DISENTANGLING ENVIRONMENTAL AND PHYSIOLOGICAL INFLUENCES ON OTOLITH CHEMISTRY USING A FLATFISH MODEL

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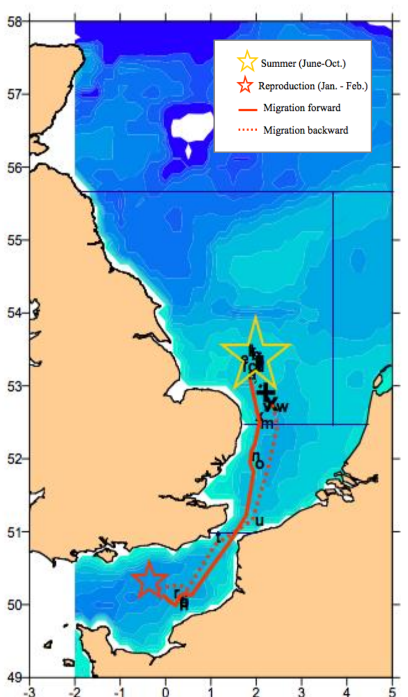
Otoliths are $CaCO_3$ 'earstones' common to all bony fish. They are excellent chronometers, but are they also reliable environmental recorders?

Otolith chemistry can potentially be used a natural tag of location & movement. This relies on incremental growth incorporating elements from the surrounding water to produce a temporally resolved chemical record of ambient conditions. If fully validated, it would be an extremely valuable tool. However, a fundamental assumption of this use of otolith chemistry is that the within-fish transport chemistry of metals is largely unaffected by physiological variations or that any such 'vital effects' are smaller than environmental effects. For some elements, this assumption is being challenged.

Plaice, our flatfish model

Wild plaice were tagged with electronic data storage tags (DSTs) that record temperature & pressure, which can be used to estimate individual tracks (Hunter *et al.* 2003).



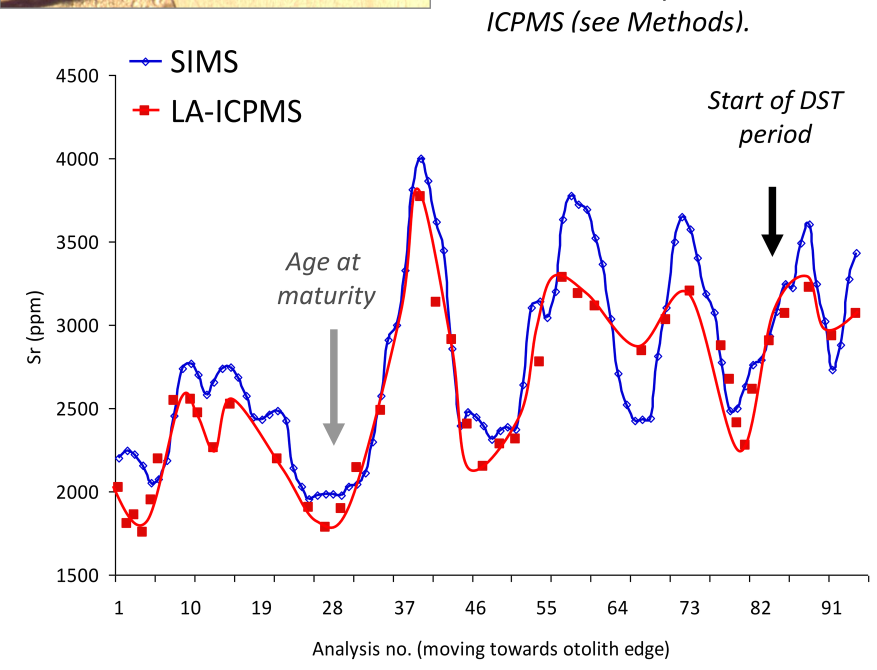
Above: Wild female plaice with a DST attached (© CEFAS).

Right: Track of a tagged female at liberty for 1 yr & recaptured within 20km of the release site (image courtesy of A Darnaude).

The otolith from the female above, who migrated more than 500 km in the year, exhibited clear Sr cycles:

Left: Photograph of the sectioned otolith shows the pits left by the LA-ICPMS analyses (see Methods).

Below: Sr concentrations across an otolith from age 4 to 9 years, as measured by SIMS and LA-ICPMS (see Methods).



