Institution: University of East Anglia

Unit of Assessment: 5 – Biological Sciences

Title of case study:

High throughput molecular fish fingerprinting assay for measuring fish stocks

1. Summary of the impact

In the early 2000s, **Taylor** and **Rico, C.** at UEA developed a molecular fingerprinting assay to identify visually indistinguishable cod, haddock and whiting eggs for the accurate assessment of fish stocks. This is essential in setting fishing quotas for healthy populations. The impacts are:

- The Centre for Environment, Fisheries & Aquaculture Science (Cefas) developed a molecular lab, with advice and assistance from Taylor and substantial funding from Defra.
- The assay was used between 2006 and 2010 in the annual Cefas assessment of cod and haddock stocks in the Irish Sea.
- The assay is also used to map cod spawning grounds in the Eastern Baltic Sea by the Swedish Board of Fisheries.

2. Underpinning research

Research initiated by Taylor and Rico, C. at UEA in the early 2000s led to the development of new molecular methods to survey and accurately distinguish between the important fish species haddock, cod and whiting, whose eggs and early stages are impossible to distinguish by visual inspection. This molecular genetic assay allows high throughput genetic identification of the species of individual pelagic fish eggs. This real-time PCR-based assay uses Taqman hybridisation probes labelled with different fluorescent dyes to simultaneously identify cod, haddock and whiting eggs in a single reaction that includes both “universal” primers (which amplify the DNA of all three species) and three species-specific probes. The assay is >95% accurate and allows large numbers of egg samples to be processed in relatively short times. For example, DNA can be extracted from individual eggs in 96-well format using either manual or robotic platforms. Genetic identification also occurs in 96-well format allowing eggs to be identified in 20 minutes using the Applied Biosystems fast – real-time PCR system (research references 1-4).

This has solved several problems and inaccuracies previously associated with fisheries stock assessments, which can be conducted using (i) catch-dependent or (ii) catch-independent methods.

(i) Catch-dependent methods require data on the mass and distribution of fish-landings. These are prone to bias due to misreporting of landings, undeclared discarding and model assumptions that are violated at low stock abundances.

(ii) Catch-independent methods rely on other forms of data not obtained from recorded catches (e.g. the abundance of eggs in the water column). They involve identifying and counting fish eggs and larvae (ichthyoplankton surveys), and can be used as an additional stock assessment tool that is largely free from catch-dependent biases. Egg production information is combined with estimates of weight-specific fecundity of the female fish, levels of atresia (degradation and absorption of oocytes prior to spawning), and the sex ratio of the population to produce an estimate of the spawning stock biomass. However, in the North and Irish Seas, cod, haddock and whiting co-exist and spawn at similar times and in similar places. Since their eggs are visually indistinguishable during the early stages of development, initial attempts to use the egg production method pooled all cod-like eggs (a mixture of cod, haddock and whiting eggs). Not surprisingly, this resulted in overestimates of cod abundance (in comparison to catch-dependent methods).

Key researchers: Ricardo Pereyra (postdoctoral research associate employed on DEFRA Grant MF0151; Martin Taylor (postdoctoral research associate employed on MF0146 & researcher/principal investigator employed on MF0156.

3. References to the research

Publications

(UEA researchers in bold)

1. **Taylor, M. I**., **Rico, I.**, Fox, C. & **Rico, C**. (2002) Species specific Taqman probes for simultaneous identification of cod (*Gadus morhua* L.), haddock (*Melanogrammus aeglefinus* L.) and whiting (*Merlangius merlangus* L.). *Molecular Ecology Notes* **2**:599-601. (48 Citations). doi: 10.1046/j.1471-8278. 2002.00269.x
2. Fox, C.*, **Taylor, M. I**.*, **Pereyra, R**., & **Rico, C**. (2005) TaqMan DNA technology confirms likely overestimation of cod (*Gadus morhua* L.) egg abundance in the Irish Sea: implications for the assessment of the cod stock and mapping of spawning areas using egg-based methods. *Molecular Ecology* **14***:*879-884. * Joint first authors. (33 Citations). doi: 10.1111/j.1365-294X.2005.02439.x
3. Fox, C. J., **Taylor, M. I**. *et al.* (2008) Mapping the spawning grounds of North Sea cod (*Gadus morhua*) by direct and indirect means. *Proceedings of the Royal Society of London* B. **275**:1543-1548. (25 citations). doi: 10.1098/rspb.2008.0201
4. Börjesson P., Jonsson, P., Pacariz, S., Björk, G., **Taylor, M. I**., and Svedäng, H. (2013). Spawning of Kattegat cod (*Gadus morhua*) - mapping spatial distribution by egg surveys. *Fisheries Research* 147:63-71. (0 Citations) doi: 10.1016/j.fishres.2013.04.012

Key grants (1998-2006)

1998-2001 DEFRA MF0146 (£166,829) Genetic identification of fish eggs by species specific DNA markers for use in stock biomass assessment**.** Awarded to Rico, C**.**

2003-2004 DEFRA MF0151 (£41,911). A field trial of genetic probes for the identification of gadoid eggs. Collaborative project with Cefas Lowestoft. Awarded to Taylor.

2004-2006 DEFRA MF0156 (£137,268). Genetic analysis of gadoid eggs from North Sea Ichthyoplankton surveys. Collaborative project with Cefas Lowestoft. Awarded to Taylor.

