Unlocking the bio-logging potential of otoliths as natural tags: Disentangling environmental and physiological influences on otolith chemistry

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Introduction

Otoliths, the calcium carbonate 'earstones' common to all bony fish, have gained increasing prominence in the published literature as **natural bio-loggers of lifetime movements**. Incremental growth, incorporating elements from the surrounding water, produces a temporally resolved chemical record of ambient conditions experienced by the fish. However, a fundamental assumption of otolith studies is that the within-fish transport chemistry of metals is either largely unaffected by physiology, or that any physiological variations are smaller than environmental variations. For some elements, this assumption is currently being challenged.

Environmental vs. physiological?

Otolith Sr (or Sr/Ca) is often used as a marker of movement
- as it is conserved with salinity,
- substitutes easily for calcium,
- and has a positive linear relationship with ambient levels

However, cycles of Sr/Ca are oft-observed in the otoliths of **marine species** (e.g. Fig. 1), where salinity and therefore Sr, should remain constant. Other 'conservative elements', such as Mg and Li, also vary within the otolith in patterns not easily explained by water concentrations alone.

So, what **other factors** might be involved?

The literature reports a number of possibilities, including
- Temperature
- Ontogeny
- Stress
- Gonad development
- Growth rate

Discriminating between these requires further validation studies

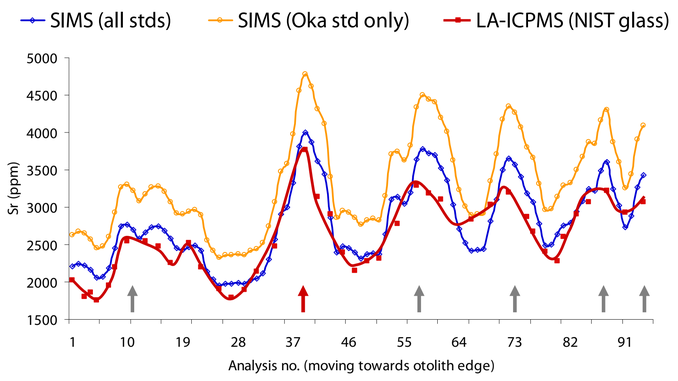

Fig. 1 Seasonal fluctuations of otolith Sr/Ca ratios in a wild female plaice, measured by LA-ICPMS and SIMS (see methods). Instrument and calibrations indicated in figure legend. Years shown by arrows, red one = age at maturity. LA-ICPMS data provided by A. Darnaude & E. Hunter (2004 unpubl.)

Presented within the context of otolith chemical profiles for wild plaice (*Pleuronectes platessa* L.), with 'known migrations' reconstructed from archival **Data Storage Tag (DST) data**, we here communicate the results of an **experimental study** investigating the relationship between blood & otolith chemistry in plaice over a reproductive cycle

Aquarium experiment

Set up

Capture
Plaice caught from the Irish Sea in March 09 and additional females from the English Channel, July 09 (Fig. 4)

Maintain
at CEFAS,,Lowestoft in 9m³ flow through SW tank. Both sets acclimatised for 3 mo

Prepare fish
Weigh, measure
Vibrio vaccination
OTC injection (Fig. 5)
PIT tag

Fig. 4 Map showing capture sites (●) & experiment location (◆) at CEFAS, Lowestoft

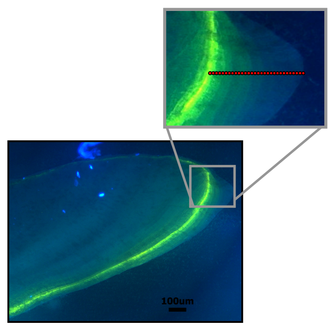

Fig. 5 Photograph of otolith under blue light, showing fluorescent OTC mark and (inset) proposed transect for LA-ICPMS and SIMS analysis (see 'Methods')

Variable	*Monitored*
Temperature Ambient, but capped at 14°C	**Daily (to 0.1°C)**
Salinity Ambient, but can fall after rain events	**Weekly (to 5 d.p.)**
Water chemistry Ambient	**Weekly (to 1 ppb)**
Diet chemistry Lugworms from 1 local beach	**Monthly (to 1 ppb)**
Contamination Clean cell used where possible, consistent procedures, acid cleaned consumables	**Blanks each sampling day**
Condition High ration diet (equiv to 2.5% BW/day)	**Fed biweekly**

Methods

June 2009 - 2010: monthly sampling

1. Fulton's condition factor

2. Female Gonadosomatic Index (GSI)

Ovary mass extrapolated from ovary area (Fig. 6, Kennedy et al 2008).

3. Blood sample

~0.4ml from caudal vein (Fig. 7). Plasma aliquots stored at -20°C.

(A) Total protein
Biuret method

(B) Trace metals ***(Ca, Sr, Zn, Cu, Mg, Se, Li, Ba, Mn, Pb)***
Inductively Coupled Plasma Mass Spectrometer (ICPMS)

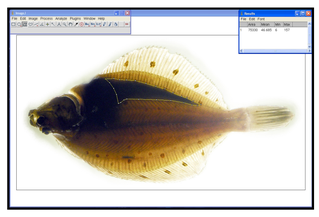

Fig. 6 Method to estimate GSI in females non-destructively, using a photo taken on a lightbox and Image J freeware to measure area of ovary shadow

Fig. 7 Sampling blood from the caudal vein of plaice. Samples were kept on ice until they could be centrifuged and the plasma pipetted off in a clean cell

June 2010

Sagittal otoliths removed for chemical analysis* by laser ablation ICPMS (LA-ICPMS) and secondary ion mass spectrometry (SIMS)

