

# Procedures for Sorting, Staging, and Ageing Eggs

GARY STAUFFER and SUSAN PICQUELLE<sup>1</sup>  
Southwest Fisheries Center,  
National Marine Fisheries Service, NOAA,  
P.O. Box 271, La Jolla, CA 92038

## ABSTRACT

Estimation of the daily production of eggs spawned is based on data for the number of eggs sampled during the plankton survey. This requires that the age of individual eggs be determined. To accomplish this, eggs of the surveyed species must be sorted out of the plankton samples, staged according to their state of embryonic development, and aged. The initial step in processing the plankton samples is removing the fish eggs and larvae and identifying the eggs of the target species. The second step is assigning each egg to a stage of embryonic development and counting the number of eggs of each stage for each plankton sample. Once all the samples have been staged, ages are assigned separately to each sample of the staged eggs based on a stage/temperature/age key, station temperature, and time of station occupancy. This procedure is subject to a number of sources of error that should be kept in mind and evaluated for each application.

## INTRODUCTION

The daily production of eggs spawned into the sea is estimated by regressing a mortality model to density data on the number of eggs at age derived from plankton samples. To generate the egg density data, the fish eggs (and larvae) of the target species must be sorted from the plankton samples, staged on the basis of embryonic development, and aged. This process can begin as soon as the plankton samples have been transferred to the laboratory. At the Southwest Fisheries Center (SWFC), this procedure for the eggs and larvae of northern anchovies is carried out by three separate groups.

## SORTING

The method of sorting eggs and larvae from the CalVET<sup>2</sup> samples are similar to the procedures outlined by Kramer et al. (1972) and Smith and Richardson (1977). The volume of plankton in CalVET samples was not measured. In this survey, plankton volumes were quite small because of the size of the CalVET net and the short duration of the tow and were not necessary for estimation of spawning biomass.

The plankton samples must be sorted by personnel trained in the identification of fish eggs and larvae particularly of those species from the survey area. Sorters are responsible for cross-checking the inside and outside labels of each sample jar as it is processed. It is critical that sample identification numbers written on all data forms filled out by the sorters match those on the sample jar labels. Sorters are responsible for picking out the eggs and larvae for all species and identifying the target species. At the SWFC, eggs and larvae of the target species are placed in 2-dr vials, filled with diluted Formalin, capped, and labeled with station identification numbers. Additional station data to be recorded on the staged egg data forms are water temperature and time of collection. These data are necessary for ageing the staged eggs at a later date.

Depending on the objectives of a particular survey, other species may also be sorted. Egg counts and possibly larval lengths of the target species are recorded on staged egg data forms. Sorting time per sample depends on the volume and quality of plankton and the quantity of ichthyoplankton in the samples. A sorter at the SWFC can process about eight CalVET samples per day. If processing of the plankton samples is on a strict schedule as it is at the SWFC, then it is advisable to sort first the samples collected at stations with the highest expected density of eggs.

## STAGING

The second step in processing eggs of the target species is the assigning of an embryonic developmental stage to each egg. More training, experience, and time are required to accurately stage the embryonic development of fish eggs. The 2-dr vials containing sorted eggs of the target species and the respective staged egg data forms are turned over to a second group responsible for staging eggs at the SWFC.

Working with each sample separately, the technician pipettes or empties the eggs in a vial into a petri dish and sorts the eggs under a binocular dissecting scope into groups for each standardized developmental stage that spans incubation from time of fertilization to hatching, as described in Moser and Ahlstrom (1985). Eggs

<sup>1</sup>Present addresses: Northwest and Alaska Fisheries Center, National Marine Fisheries Service, NOAA, 7600 Sand Point Way NE, Seattle, WA 98115.

<sup>2</sup>For a description of the CalVET net, see Smith et al. (1985).

with disintegrated embryos are often difficult to stage and require special attention (see Moser and Ahlstrom 1985). In some samples, as much as 50% of the eggs could be disintegrated and difficult to stage. The number of eggs in each stage group are counted and recorded on the staged egg data sheet. The number of eggs for which it is impossible to assess the embryonic condition must be recorded also. The sum of the number of eggs staged should equal the earlier sorter's count. If not, then all groups should be recounted and any discrepancies accounted for and noted. When the staging of a sample is completed, all the eggs must be returned to the vial and the sample archived in a storage area.

