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The Propagation of Cod *Gadus morhua* L.

# BIOCHEMICAL GENETIC IDENTIFICATION AND POPULATION GENETIC STUDIES OF MARINE FISH EGGS*

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## ABSTRACT

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The visually indistinguishable eggs of various fish species may be identified by species-specific zymograms. Polymorphic loci in identified eggs permit estimation of allele-frequencies for use in population structure analyses. General principles and practical procedures in such work are described, with references to results from recent applications. Potential applications in cod aquaculture are considered.

## INTRODUCTION

Traditionally, identifications of marine fish eggs are based on visual characteristics like size, texture, shape, peri-vitelline space, oil-drops, and embryo pigmentation (Russell, 1976).

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Early development stage eggs of several Norwegian gadoids and flatfishes differ only in diameter, which overlaps extensively between species (Russell, 1976; Mork et al., 1983.

Recently, methods were described by which pelagic fish eggs could be identified by means of species-specific tissue enzyme zymograms which were diagnostic in the investigated species; 11 gadoids and 6 flatfishes commonly occurring in Norwegian waters (Mork et al., 1983).

The analytical methods used; isoelectric focusing and specific histochemical staining, proved very sensitive and required minimal amounts of protein for egg identifications. This allowed the scoring of enzymes additional to the diagnostic protein in spare extracts from identified eggs, and was utilized in a population genetic study of three gadoid species by Mork and Sundnes (1983). The present report gives a summary of the general principles for and techniques used in such work, with references to some practical results and suggestions for additional areas of application.

## MATERIALS AND METHODS

### Egg collection, preservation and preparation

Live eggs collected with plankton nets or pumps are adequate for enzyme analyses. It is essential, in order to prevent enzyme inactivation, to avoid exposure to high temperatures. This applies to all stages of egg handling. Immediate submersion and storage in liquid nitrogen (-196$^{o}$C) pending the electrophoretic analyses is recommended. A few minutes before analyses, the eggs are thawed at 0-4$^{o}$C and macerated in 20-50 µl of cold distilled water or a 1% aquous solution of the carrier ampholyte to be used in the focusing gel. Centrifugation is not necessary. Only part

of the egg extract should be used for identification, leaving spare extract available for analyses of additional enzymes.

## Analytical procedures

The choice of the electrophoretic method is important when dealing with the small quantities of proteins present in most marine fish eggs. We have found that isoelectric focusing in polyacryleamide gel (IFPAG) is superior to starch gel electrophoresis with respect to ease of procedural standardization, band -resolution and -sharpness, and sensitivity. For isozyme detection, it is recommended that high concentrations of substrate and co-factors in the incubation solutions should be used. More detailed information on procedures in IFPAG and histochemical detection of various enzymes are given in Mork et al. (1983), Mork and Heggberget (1984), and Mork and Sundnes (1983).

## Diagnostic enzymes

The first step in establishing a diagnostic key to egg species will be an investigation of the zymogrammatic expression of various enzymes in tissues from adult specimens in the relevant species. A wide range of enzymes can be detected by histochemical methods (cf, e.g., Harris and Hopkinson, 1976, for specific staining recipes). Since some species, particularly those which are closely related, may display identical zymograms for some enzymes, it may sometimes be necessary to study more than one enzyme in order to identify all eggs in a particular sample. This is one reason for not using all of the individual egg extracts in the initial analyses.

The next step will be to check which of the candidate enzymes are sufficiently expressed in eggs in early developmental stages to be detected zymogrammatically. This test

is performed on eggs of known origin, preferably from controlled crossings in the relevant species, which also allows simultaneous checks on the possible occurrence of unique embryonic enzyme loci, and the genetic control of the characteristics under study.

