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REPORT OF THE WORKING GROUP  
ON

*Modern approaches to assess  
maturity and fecundity of  
warm- and cold-water fish and squids*

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## Preface

Research on reproduction of resource species is an essential element in the evolution of fishery science for several reasons. For example the age or size at first reproduction is a driving variable in most stock assessment models, which directly affects estimates of stock productivity. Similarly annual age- or size-specific reproductive effort is a critical variable in stock and recruitment models and in understanding recruitment variability. Reproductive analysis is also an essential element in egg and larval production models used to estimate fish biomass, as part of very widespread survey techniques used to monitor biomass of fish stocks independent of commercial fisheries. While such parameters are routinely estimated in fishery science, investigation of the scientific basis for such estimates is a long neglected subject area. Many of the basic measurements and concepts have remained unchanged over the last hundred years, underlying major assumptions are seldom discussed or investigated although the potential for error or bias are substantial. Today fishery analysis often uses reproductive parameters based on concepts and protocols of the early 20<sup>th</sup> century conceived long before the dawn of the computer age to feed into increasing complex numerical stock assessment models. Over the last 15 years some advances have been made in fishery-based reproductive measurements, such as daily fecundity. These advances have fundamentally altered thinking regarding reproductive rates and raise fundamental questions regarding the soundness of traditional reproduction measurements and protocols, used world-wide in fisheries today. Existing fishery manuals for reproductive rate measurements for fishery scientists around the world are also long out of date.

Our intentions with this Workshop were to improve the overall quality of reproduction data used in assessments of marine stocks and egg production surveys world-wide by reviewing present practices, and establishing a new standard for routine reproductive measurements in fishery science.

Specifically the Workshop should:

- ❑ Review present practices in the reproductive science that support fishery assessment and surveys around the world.
- ❑ Identify key topics and set priorities for research that will improve accuracy of reproductive information used in fishery stock assessments and biomass surveys. In this process consider the major potential biases in reproductive parameters used for fishery assessments and surveys; identify cost effective measures that could improve precision, reduce bias and costs. Identify what we know and what we do not know regarding key life table reproductive parameters.
- ❑ Recommend new standards for measurements and procedures for fishery-based reproductive work.
- ❑ Produce workshop report that covers: review chapters on specific topics, and various “experience papers” which are scientific contributions from various countries, consistent with the general theme but limited to specific observations on a particular species in a particular country.

Both experts in fish reproduction as well as fishery scientists that use reproductive information were invited to the Workshop. The 21 participants were selected from different countries and continents and formed an outstanding group of people with very broad competence and insight (see List of Participants).

We were most pleased with the outcome of the Workshop and hope this Report will attract attention among scientists and technicians working within this important field of applied fish and squid reproductive biology.

The Editors would like to thank the Institute of Marine Research, Bergen and The Research Council of Norway (project no. 140267/120) for supporting this Workshop.



# Plenary document

This document is based on the results of the open discussions between members of the Workshop during the length of the meeting (see List of Participants). The list of topics was to some extent ranked by an increasing complexity in the protocol needed to carry out the work successfully. Thus, we decided to start with the use of visual maturity stages and end with the estimation of realised fecundity. Recommendations are included for each topic.

## GONAD GRADING KEYS BASED ON MACROSCOPIC CHARACTERISTICS

It was agreed on that macroscopic stage systems have several shortcomings, but are very simple in use, i.e., fast and cheap, and already exist in numerous time series; they are here to stay.

However, to be used on females the classification must:

- ✓ Be able to discriminate hydrated oocytes/gonads with running eggs as a separate class
- ✓ Have one class that clearly has visible yolked oocytes
- ✓ Have a clearly immature ovary class: oocytes not visible

and the ovary being completely translucent  
 ✓ All else are “other”

Male gross anatomical classification may start with:

- ✓ Immature – translucent
- ✓ Other – no milt in sperm duct
- ✓ Spawning – milt running or present when testes are bisected with a knife

For both genders, colour of the gonad is not a basic feature to catalogue maturity stage.