4. Details of the impact

The assay developed by Taylor and colleagues was used to identify pelagic cod-like eggs collected in ichthyoplankton surveys in the Irish and North Seas by International government-funded fisheries institute partners from England (Cefas), Scotland (Fisheries Research Services), Netherlands (Institute for Marine Resources and Ecosystem Studies), Norway (Institute of Marine Research), Denmark (Danish Institute of Fisheries Research) and Germany (Leibniz Institute of Marine Sciences) and coordinated by the Planning Group on North Sea Egg Surveys of the International Council for the Exploration of the Sea (ICES) (research references 1-3). ICES is an intergovernmental organization whose main objective is to increase the scientific knowledge of the marine environment and its living resources and to use this knowledge to provide advice to competent authorities. The ICES network comprises > 4000 scientists from almost 300 institutes with 1600 scientists participating in activities annually.

The Irish Sea survey revealed the spawning grounds of cod, haddock and whiting, demonstrated that a spawning stock of haddock existed in the Irish Sea and indicated that previous estimates of cod stocks using the annual egg production methods (AEPM) were biased upwards due to inclusion of haddock and whiting eggs in the calculations. The North Sea survey also identified the current spawning grounds of cod, confirming that major historical spawning grounds for North Sea cod were still active in 2004 but that some localized populations may have been reduced to levels where it was difficult to find cod eggs in the plankton. These populations could be at particular risk of severe depletion and were identified as potentially requiring targeted conservation measures (corroborating source D)

In 2005, the assay was adopted by Cefas after they set up a molecular lab with funding from DEFRA and assistance with design and specification from Taylor. Cefas then implemented the

assay in routine pelagic egg surveys to provide a more accurate use of AEPM for stock assessments in the Irish Sea (corroborating sources A-C). By their own assessment, Cefas have repeatedly used this methodology very successfully for large scale egg surveys in the North and Irish Seas (corroborating source D).

Cefas also used the assay successfully in the 2008 and 2010 egg surveys in the Irish Sea for which 'cod-like' eggs were genetically analysed, and the annual egg production method was conducted to estimate stock size for cod in the Irish Sea (corroborating sources C and E). Since cod fishing is effectively banned in the Irish Sea, this methodology has been essential for Cefas to be able to accurately measure the recovery of cod stocks in this area. A letter of support, from the Chief Scientist at Cefas, states that the assay developed by Taylor '*represents a very significant contribution to the sustainable exploitation of cod stocks around UK waters*' (corroborating source F).

The assay has also been implemented in an egg survey at an International level in the Baltic Sea by the Swedish Board of Fisheries (now under the control of Swedish University of Agricultural Sciences SLU) (http://sustainability.formas.se/en/Issues/Issue-2-May-2011/Content/In-brief/Swedish-Board-of-Fisheries-rd-transferred-to-SLU/) and a letter of support confirms the impact of the research for fisheries conservation - '*the assay represents a significant contribution to catch-independent fisheries monitoring*'. The assay will be used again in this year's analysis of the 2013 Swedish ichthyoplankton survey (corroborating source G).

5. Sources to corroborate the impact

A. DEFRA Final Report on Project MF160 "**Pilot study for fishery-independent monitoring of cod recovery in the Irish Sea by means of egg production surveys**"
Held on file at UEA and available at:
http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=14322

This shows how the assay was used by Cefas to estimate spawning stock biomass for cod in the Irish Sea using the results of the assay developed by Taylor.

B. The Cefas website: http://www.cefas.defra.gov.uk/our-science/fisheries-information/surveys/estimating-spawning-stock-biomass-using-egg-surveys.aspx.

This web site provides information about how and why Cefas carry out egg surveys and why Taylor's method is so important

*This Cefas website also gives details of how Cefas, in conjunction with **Taylor** at UEA, developed gene-probes to identify the eggs of cod, whiting and haddock, and how these have been used very successfully in large-scale egg surveys of the North Sea and the Irish Sea. They identify the key references as Taylor et al. (2002) and Fox et al. (2005) (see Section 3, Refs 1 and 2].*

C. **The Marine Fisheries Science Yearbook**
A copy is held on file at UEA and available at:
http://archive.defra.gov.uk/environment/marine/documents/science/marine-fish-year.pdf

This details aspects of this work on page 14 and states:
"Working with the University of East Anglia, Cefas has developed genetic probes to identify cod, haddock and whiting eggs that have previously been difficult to distinguish visually…."

D. Scottish Industry / Science Partnership (SISP) Report No 03/08: **Identifying Critical Spawning and Nursery Areas for North Sea Cod; Improving The Basis for Cod Management**
A copy is held on file at UEA and available at:
http://www.scotland.gov.uk/Uploads/Documents/SISPReportNo0308.pdf

This report cites Fox et al 2008 (see Section 3, Ref 3] and shows how the information was used to compare past and present spawning grounds of cod and also to compare adult and juvenile densities in different places in the North Sea with egg survey data.

The report confirmed that some of the major historical spawning areas for the North Sea cod are still active, but some localised populations may now be reduced. These populations could be at particular risk of severe depletion and may require targeted conservation measures.

E. Maxwell D.L, Armstrong M.J., Beggs S. and Aldridge J.N. (2012). Annual egg production estimates of cod (*Gadus morhua*), plaice (*Pleuronectes platessa*) and haddock (*Melanogrammus aeglefinus*) in the Irish Sea: the effects of modelling choices and assumptions
Fisheries Research, Volumes 117–118, Pages 146-155. doi: 10.1016/j.fishres.2011.09.005

This gives Cefas egg production estimates in 2008 in Irish Sea. Cites Fox et al 2005 and 2008, on which Taylor is joint first author, and co-author respectively [see Section 3, Refs 2 and 3].

F. Letter of support from the Chief Scientist at Cefas, held on file at UEA, in which it is stated that:

the assay developed by Taylor "represents a very significant contribution to the sustainable exploitation of cod stocks around UK waters".

G. Letter of support from the Head of Stock Assessment Unit, Swedish Fisheries, held on file at UEA, in which it is stated that:

'the assay represents a significant contribution to catch-independent fisheries monitoring'