ICES ASC 2007 Theme Session Q24

Using genetic probes to identify gadoid eggs in surveys to monitor the Irish Sea cod stock under the EU recovery program

freya.goodsir@cefas.co.uk

Freya Goodsir, Mike Armstrong, Michael Godard, Natasha Taylor and David Stone
Centre for Environment, Fisheries & Aquaculture Science, Lowestoft and Weymouth www.cefas.co.uk
Clive Fox
Ecology Group, Scottish Association Marine Science, Oban www.sams.ac.uk
Pieter-Jan Schon
ABFI, Belfast pieter-jan.schon@afbini.gov.uk
Martin Taylor
School of Biological Sciences University of Wales, Bangor m.taylor@bangor.ac.uk

Introduction

Early stage eggs of cod (*Gadus morhua* L.) are impossible to visually differentiate from those of haddock (*Melanogrammus aeglefinus* L.) or whiting (*Merlangius merlangus* L.). This project uses Taqman species specific probes in multiplex real-time PCR chemistry to distinguish between these three species as part of the Irish Sea egg production monitoring which aims to evaluate the status of the Irish Sea cod stock during the EU stock recovery program.

Methodology and analysis

Cod-like eggs (those lacking oil globules and in size range 1.1-1.75 mm dia) were collected during five ichthyoplankton surveys during the spawning season in 2006 using Gulf VII high-speed plankton samplers (Figure 1). At each plankton station, a subset of the eggs caught was fixed in individual vials filled with ethanol and brought back to the laboratory. Samples were coded into tubes and individual stations were laced with controls (known species eggs).

DNA was extracted and purified using Quiagen DNeasy tissue kits. Universal primers and Taqman species-specific minor grove binding (MGB) probes were used in a multiplex high through-put assay and then processed on an Applied Biosystems 7900 real-time sequence detection system. (Taylor *et al.* 2002)

DNA was amplified over 40 cycles of denaturing, annealing and extension, using universal cycle conditions (Figure 2). The 7900 software detects the reporter dye fluorescence during real-time. As soon as a reporter dye is stimulated, a signal is produced (Figure 3). The end point of the PCR was then analysed which indicated whether the sample was cod, haddock, whiting or a null result. For quality control purposes, the locations of the control samples in each 96-well plate were not revealed until the results of the analysis had been completed.

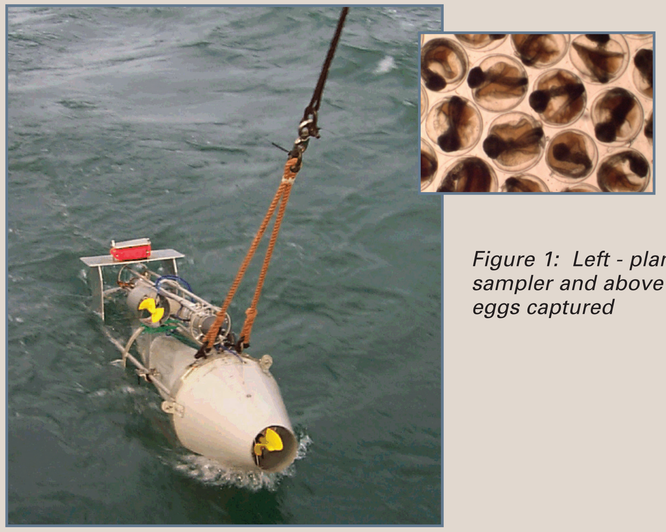

Figure 1: Left - plankton sampler and above - cod-like eggs captured

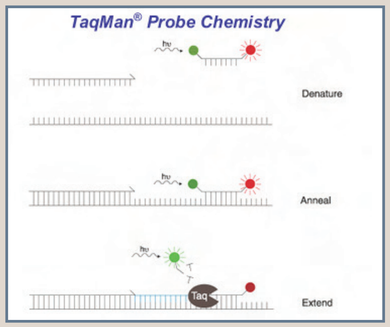

Figure 2: TaqMan chemistry process in real-time PCR
© 2003-2007 Baylor College of Medicine