Taken together, the classification system may look like this:

Reference number	Maturity stage	Females	Males
M1	Immature	Tube like, transparent	Flat and solid, transparent
M2	Maturing	Vitellogenic oocytes visible	White and firm
M3	Spawning	Hyaline oocytes visible, or running eggs	Running milt or milt present in central collecting duct
M4	Early maturing / spent	Firm or flaccid – no discernible yolked eggs	White, no milt present in central collecting duct

Note that this is a general case and cannot be applied to all species, e.g. male sole, which show reddish M2-gonads and do not run milt to any extent in M3; may need a special key with histological validation. Also, *an additional code must be included for each fish to cross reference with other biological information, time, location and mode of sampling (commercial or research vessel catch).*

### Alternative approaches

- Histology (formally speaking this is not an alternative; it is considered to the best)
- Oocyte size frequency distributions including whole mount examinations
- Male and female GSIs (gonadosomatic indices)
- Fat content (especially for small pelagic fish)

### Recommendations

1. Assess uncertainty in these coarse macroscopic keys to derive confidence limits (e.g., to comply with EU data regulation)
2. Validate indices, e.g. redness - time at stage
3. Additional research on developing new methods of maturity classification, e.g. selective staining and hormone assays.
4. If GSI will be used, try to validate it. Calculations of monthly averages of GSI stratified by length could be useful in studies of the effect of size on the timing of the spawning season.

### AGE OR LENGTH SPECIFIC MATURITY VALIDATION

The objective here is to develop a maturity age or length schedule. An additional important benefit would provide information on reproductive strategies and reproductive effort.

### Recommendations

1. For all new species or stocks, the 1<sup>st</sup> step should be to conduct a detailed histological study with respect to fish age or length classes.
2. For maturity validation on existing species, all technical available means e.g. histological, oocyte diameter, GSI, visual identification of yolk and hydrated oocytes should as far as possible be used to calibrate the gross anatomical system in use to develop and provide a probability of correct classification.
3. Some grading systems are so inaccurate and imprecise that it is a waste of time to validate them.
4. Samples taken must be representative of the stock, both spatially and temporally.
5. Cross validation between research groups working on the same stock or on different stocks of the same species should be undertaken with the objective to develop a unified grading system.

With reference to the annual timing of samples for measuring maturity:

6. Must be done during spawning season
7. Sampling early in the spawning season is preferred to reduce risk of misclassification
8. Routine sampling outside the spawning season is still going on, but is often of limited use except when it is used to clarify when the spawning period is going to start.
9. Try to carry out a stratified sampling by fish size to indicate reproductive potential by length groups.

### FECONDITY AND MATURITY METHODOLOGY

#### General recommendations

1. A set of standards should be established for estimation of maturity and fecundity schedules.
2. Measurements of maturity and fecundity require histology to be accurate.
3. Studies based on historical time series are valuable but the precision of the assessment method should not be ignored.
4. The linkage of reproductive potential and the dynamics of the stock and effects of the environment are poorly understood and require experimentation and modelling.
5. Spawning biomass should be considered a poor index of the reproductive potential of the stock (cf. condition and age effects on realised egg production)
6. A thorough revision of terminology used in reproductive studies is required and should be incorporated in any relevant manual.

#### Important questions

- Under what conditions should the Batch Fecundity Method be applied
- Under what conditions should the Potential Annual Fecundity (PAF) Method be applied

#### Specific recommendations

Evaluate reproductive annual strategy before you select and apply fecundity methods. This should include:

1. If ovaries with hydrated oocytes are available determine batch fecundity in relation to spawning season.
2. Determine oocyte size frequency distributions through the reproductive cycle (annual if possible)

Measure extent of atresia throughout the season, and then note:

3. Spatial and temporal sampling should be included in experimental design.
4. Quantify, using histological or stereological techniques, role of atresia in regulating potential fecundity through the annual cycle.



5. Discriminate between pre-spawning and spawning stages.
6. Establish oocyte development growth curve.

Do both batch and potential annual fecundity assessments if possible and evaluate which is the most accurate. If you cannot do both, do the one that is most practical to do.