## AGEING

Once all the station samples have been staged, the completed staged data forms can be turned over to a team which assigns ages to the staged eggs. Although this group of two or three people need not be skilled in identification of eggs and larvae or egg stages, they must understand the daily spawning cycle and the effect of temperature on development rate. It is recommended that each team member age the eggs for all stations independently of the others. This serves as a check of potential ageing errors and biases that could result from subjective decisions on likely ages of eggs for which the day of spawning is not obvious.

Assigning ages to eggs based on the stage of embryonic development is a relatively straightforward procedure if spawning occurs within a brief time interval during the 24-h daily cycle and if the duration of egg stages is less than a day for the range of temperatures observed during the survey. If either one of these conditions is not the case in any particular application, then the procedure described here will have to be modified in order to estimate daily rate of egg production in the sea.

Before the ageing step can begin, the team must specify the hour of peak spawning within the daily cycle to determine time zero for egg development. This can be determined from the time of day that newly fertilized eggs appear in the egg collections and from observations on the spawning behavior or condition of the adults. A stage/temperature/age key as described in Lo (1985, fig. 2) must be available for the target species of the survey. This key represents embryonic growth curves for each developmental stage, relating time to develop with temperature. From these curves, the time or age from fertilization to time of collection can be estimated given the stage of development and station surface temperature, which is assumed to be the temperature at which the egg was incubated. The age of an egg can be calculated by estimating its age in whole days and then adding on the portion of a day that has elapsed between peak spawning hour and the time of day the sample was collected. For northern anchovies off California, the peak spawning hour is set at 2200. Thus eggs need be aged only to the nearest day and then adjusted for the portion of the day between sampling and peak spawning.

The benefit of having all spawning occur in the same short interval each day is that the ages of the eggs within each sample are separated by 24-h increments. Because the duration of any particular stage is much less than 24 h, the distribution of eggs of a single sample over the different developmental stages should form distinct groups or modes with unrepresented stages separating each group. One can then assume that each group is separated by 24 h. This pattern is extremely helpful in assigning whole-day ages to groups of eggs within a sample. However, this pattern becomes blurred for samples taken from colder temperatures where the duration of each

stage is longer and the modes or groups of eggs may overlap. This pattern is also less clear for the advanced stages because the ages are assigned with less precision. The longer the incubation time, the more inflated the variance in the age key becomes as the effects of variability in growth rate and environmental factors accrue.

These two problems are partially avoided by truncating the data series and eliminating the eggs older than a specific age. The primary reason for doing this is to avoid the time period when eggs begin to hatch into larvae and are no longer available for sampling. Truncation of egg density data for eggs older than the age at which the onset of hatching is expected for the warmer temperatures encountered on the survey insures that mortality is the only process contributing to the decline in egg abundance. This also eliminates eggs for which the procedure of assigning ages has the greatest uncertainty.

For the northern anchovy surveys conducted by the SWFC, time was saved by taking temperature data at the surface only, at most stations. The recorded temperature data represent the maximum incubation temperatures that eggs would likely encounter in the upper mixed layer, where anchovy eggs are found. In a few cases, eggs could have been assigned to either one of two spawning days based on the age key. In these cases, the older age was assigned based on the assumption that the egg was probably incubated at a temperature colder than the surface waters. However, the majority of eggs closely corresponded to the predicted age suggested by the embryonic growth curves and by the separation of egg-stage modes.

Plankton samples collected during the spawning hours will under-sample the new eggs until spawning is completed for the daily spawning period. To avoid underestimating daily egg production from the mortality model, egg densities of the youngest ages exclude samples collected during the spawning period. For northern anchovies this period was between 1800 and 0200. These samples do provide data on eggs older than approximately one-half day (for further discussion see Picquelle and Stauffer 1985).

After assignment of ages by all team members working independently of one another, the staged egg data forms were sorted into convenient time and temperature strata. The team as a group examined the data forms for each stratum for consistency among samples in the assigned age of each stage. At this time, discrepancies between the ages assigned by different team members and among stations with similar conditions were discussed and rectified. This final comparison is very important to minimize any differences among the team, to reduce errors, and to insure that the determinations from the age key are consistent throughout the ageing exercise.