Otolith strontium (Sr) is often used as a geographical marker because it is positively related to water Sr. But in the sea, salinity & Sr are almost constant.

So what else (apart from water chemistry) might be causing these fluctuations?

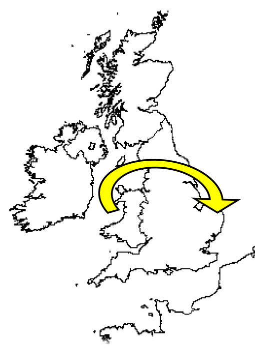
- Temperature
- Ontogeny
- Stress
- Gonad development
- Growth rate

All feature in the literature, but more validation work needs to be done to discriminate between them

Here, we communicate results from an experimental study investigating the relationship between blood and otolith chemistry over a full reproductive cycle in plaice (*Pleuronectes platessa*)

Methods

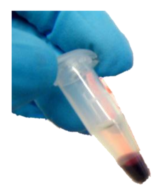
Capture *and transport plaice from Irish Sea to CEFAS aquarium, Lowestoft* → **Maintain** *9m³ flow-through SW tank (ambient). Acclimatised for 3 mo* → **Prepare** *Vibrio vaccination, PIT tag, OTC injection*



- Fish fed local source of lugworms
- Salinity recorded weekly
- Temperature recorded daily
- Fish sampled every month (June 2009-10)
- Half the fish treated with slow-release GnRH (Jan 2010) in case of reproductive dysfunction

Used to predict otolith $\delta^{18}O$

Blood chemistry



~ 0.4ml blood sampled from caudal vein. Plasma stored at -20°C

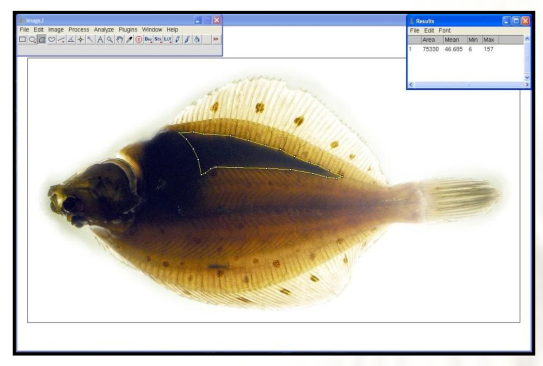
Trace metals
Prep in Class 100 clean room. Analysis by High Resolution Inductively Coupled Plasma Mass Spectrometry (HR-ICPMS)

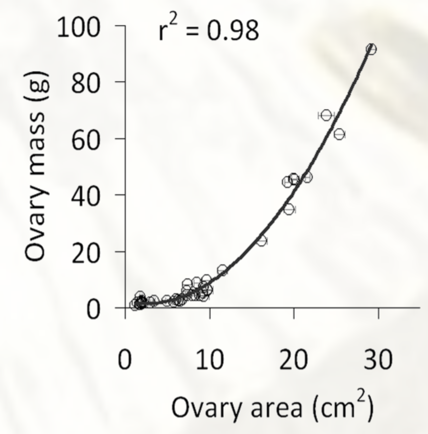
Total protein & albumins
Biuret & bromocresol green tests

Physiology

a) Fulton's condition factor (TW/TL³)

b) Female gonadosomatic index (GSI)

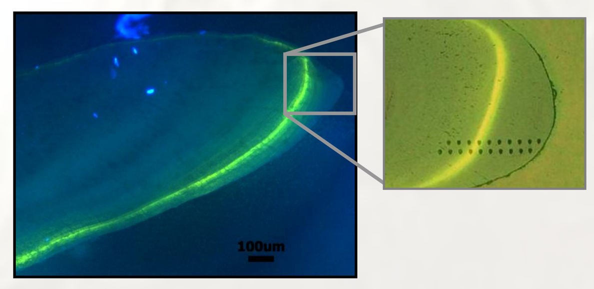




Ovary mass extrapolated from ovary area using Image J freeware (Kennedy et al 2008).

