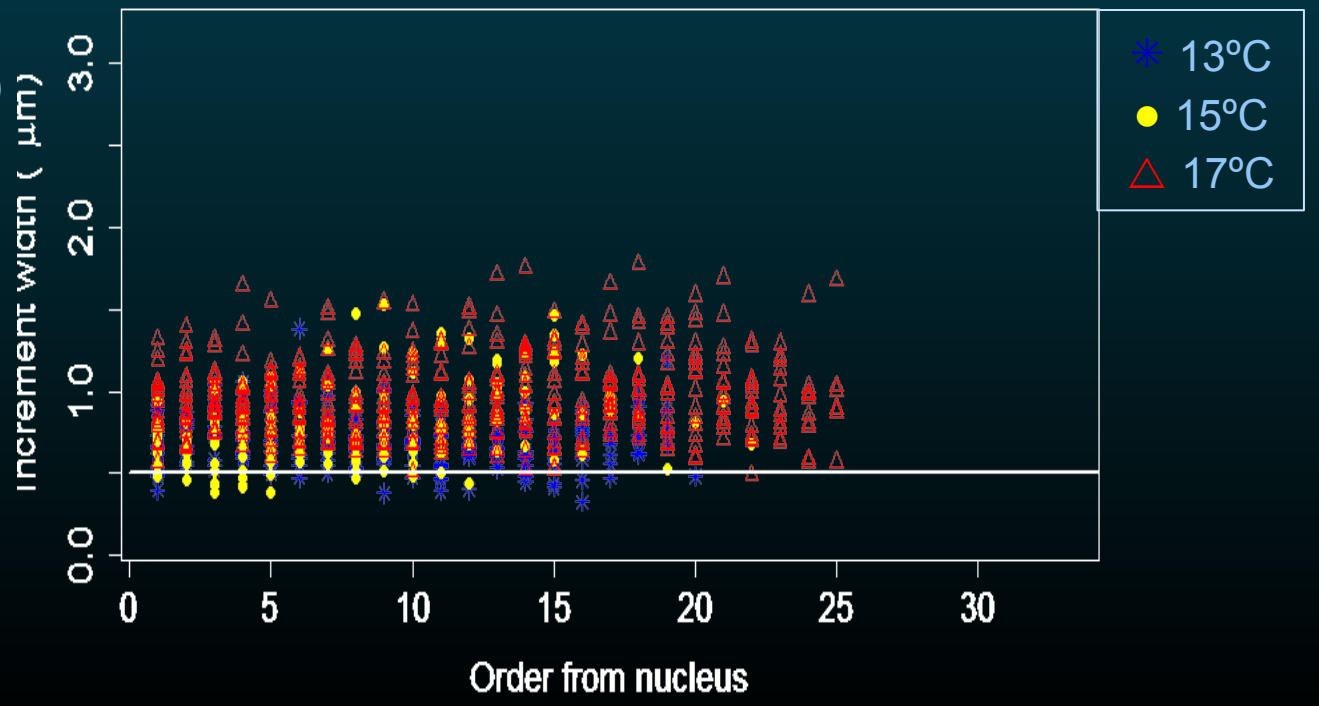
- Bigger larvae → wider otoliths (13ºC with smaller larvae and otoliths VS 17ºC larger larvae and otoliths).
- Real age vs otolith estimation
  - ✓ overestimation for newly hatch larvae → embryonic increments
  - ✓ underestimation for larvae older than 3 dph (mostly for 13 & 15ºC) contrary to 17º
  - ✓ However, high inter-individual variability within groups