One should identify the oocyte size frequency distribution at the onset of the spawning season, and if there is a clear hiatus between the advanced stock from the other oocytes apply the PAF method. The mean diameter of the advanced stock of oocytes should be measured and if it does not increase during the spawning season the PAF method should be rejected. If there is a high incidence of atresia, extensive laboratory studies and field work will be required for an accurate estimate of PAF.

The batch fecundity method should be used when no clear hiatus exists.

#### Clarifications of methodological problems

##### *The Batch Fecundity Method*

- ✓ Lose hydrated oocytes due to catching and handling, which could bias sample for hydrated females.
- ✓ Since the duration of the hydrated stage is short, it may be difficult to obtain sufficient numbers of specimens for estimating frequency of spawning. Also, frequency of spawning is quite variable and requires a large number of positive sets or hauls.

##### *The Potential Fecundity Method*

- ✓ If there are high atresia rates, these have to be quantified and the atretic duration known.

- ✓ Potential is not realized; potential and realized fecundity could be substantially different.
- ✓ Recruitment problem of oocytes to enhance potential fecundity.
- ✓ Verify potentially fully formed ovaries (prespawning) and not yet lost eggs (not spawned).

##### *Atresia and POFs*

Steps for quantifying atresia:

1. Define alpha atretic classes to be used to estimate losses to all oocytes included in potential fecundity.
2. To measure atretic losses the alpha stage is the only reliable stage.
3. The later stages of atresia should be identified to increase period of detection for maturity classification (as for instance immature vs spent fish).
4. Intensity and prevalence of atresia should be assessed carefully from the time the potential fecundity is made until spawning is completed.
5. Estimate intensity of atresia based on
  - a. the Disector method or the Weibel method (see review of Andersen, this volume)
  - b. whole oocyte preparation might be used to quantify atresia if the time at stage can be determined.
6. Estimate duration of atretic stages in relation to temperature.
7. Determine the oocyte growth curve from the time of sampling until the time of spawning.

Steps to quantify and use POFs as markers of spawning activity:

1. Define POF classes based on their morphology and or size in relation to the time following ovulation.
2. Estimate duration of POF stages in relation to temperature.

#### FUTURE RESEARCH

- ❖ Initiate research on and develop new techniques, including biochemical, to separate developing ovaries from post spawning ovaries.
- ❖ Develop methods to identify and age postovulatory and atretic follicles in non-histological tissue preparations
- ❖ Develop criteria to separate immature, inactive, and active testes.
- ❖ Develop volume fraction method for assessing maturity in males and females.
- ❖ Estimate daily spawning fraction by age or size groups.
- ❖ Is reproductive output proportional to fish weight or age?



# Unbiased stereological estimation of cell numbers and volume fractions: the disector and the principles of point counting

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## ABSTRACT

Modern stereology provides tools for obtaining unbiased, quantitative estimates of number, volume, area or length in microscopy. Methods for estimating number and volume fractions are presented in this paper, based on information available in the literature.

## INTRODUCTION

The need for estimates of cell numbers is responsible for a large portion of the workload in many fisheries-related reproductive biology studies. New image analysis methods (Thorsen & Kjesbu 2001), or the traditional gravimetric or volumetric methods (see e.g. Bagenal 1978) are in most cases adequate and, indeed, often the simplest solution. Nevertheless, situations exist in modern fecundity analysis, as well as in a number of histological studies related to maturation, where such approaches are not suitable. Examples include estimates of oocyte numbers in early maturing fish, and potential fecundity estimates in species or individuals with high levels of atresia. In these cases, the small size of the cells as well as the lack of reliable whole-cell methods for detecting specific developmental stages or atresia prevent the use of simple methods, and using them may bias the results. Stereological methods for estimating particle numbers have been available for several decades. Weibel (1979) describes methods used prior to the disector, and Emerson *et al.* (1990) describe an application in fisheries biology. However, the first (and so far only) *shape independent* method was described for biological microscopy as late as in 1984 (Sterio 1984; Gundersen *et al.* 1988a; Mayhew & Gundersen 1996), *the disector principle*. This principle enables unbiased counts to be made of any particle, regardless of its shape or size. The use of stereological

methods for counting particles based on histological sections is imperative, as the use of simple profile counts will bias the results severely and variably. The size of the bias introduced is highly dependent on the shape and size distribution of the cells or particles being counted.