Otolith chemistry

Sagittal otoliths sectioned for trace metal (Ca, Sr, Ba, Mg, Li, K, Cu, Zn, Mn) and $\delta^{18}O$ analysis by laser ablation HR-ICPMS & secondary ion mass spectrometry (SIMS)



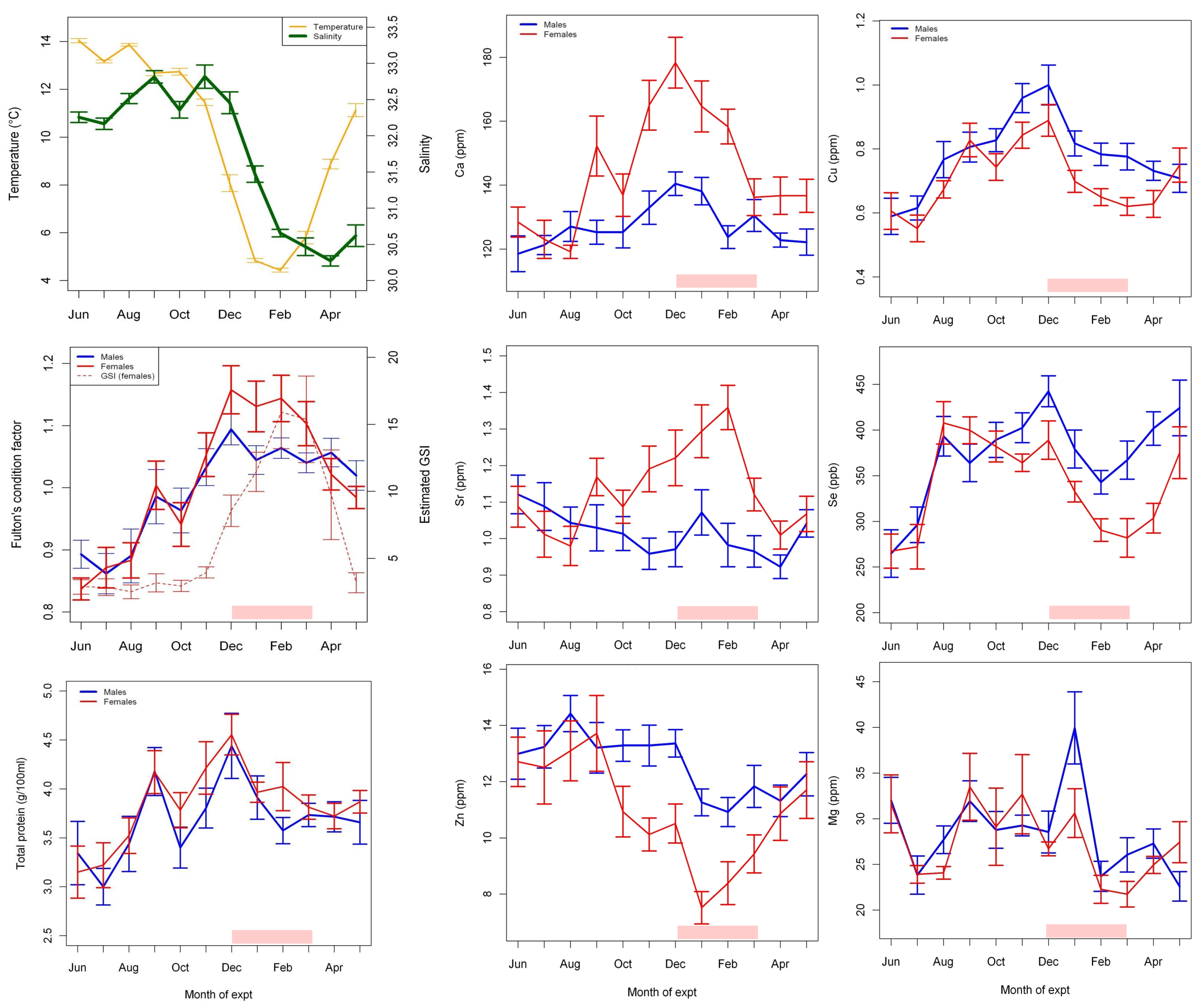
Sectioned otolith showing OTC mark (=expt start) and 20μm spots made by SIMS for $\delta^{18}O$ analyses. Spot sizes for trace metals were 8μm on SIMS and 35μm on LA-HR-ICPMS