## RESULTS

### Egg identifications

A study on the zymogrammatic expression of LDH (Enzyme Commission No. 1.1.1.27) in a total of 17 relevant gadoid and flatfish species from Norwegian coastal waters showed that the white skeletal muscle predominating locus, which was sufficiently expressed in eggs at all stages, was diagnostic. Egg LDH analyses thus successfully solved the traditional identification problems in routine pelagic egg samples taken in northern Norway coastal waters during and shortly after the spawning of the Arcto-Norwegian cod stock (Mork et al., 1983). A total of seven species were represented by their eggs in the samples investigated. In order of their relative abundance they were; cod, Norway pout, haddock, coalfish, plaice, dab, and long rough dab. By stratified sampling with plankton pumps a marked difference in the vertical distribution of eggs from some species (e.g. cod and Norway pout) could be demonstrated. There was also a geographical variation in the relative abundance of eggs from different species (Mork et al., 1983).

### Population genetic studies of pelagic eggs

When the enzyme locus used for identification purposes is polymorphic, which occurs not infrequently, eggs genotyped in the identification process may simultaneously form a basis for the estimation of population allele fre-

quencies. Mork and Sundnes (1983) utilized polymorphisms at the "identification" LDH locus in eggs from Norway pout and haddock for such purposes. They also scored genotypes at a polymorphic IDH (E.C. 1.1.1.42) locus, by using spare extracts from identified cod eggs, and compared allele frequencies in samples from different locations.

## DISCUSSION

### Egg identifications

The present repertoire of species whose eggs can be recognized by LDH zymograms is not quite complete for use in Norwegian waters, although it is probably sufficient for most practical purposes. The species list can probably be significantly extended with only limited effort. By the use of several loci it might be possible to construct a complete biochemical genetic key to the species identity of pelagic fish eggs. However, we see no immediate need for a change of methods in cases where visual identifications work well. Rather, biochemical identification provides a valuable alternative in special cases, for instance the notoriously difficult visual discrimination between eggs from cod and haddock, and to a certain degree some other gadoids and flatfishes in Norwegian waters. In combination with diameter measurements, the biochemical identification may also be used to update the species egg diameter distributions currently in use for visual identification. There is reason to suspect that these distributions, which may be based on a limited number of measurements, or measurements in eggs from a limited number of parents, may not be sufficiently representative for their own population, and definitively not for other populations.

## Population genetic studies of pelagic fish eggs

Each fertilized egg is a member of the population, and egg samples may provide reliable estimates of genetic population parameters. However, as discussed by Mork and Sundnes (1983), special care should be taken to check that the egg sample is representative of the population as a whole; under certain circumstances there is an obvious risk that eggs from only a few females are collected in a batch, for instance during fixed depth sampling (plankton pump) during or shortly after egg release.

When planktonic egg samples can be considered as representative, they provide a simple, rapid, and inexpensive method of collecting and processing large numbers of individuals for the estimation of population allele frequencies. Due to the limited amounts of proteins available in eggs, the number of loci which can be scored in each individual will be lower than in more developed specimens. Detailed studies have yet not been performed on this aspect, but in pilot experiments we have scored five different enzymes (totalling seven loci, of which four were polymorphic) in individual cod eggs. Thus, there seem to be a potential for routine use of pelagic fish eggs in population genetic studies.

## Applications in cod aquaculture

As shown by Mork et al. (1983), egg zymogram techniques are a rapid way of controlling the heredity of possible genetic markers by analyses of egg batches from controlled crossing. In this way the time needed to get the desired information from the crossing is minimized. Presumably, a cod aquaculture industry would imply a large scale production of fry for use in propagation. Possibly also, selection programs will be applied in order to increase individual production rates. In this situation there is

a danger of unwanted inbreeding and thus loss of genetic variation. The importance of maintaining sufficiently high levels of genetic variation in hatchery populations has been stressed by several authors (cf, e.g., Hynes et al., 1981, and references therein). The most direct measures of levels of genetic variability are obtained by electrophoretic methods, which thus allow a monitoring of the inbreeding status of laboratory stocks. For obvious reasons is would be desirable to perform such measurements as early as possible after the creation of a filial generation. Here multi-locus analyses of eggs may provide a nearly instant control of the amount of genetic variation in offspring batches.

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