Stereological estimates of cell or particle number tend to be rather labour-intensive and complicated. Estimates of volume and volume fraction, on the other hand, are easily obtained stereologically, and good methods have been known for a long time (see Weibel 1979; Gundersen *et al.* 1988a; Howard & Reed 1998). Before point counting methods (like the one described below) were described, people have used a number of solutions. This includes drawing an image of the tissue on paper, cutting out the regions of interest, and weighing the pieces of paper, thereby obtaining fractions of individual histological or cellular compartments! As will be discussed, software-based image analysis methods that, in theory, should give unbiased results, may nevertheless be biased due to the interpretation of tissue boundaries.

So, what is stereology? Stereology may be considered a set of mathematically well-defined tools for the estimation of volume, area, length or number. These tools can be applied in almost any field, be it satellite photography, geology, or fish biology. This brief presentation covers two methods for use in quantitative biological microscopy. Please consult the cited literature for details before starting to use the methods described. The excellent introductory text by Howard & Reed (1998) is a good place to start, and contains references to most major publications in the field. It will be referred to extensively in this presentation.

**METHODS**

**Sampling**

All stereological methods require random samples. As a consequence, it is necessary to use a design-based sampling scheme, unless it is known that the structure (e.g. an ovary) from the species being studied is homogenous. The fractionator, a method for obtaining random, quantitative samples from a large structure (population, individual, organ etc.) (Gundersen et al. 1988a; Howard & Reed 1998) is probably the best sampling scheme available. Small biopsies from a more or less fixed position in the ovary may only be used for stereological estimations when the ovary is homogenous (this should be tested by a design-based method like the fractionator).

**Volume fraction estimation**

Volume fractions are the easiest estimates to obtain from sections. Only a single, random (not arbitrary) section is needed, and there is no need to compensate for shrinkage or section distortion. In short, a point grid (e.g. one similar to that in Fig. 1) is placed randomly on sections through the tissue. Placing a randomized grid on a section is equivalent to placing random points within the three-dimensional tissue. It should be realised that if one point in the grid is randomised in 3D, then every point in the grid is randomly placed. Such a system, where one point is randomised, and the remaining points are systematically placed in relation to this random point, is called a *systematic random selection*. This systematic random sampling scheme is the most efficient in reducing the sampling error in the estimate (Howard & Reed 1998). To estimate the volume fractions of tissue components, the number of points in the grid hitting

various structures are counted. The volume fractions are then estimated as:

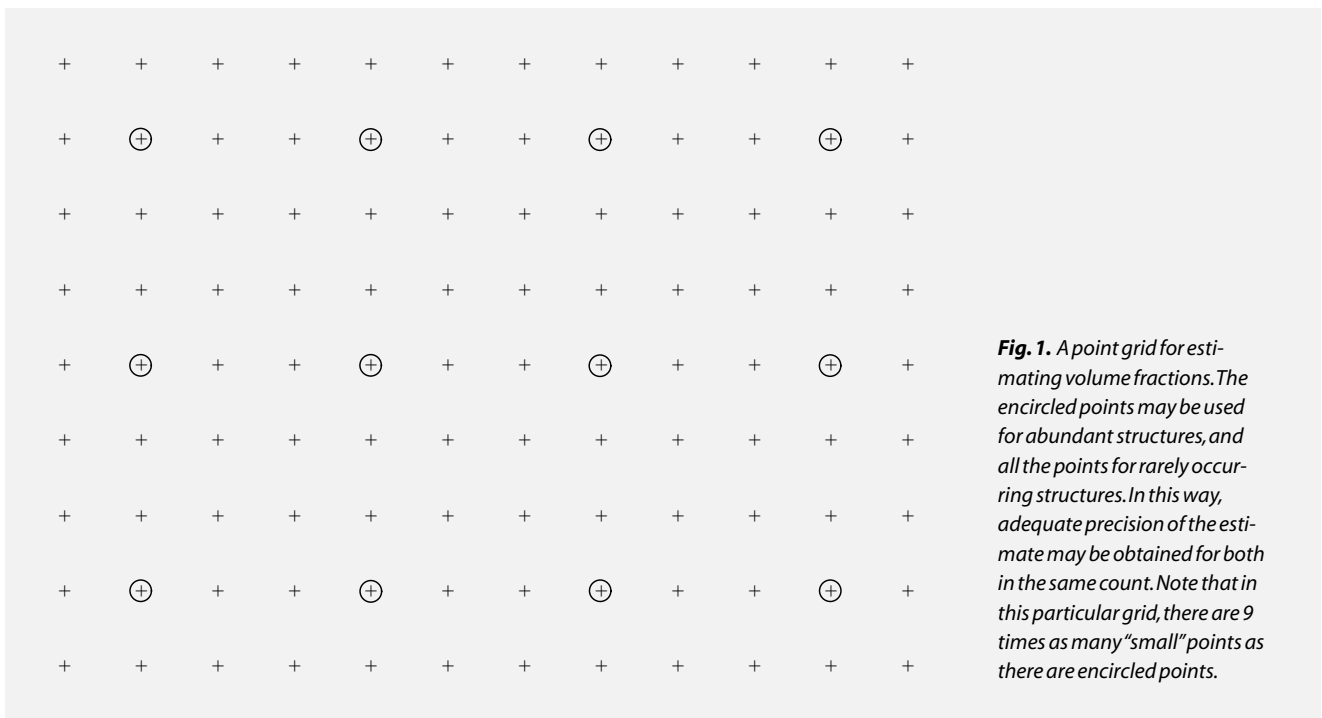
$$est V_{(a,ref)} = \frac{\sum_{i=1}^n P_{(a)i}}{\sum_{i=1}^n P_{(ref)i}}, \text{ where} \tag{1}$$

$est V_{(a,ref)}$  is the volume fraction of structure  $a$  in the reference space,

$P_{(a)i}$  is the number of points falling on (“hitting”) structure  $a$  in field  $i$ , and

$P_{(ref)i}$  is the number of points falling on (“hitting”) the reference space in field  $i$ .

Please note that the points hitting the structure of interest must be added together over all sections and fields before being divided by the sum of points “hitting” the reference space. If this division is made for each field, and the results averaged, the estimate may be wrong. This is because both the numerator and the denominator may vary between counting field. As a result, too much weight will be given to the fields with little reference space, if the division is done for each field. The term reference space represents the structure in which another structure is to be quantified. Examples include: the animal (e.g. the fraction of the liver in the individual), an organ (e.g. the fraction of hepatocytes in a liver) or a cell type (e.g. the fraction of nuclei or lipid droplets in the hepatocytes).



**Fig. 1.** A point grid for estimating volume fractions. The encircled points may be used for abundant structures, and all the points for rarely occurring structures. In this way, adequate precision of the estimate may be obtained for both in the same count. Note that in this particular grid, there are 9 times as many “small” points as there are encircled points.

The number of points to be counted is usually low. In most cases about 200 points “hitting” the tissue is sufficient to reach 5% CE (coefficient of error) in the estimate. It is best to use few points and several fields. E.g.: Using four small tissue pieces (all four may be embedded in one mould), taking four fields from each, you only need about 12 points in each point screen. This kind of screen may easily be printed on A4 paper, and photographed at a suitable reproduction size (preferably using lithographic film). The film (must be positive!) with the point screen may be cut circular and placed in the visual or photo/video ocular of the microscope. The XY-table on the microscope may then be used to move the screen systematically over the tissue.

If a structure has a low occurrence in the tissue, a screen like the one in Fig. 1 may be used. The encircled points are then used for estimating the reference space, and all points for estimating the infrequent structure. By using more points for the rare structure, a reasonable CE of the estimate is obtained, while using the encircled points for the reference space ensures that no more time than necessary is spent counting (for the grid in Fig. 1, one must not forget to compensate for the fact that there are 9 times as many points in the fine grid as in the coarse grid!).