Cameca 4f SIMS at the Edinburgh Ion Microprobe Facility

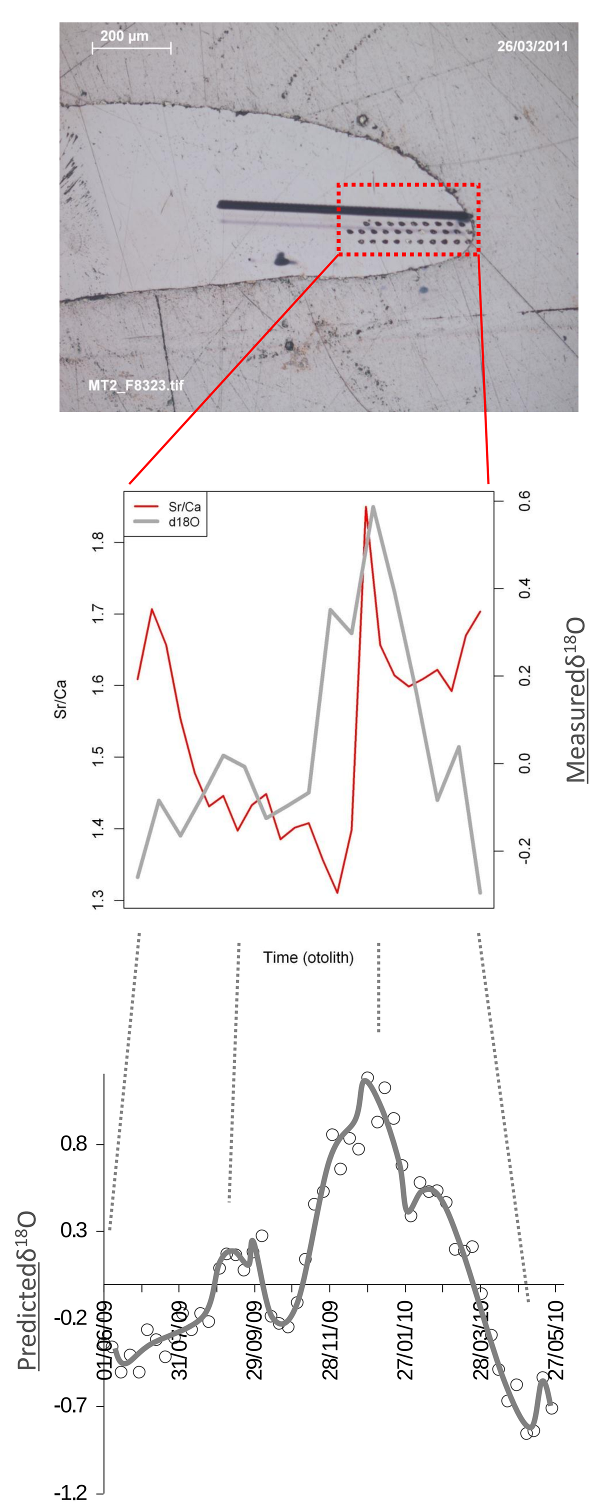
Results

Physiology

Blood chemistry



Otolith chemistry



The otolith trace metal and $\delta^{18}O$ data analysis is in the early stages, but distinct patterns in otolith $\delta^{18}O$ will provide an intra-annual timeline within each otolith to precisely match otolith material with the other variables. We are currently developing a method in R to match curves in an automated manner.

- Fish exhibited clear seasonal changes in physiology, in keeping with observations from wild fish.
- Blood Ca and Sr were higher in females, and peaked during the spawning period (pink bar), although Ca peaked at the start of the spawning period, following vitellogenesis, while Sr peaked with GSI.
- Blood Zn exhibited a clear negative relationship with GSI in females
- Blood Cu, Se (and possibly Mg) appeared correlated with condition & total protein concentrations, peaking just before the spawning period, although generally remaining higher in the males.

Conclusions

Through a combination of time-resolved *in situ* DST records and experimental observations we hope to quantify and partition environmental and physiological influences on otolith chemistry. At this stage, the experimental data generally just reassure us of 'normal' reproductive functioning in the experimental population, however many of the elements measured have not been described previously in fish blood. Modelling the transport chemistry of trace metals from water to blood, and blood to otolith remains an exciting area for us to explore.

If we can ascertain how otolith elements record ambient conditions it would greatly enhance their potential to geolocate individual fish in time and space. If some elements are shown to be primarily controlled by physiology it reduces their value as a marker of movement, but they could prove useful for estimating age-at-maturity & spawning frequency, both key parameters in stock assessment & fisheries management.

Acknowledgements

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