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Estimation of potential and realised fecundity of fish

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1.1 Relevance to fisheries science

A key assumption in the rational exploitation of fish stocks is that fishing mortality does not adversely effect recruitment of new spawning stock. To some extent this depends on the spawning stock biomass in absolute terms but it has also become apparent that population egg production is a more robust indicator (Marshal et al 1998) of recruitment. In order to understand why egg production is a better indicator of recruitment we need therefore to understand the relationship between female size and realized fecundity defined in both absolute and qualitative properties that effect egg survival. An additional reason to estimate fecundity is to use the data in the determination of spawning stock biomass independent of commercial fisheries based on three approaches: the annual egg production method (Lockwood *et al*/1981), the fecundity reduction method (Lo *et al*/1992) and daily egg production method (Lasker 1985). To apply each method it is essential to assess the standing stock of fecundity and measure the dynamics of egg production per unit of time by tracking the regression of post ovulatory and atretic follicles.

1.2 What has been done so far, traditions, reviews and short falls

Two recent reviews (Murua *et al*/2003, Kjesbu *et al*/2004) have described the science behind determination of fecundity linking this with the pioneering work of Hunter *et al*/(1989) and other older methods to recommend best practice to carry out the work. Both reviews advocated the use of formaldehyde to preserve the ovary for analysis and described the methods used in the past to quantify the standing stock of fecundity and the dynamics of egg production from the product of spawning frequency and batch fecundity (quantity of eggs released in a spawning event). Many marine fish, especially those species caught in European waters are very fecund, broad cast spawners producing thousands or millions of eggs from their ovary which must be identified amongst an even larger population of reserve of cells (Greer Walker *et al*/1994).
The review of Kjesbu et al also considered what advances would improve the accuracy and reduce the costs associated with estimating population fecundity and this stimulated much of the work described here and other presentations at this theme session of the 2005 annual Science conference.

2 Recent advances

2.1 Sampling the ovary

A new method to aid the collection of gravimetric sub samples (25, 50 ,100 and 200 mg) from the ripe ovary with a solid displacement pipette (Drummond Scientific Co Wiretrol II) will be demonstrated. Supporting information on the advantages gained will be presented: reduction in the use of toxic preservatives, lower storage requirements and costs of sample distribution to international partners involved in the assessment such as the ICES coordinated Mackerel and horse mackerel triennial survey. Further information on the accuracy and repeatability will be provided in the presentation by Van Damme *et al*/ also in this theme session.

2.2 Tribulations interpreting histological sections and unbiased stereolgy 'the dissector method'

When analyzing histological slides small cells have a smaller chance of being counted than larger cells. Thus when counting cell types that are of different size it is important to account for this. When estimating the intensity of atresia in ovary samples the size of the cells being counted is of different sizes. The stereological method ensures that all cells have an equal chance of being counted by analyzing pairs of sections in parallel planes with a distance of about 1/3-1/4 of the minimum cell diameter (Mayhew, 1992). Using a three-dimensional model (Mayhew, 1992) the cells are counted when

found on the slide being analyzed (the "reference") but not on the next slide (the "look-up"). We will present some slides that demonstrates how this can be done practically.

2.3 Analysis of whole mounts: what they can tell us and their present limitations

A presentation will be given illustrating the preparation and interpretation of stained sub-samples of ovary tissue to aid automatic counting and measurement of developing follicle size distributions. Examples of the appearance of atretic and post ovulatory follicles in dispersed fragments ovary tissue (whole mounts) will be compared to their appearance in histological section illustrated in cod and hake. This provides a means to quantify rates of spawning and follicle regression and therefore working towards replacing expensive histology. Although we can show how morphology changes we cannot, as yet, show how fast the process occurs with the required precision to determine spawning rates or loss of fecundity through follicular atresia.

2.4 Acquiring and storing information through image analysis

Image analysis software is increasingly being used in fecundity analysis supported by continuing improvements in resolution of the hardware (digital camera, Pc, monitor and motorized stage) albeit with a high level of cost.

Software suitable for this type of work is available both from commercial vendors and from research institutions developing software for freeware distribution. Among the freeware software the ones from NIH (http://rsb.info.nih.gov/ij/) are probably the most commonly used. ImageJ (programmed in Java for Windows, Mac and Unix) is probably the one that currently is most actively developed and probably also the program that have the largest user base. The older variant called NIH image (only available for Mac) is not developed anymore, but derivatives of this program are still developed under the names of ObjectImage and ImageSXM by others. All these programs have been found to be suitable for the kind of work discussed in this communication.

The features of a commercially available software Myrmica (Pilkingtom image analysis systems) will be illustrated. This includes: a light meter to show white balance and intensity, automated object counting and measurement, automatic archiving of images and analysis outputs (overlays) for quality assurance, multiple measurement options: lines, angles, polygons, chains, areas etc. The system can be interfaced with a motorized stage to take measurements at high resolution over distances much larger that the field of view with annotation of the image montage as required.

One of the recent developments in image analysis teqniques for fecundity work is the *autodiametric fecundity method* (Thorsen and Kjesbu, 2001). Using this method the potential fecundity is estimated by particle size analysis of pictures of whole mount ovary samples. The method has proven to be fast compared to traditional methods and also have the advantage that, ones established, it is not based on gravimetric subsamples.

3. Future progress and topics we need to research.

To get numbers on realized fecundity is sometimes very difficult without doing spawning experiments in tanks. This is especially true for the so-called indeterminate spawners that continue to recruit vitellogenic oocytes during the spawning period. Horsemackrel is an indeterminate spawner and plans are to start spawning experiments in Matre, Norway in the coming season. This may give important data for the assessment of the horsemackrel in the North West Atlantic. The size of this stock is currently estimated from the number of pelagic eggs found during triennual surveys in the spawning period. The presentation will be illustrated by a series of pictures of aquaculture facilities that can be used for fecundity studies.

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