#### Estimation of the reference space ( $V_{ref}$ )

One very important condition must be fulfilled for volume fractions to be meaningful: the volume (in some cases the weight is sufficient) of the reference space must be known! If you don't know the size of the reference space, a change in volume fractions may just reflect a change in the size of the organ or the amount of another component, and not a

change in the number or size of the cells examined. A good example is the fraction of non-maturing oocytes in the ovary. As maturation proceeds, the fraction of these in the ovary will decrease dramatically, a change not related to the amount of immature oocytes, but to the expansion of the reference space (i.e. the growth of maturing oocytes). This is often referred to as the “reference trap” (see Howard & Reed (1998), and references therein for several more examples), and may cause the wrong conclusions to be drawn. For many fisheries-related studies, the weight of the ovary may be used as the reference space estimate, if necessary with a compensation for weight per unit volume. If, however, one is trying to identify volume fractions of mature, lipid-rich oocytes in comparison with early maturing oocytes (within or between ovaries), there is likely to be a difference in specific gravity between the two cell populations being compared. It may then be necessary to estimate the true volume of the ovary using the Cavalieri principle, in combination with a point counting method. Note that whereas all modern literature refer to this as the Cavalieri-principle, a look at a 1653 edition of the original reference (Cavalerio 1653), implies that it should be called the Cavalieri-principle, the mans name being P. Bonaventura Cavalerio (I'm not sure if the *P.* is an initial or a title). A modern implementation of the Cavalieri priciple may be found in Howard & Reed (1998, cited as the Cavalieri method).

#### Counting cells

##### Two-dimensional (2D) counting

Counting rules commonly used in 2D are not always unbiased. For counting blood cells or algae, a counting chamber is often used, with a counting scheme like the one shown in

**Fig. 2.** 2D counting frames.  
 a) A commonly used, biased counting frame.  
 b) An unbiased counting frame.  
 Full drawn lines are exclusion lines (“forbidden lines”), and particles crossing these are not counted. Dotted lines are inclusion lines (“allowed lines”), and particles crossing these are counted. In the unbiased counting rule in b), particles crossing the inclusion lines are only counted if they do not cross any of the forbidden lines. In a), particles crossing both the inclusion and exclusion lines may, or may not be counted, depending on the rule applied. If tessellated over the tissue, it will be clear that the frame in a) counts some particles more than once, and is therefore biased. The frame shown in b) allows each particle to be counted once and only once, and is therefore unbiased.