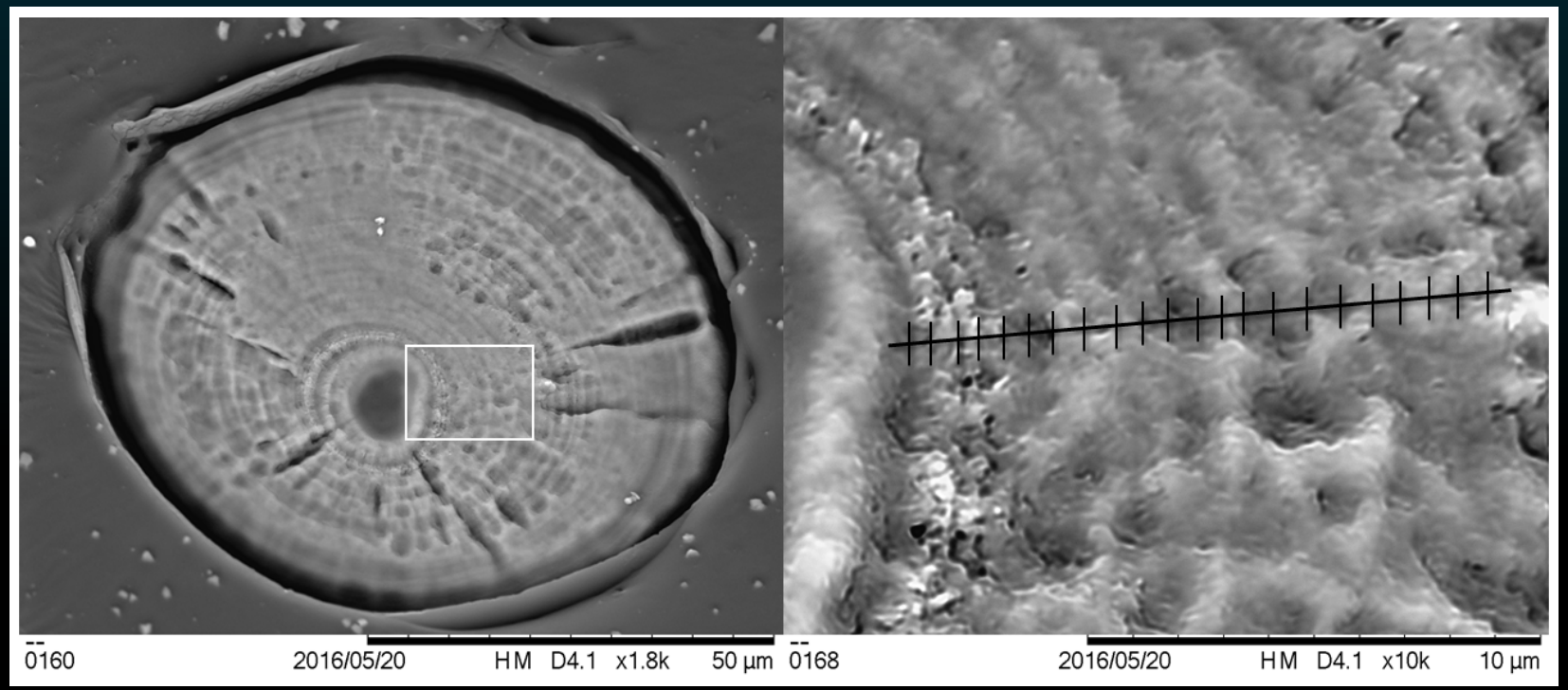
Introduction | Objectives | Methods | **Results** | Discussion

- ✓ 13ºC with smalller increment widths (0.68 µm ± 0.16 SD)
- ✓ 15ºC with increment widths of 0.90 µm (± 0.33 SD)
- ✓ 17ºC with increment widths of 0.97 µm (± 0.24 SD)



- ➢ 0-20 dph, larvae at 13 and 15ºC with increments below 0.5 µm while for larvae at 17ºC mostly above.
- ➢ High variability of width from the nucleus to the outer region where for larvae at 13ºC there was no significant difference between increments.

- SEM confirmed daily otolith increment deposition
  - ✓ Range of widths between 0.28 and 0.89 µm (0.56 µm ± 0.19 SD)



- At 13ºC growth was significantly lower as well as deposition rate
  - Higher temperatures acelerate metabolism increasing growth rates and reducing development time

→ Active swimming and search for food starts earlier

- At hatching, larvae and otolith nucleus size did not differ between temperatures while otolith diameter increased with temperature.
  - Otolith growth directly related to metabolic rates while larvae size-at-hatch is also a result of maternal effects

→ Temperature affects increment spacing

- Otolith microstructure analysis seems to be a powerful tool based on the assumption of daily increments deposition

↓

Environmental conditions can affect the accuracy of age estimations

Overestimation (embryonic increments)

Underestimation

- Non-daily otolith increments observed with light microscope were found in other studies often attributed to food conditions, photoperiod and temperature
  - 15ºC with no food limitation, otoliths show less than one increment per day at these low temperatures, and ages have been underestimated in wild larvae (overestimating growth)
    - → situation also described for anchovy by Aldanondo *et al* (2008), only validating for temperatures above 17ºC

Critical for sardine age estimations

- Environmental conditions and ontogeny influence the number of unreadable increments
- Method limitations: microscope resolution and / or observer experience → **SEM is not viable**
- Accurate age estimations crucial for back calculations of birth date studies

Error correction higher as lower the temperature

Thank you