The ageing step can be automated using a computer. Based on the stage/temperature/age key, a reference table can be constructed and included in a computer program. The table should contain the estimated ages of eggs for each of the 11 stages at temperatures ranging from 10° to 22°C, sampled at any time throughout a 24-h period. Ages are assigned to stages according to the reference table.

The advantages of the automated system are twofold. 1) It saves time: the current manual system requires about 1 wk of manpower to age the eggs, enter the data, and build the data files for the estimation of daily egg mortality and egg production; the automated system requires one-half day at most to process the staged eggs and produce a daily egg mortality curve. 2) It standardizes the method: the automated system eliminates the subjectivity of human judgment and variation resulting from operator error, thus the accuracy of the egg production and the egg mortality estimate can be improved (see Lo 1985).

## ASSUMPTIONS AND SOURCES OF ERROR

1) Anchovy eggs are easily distinguished because of the oblate spheroid shape. As a result, the sorting procedure for anchovy eggs is quite accurate and relatively efficient. The sorting of spherical eggs of other species may require more skill and resorting to check for accuracy if there is a potential confusion with other species.

2) Often a high fraction of the fish eggs have disrupted or disintegrated embryos. In these cases, the developmental stage must be determined by additional criteria. For the northern anchovy example, it was assumed that this condition occurred during the plankton tow and that eggs in this category had a similar mortality history. The validity of this assumption should be examined in each application of the egg production method.

3) The assumption that 100% of the eggs are retained by the plankton net should be tested. Also, the catching process and fixation may stimulate the eggs to hatch.

4) The mortality model assumes that all egg stages including unfertilized eggs have the same rate of mortality. If unfertilized eggs make up only a small fraction of the total eggs, and if they persist in the water column for <1 d, then it is probably sufficient to assign them as 1-d-old eggs. This ignores the bias in the estimate of daily egg production created by the different mortality rates of unfertilized eggs and the inability to age unfertilized eggs. On the other hand, if unfertilized eggs are not an insignificant fraction of the total, then the egg production mortality model must be modified to account for them.

5) The ageing of staged eggs is facilitated by a short spawning interval within a daily period. Assigning ages to embryonic stages will become less reliable as the spawning interval makes up a greater fraction of the daily period. Stage durations of <12 h at the usual incubation temperature facilitates the assigning of daily ages to staged eggs. If embryonic development is slow, which is often the case at low temperatures, then there is a good possibility that eggs at a single stage could have resulted from more than a single spawning episode. If this occurs, then the arbitrary stages described here must be divided further.

6) The duration of an egg stage is a function of the incubation temperature. The measured temperature at a plankton station may not be the actual temperature at which the collected eggs were incubated within the water column. The temperature measurement must be representative of the temperature at which the majority of the eggs incubate. This requires a study of the vertical distribution of eggs compared with the temperature depth profile.

## LITERATURE CITED

- KRAMER, D., M. M. KALIN, E. G. STEVENS, J. R. THRAILKILL, and J. R. ZWEIFEL.  
1972. Collecting and processing data on fish eggs and larvae in the California Current region. U.S. Dep. Commer., NOAA Tech. Rep. NMFS Circ. 370, 38 p.
- LO, N. C. H.  
1985. A model for temperature-dependent northern anchovy egg development and an automated procedure for the assignment of age to staged eggs. In R. Lasker (editor), An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, *Engraulis mordax*, p. 43-50. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 36.
- MOSER, H. G., and E. H. AHLSTROM.  
1985. Staging anchovy eggs. In R. Lasker (editor), An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, *Engraulis mordax*, p. 37-41. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 36.
- PICQUELLE, S., and G. STAUFFER.  
1985. Parameter estimation for an egg production method of northern anchovy biomass assessment. In R. Lasker (editor), An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, *Engraulis mordax*, p. 7-15. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 36.
- SMITH, P. E., and S. L. RICHARDSON.  
1977. Standard techniques for pelagic fish egg and larva surveys. FAO Fish. Tech. Pap. 175, 100 p.
- SMITH, P. E., W. FLERX, and R. HEWITT.  
1985. The CalCOFI vertical egg tow (CalVET) net. In R. Lasker (editor), An egg production method for estimating spawning biomass of pelagic fish: Application to the northern anchovy, *Engraulis mordax*, p. 27-32. U.S. Dep. Commer., NOAA Tech. Rep. NMFS 36.