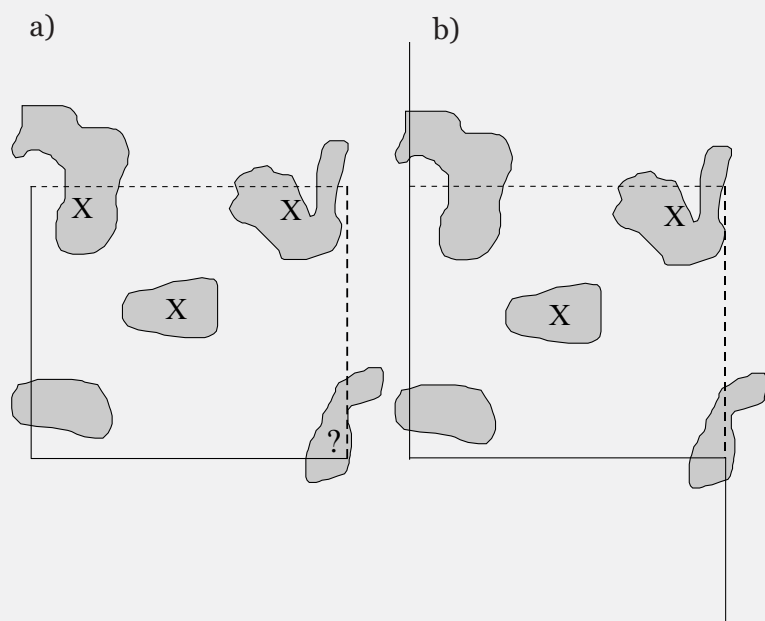


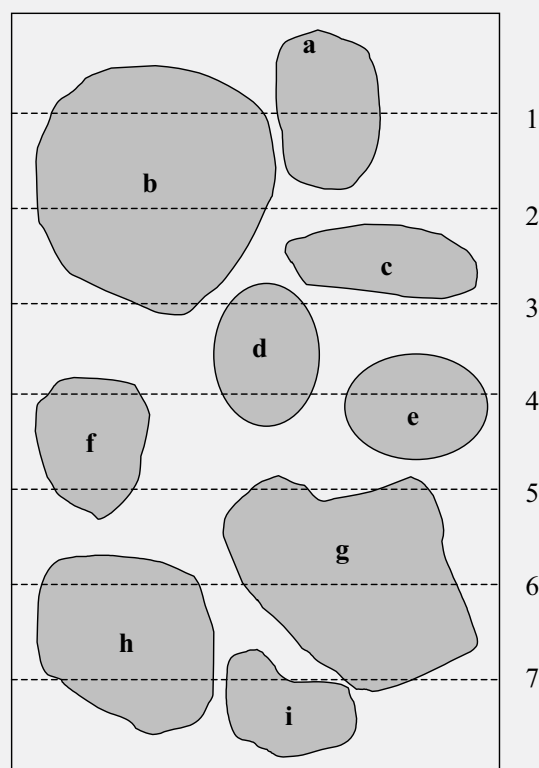
Fig. 2a. This does not result in unbiased counts, since cells or particles may be counted more than once if a tessellation of these counting frames is placed on the sample. The frame in Fig. 2b does give unbiased results (Gundersen et al. 1988b), and should always be used. It should be noted that a *projection* of whole cells (like a whole mount preparation of oocytes) is basically a 2D situation, since each cell or particle can only produce one image profile or silhouette. Consequently, a 2D counting frame may be used. It is necessary to make sure that all cells or particles are identifiable in the projected image. No overlapping particles are allowed unless they can be distinguished from each other in the image. Counting cells in sections, however, brings up the need to deal with three dimensions, and a 3D counting principle must be employed.

#### Three-dimensional (3D) counting (the disector)

Particles or cells exist in a three-dimensional environment. For practical purposes, the thin resin or paraffin sections normally used in histology are two-dimensional; they are approximately planes. A plane cannot be used as a probe to sample number. The reason for this is that any particle in a 3D structure (such as a tissue) has a probability of being represented by a profile in the section plane, which is proportional with the particle height normal to the plane. This is illustrated in Fig. 3: large particles or cells appear in a larger portion of the sections through the tissue, and produce more profiles. A requirement for a count to be correct is that all particles are given the same probability

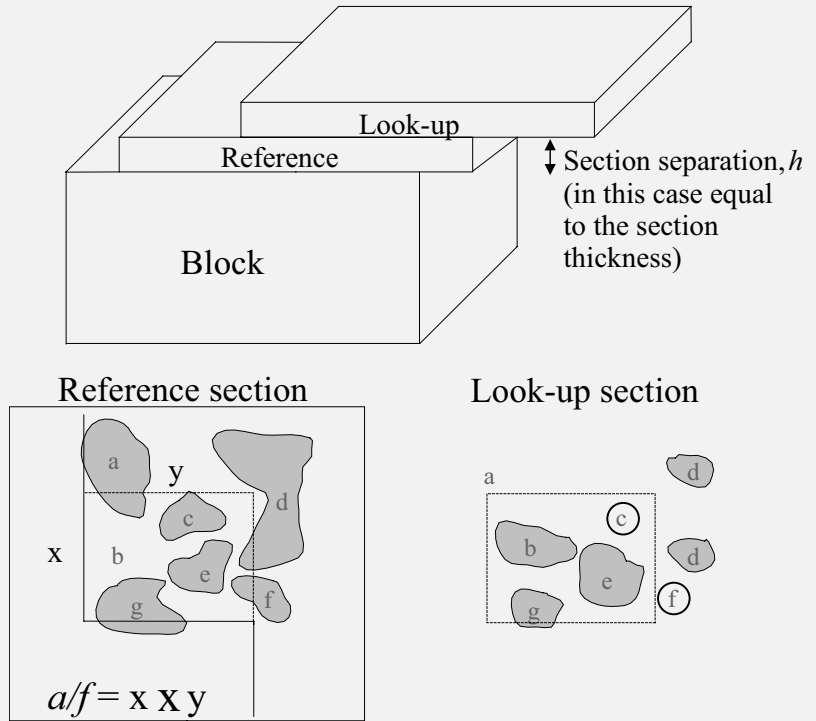
of being counted. If we count profiles in a single section, that requirement is violated. So, to sample number in a 3D environment, a volume must be used as the probe. In 1984, a method was published, which has since become the standard for stereological counting: *the disector principle* (Sterio 1984, Gundersen et al. 1988a). The *physical* disector makes use of two consecutive sections from the tissue. There is also an *optical* disector, which uses a single, thick section. Within this thick section, a volume is sampled by focusing through the specimen (optical sectioning) and cells or particle are counted as they come into focus. In this presentation, only the physical disector will be described. However, if working with small cells or particles, then the optical disector is much less labour intensive, and should be preferred over the physical one.