** Metal concentrations (same metals as above) by LA-ICPMS & SIMS and $\delta^{18}O$ by SIMS.*

Results

Physiology

- Female GSI estimates range from **0.3 to 32.5** based on ovary area vs. mass calibration ($r^2 = 0.92$, $p<0.001$).
- Positive correlations between condition & total protein **(males: $r^2 = 0.37$, $p<0.001$; females: $r^2 = 0.25$, $p<0.001$)**, and estimated female GSI (ln-transformed) & total protein **($r^2 = 0.14$, $p<0.001$)**
- Seasonal changes in physiological parameters, particularly around the spawning season (Jan-Apr), however, the English Channel females were atypical as almost all of them were immature (Fig. 8)

Blood & otolith chemistry

Trace metal concentrations *Results pending due to ongoing method development and technical issues. However, preliminary analyses revealed otolith Sr/Ca cycles in wild fish and good agreement between SIMS and LA-ICPMS (Fig. 1).*

Otolith $\delta^{18}O$
Seasonal patterns in otolith $\delta^{18}O$ were observed that can be wiggled-matched with predicted $\delta^{18}O$ (calculated from water T & S; Hoie et al. 2004) to produce a temporal model of otolith growth. This allows time-matching of otolith analyses with environmental & physiological data (Fig. 9). Issues sourcing matrix-matched SIMS standards meant that measured $\delta^{18}O$ was not necessarily accurate but precision was within 0.2‰

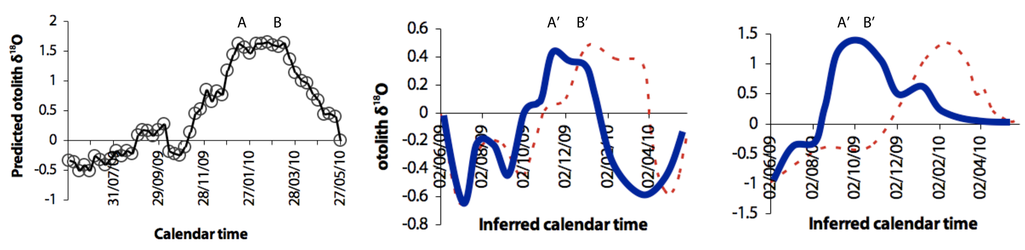

Fig.9 Predicted (left) & measured otolith $\delta^{18}O$ for two experimental fish (centre, right). Measured $\delta^{18}O$ is displayed raw (thick blue line) & wiggled-matched using points A & B from the predicted data (dotted red line). NB. The timing of points A & B coincide directly with the winter spawning season (January - March).

Fig. 8 Changes in condition, GSI, and plasma protein in plaice from the Irish Sea (IS) & English Channel (EC)

DST experiment

From 1997 to 2004, wild male & female plaice were tagged with archival DSTs that record temperature & pressure (Fig. 2). Individual tracks were determined using the Tidal Location Method (Hunter *et al.* 2003), while predicted otolith $\delta^{18}O$ was estimated using recorded temperature and modelled salinity (see www.getm.eu) and the equation of Hoie *et al.* (2004) (Fig. 3).

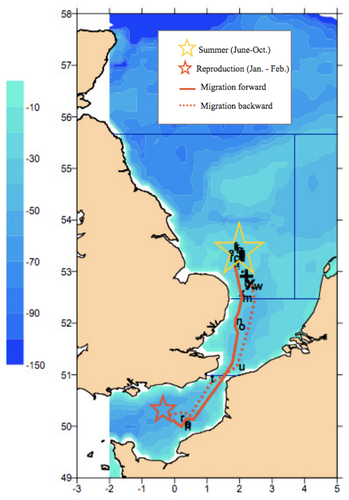

Fig. 2 Above: Wild female plaice with a DST attached (© CEFAS). Right: track for a DST tagged female at liberty for 1 year. She was recaptured within 20km of her release site. The associated otolith chemistry for this particular fish is displayed in Fig. 1 and 3.

Otolith trace metal concentrations and $\delta^{18}O$ were measured by SIMS (see 'Methods'). Predicted and measured $\delta^{18}O$ were compared to determine the DST period and produce a temporal model of otolith growth to synchronise otolith analyses with geolocations and environmental conditions experienced during time-at-liberty (Fig. 3).

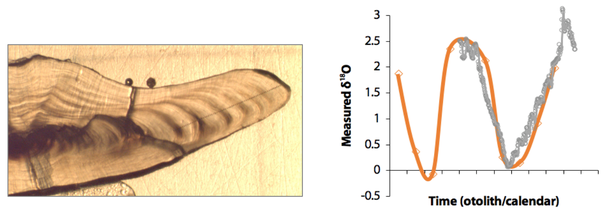

Fig. 3 Otolith from featured female with LA-ICPMS analysis pits (data displayed in Fig.1). Measured (orange line) and predicted (grey line) $\delta^{18}O$ generally correspond, allowing DST 'start time' to be located on the otolith and implying relatively constant otolith growth during the DST period.

Conclusions

Using a combination of *in situ* and experimental data we hope to quantify environmental and physiological effects on otolith chemistry, and better understand which markers can be used as 'biologgers' of fish movement in the open ocean. Such markers have great potential in the realm of fisheries management. For example, predicting stock recovery and responses to environmental change requires extensive knowledge of population structure and connectivity, which is logistically difficult to attain in the marine environment.

If some otolith elements are found to be controlled primarily by physiology, while it might reduce their worth as a marker of movement, they might prove to be useful 'physiological biologgers' with which to estimate age-at-maturity and spawning frequency, both key parameters in stock assessment and fisheries management.

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