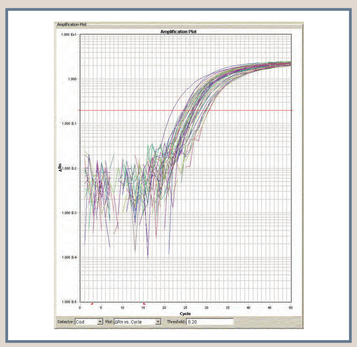

Figure 3: Real-time PCR amplification curve

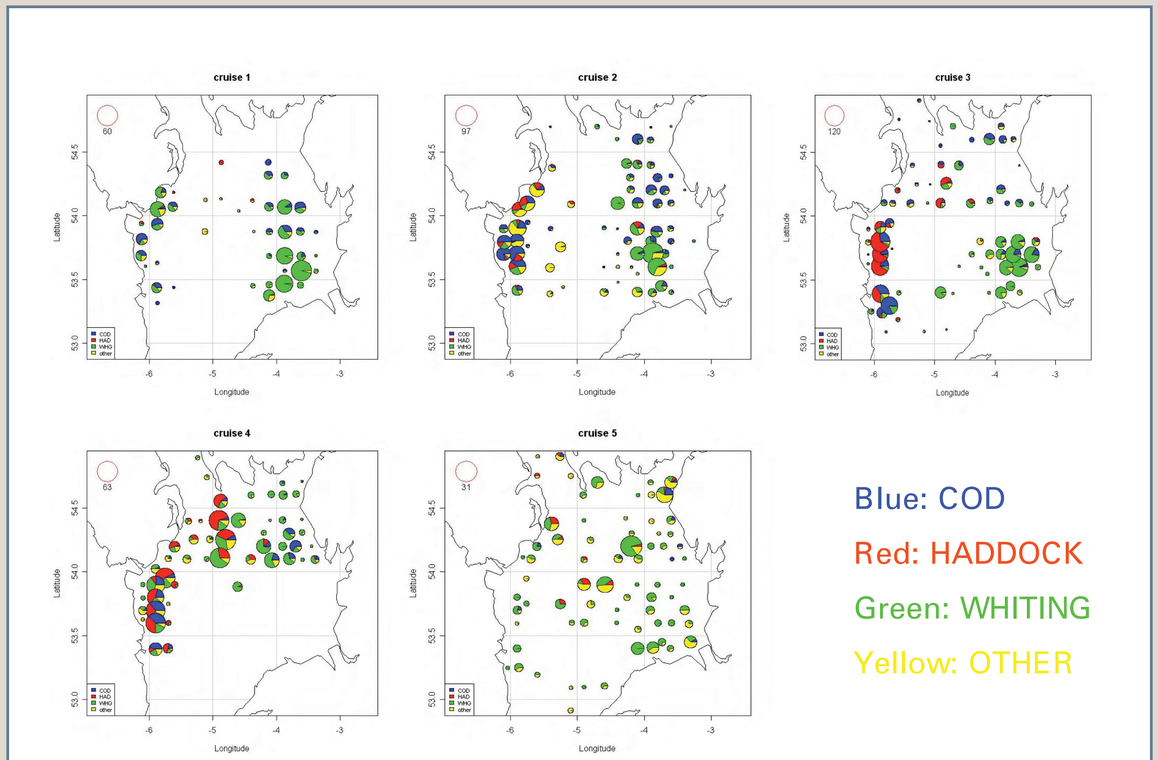

Figure 4: DNA Species compositions by station

Results

Analysis of 514 blind control eggs interlaced with the field-sampled eggs indicated >95% accuracy in determining species composition of cod, haddock and whiting. Out of 4700 eggs collected on the surveys, 23% were identified as cod, 15% as haddock and 42% as whiting, and approximately 20% were other species. Unidentified eggs are sequenced on an Applied Biosystems 3100 genetic analyser to confirm their species status.

The distribution and relative abundance of eggs of cod, haddock, whiting and other species at different stations in the Irish Sea is shown in Figure 3. The DNA results will be used in estimating the proportions of total cod-like eggs to be assigned to each target species. Total production of cod eggs will be used to estimate the stock size in the Irish Sea by means of the Annual Egg Production Method (see Armstrong *et al.* ICES CM 2007/Q25, this symposium).

Conclusion

- This method has demonstrated 95% accuracy in determining species composition of cod, haddock and whiting in field samples taken from the Irish Sea.
- Out of a total of 4700 eggs collected on the survey, 23% were identified as cod, 15% were identified as haddock and 42% were identified as whiting.
- 20% of the field survey eggs were not identified as cod, haddock, or whiting. These may be eggs of other species, or eggs with a low or poor template DNA quality. Further work will fully sequence the Mitochondirial cytochrome b gene fragment from the DNA of unidentified eggs to confirm their identity.

Acknowledgement

This work was supported by Defra project MF0160, in collaboration with Cefas Technology Ltd (CTL).

Authors wish to thank the plankton team for their hard work and support during this project.

Reference

Taylor *et al.*, (2002) Molecular Ecology Notes, 2(4), pp. 599-601