The physical disector requires two consecutive sections from the tissue. The distance between the sections must be known. As a result, it is necessary to keep track of shrinkage/swelling throughout the processing of the tissue and sections (this is not always necessary if only addressing relative numbers of particle populations). The distance between the sections must never exceed the size of the smallest cell or particle to be counted, and should generally be chosen at about 1/4 to 1/3 of the smallest particle size (e.g. if the smallest cell in the tissue is 35  $\mu\text{m}$ , then the two sections should be no more than about 10  $\mu\text{m}$  apart). Please consult the cited literature for details in this procedure. The two sections are then aligned, side by side, and a 2D counting frame is placed



**Fig. 3.** Particles are sampled with a probability that is proportional with the height of the particle normal to the section plane. In the figure, a set of particles in 3D is seen as a simplified projection from the side. If sections are taken at constant intervals through the tissue (dashed lines), each particle will appear in anything from none to several sections. The number of profiles generated is proportional to the height of the particle normal to the section plane, producing a volume-weighted estimate (for isotropic tissues and/or randomly oriented sections). This is quite different from number weighting, which gives all particles the same chance of being counted. As a result, simple section profile counts are not suitable to estimate the number of cells in a tissue or organ.

**Fig. 4.** The disector principle. The disector describes a volume of the tissue. Only if a profile is seen in one section, but not in the following section, is the corresponding cell or particle counted. The result is an estimate of the number of cells per unit volume (Equation (2)), and the total number of cells in the organ may easily be estimated by multiplying the numerical density by the volume of the organ (Equation (3)). Note that the area per field ( $a/f$ ) is the area of the disector frame at the level of the tissue. In the figure, particles  $c$  and  $f$  (encircled in the look-up section) will be counted. Particle  $a$  also disappears, but touches the forbidden line, and is not counted. If the disector is applied “backwards”; particle  $b$  is only found in the right section, and is counted. (If the disector is applied in both directions, it is important to remember that this represents two disector pairs, and therefore has a total volume  $2 * a/f * h$ ).



on one of the sections. To aid in comparing the sections, a frame may also be placed on the other section. This is not used in the counting, just to make it easier to compare the sections. If a cell or particle occurs in the first section (reference or counting section), but not in the other (look-up or control section), it is counted. If it occurs in both sections, it is not counted. To increase the efficiency of the counting, the sections may be counted in both directions ( $a \rightarrow b$  and  $b \rightarrow a$ ), giving a disector volume twice as large as the volume described by the section separation and the counting frame. The procedure is illustrated in Fig. 4.

The number of particles or cells per unit volume is estimated as:

$$estN_v = \frac{1}{(a/f) * h} * \frac{\sum_{i=1}^n Q_i}{\sum_{i=1}^n P_i}, \text{ where} \tag{2}$$

- $estN_v$  is the number of particles per unit volume,
- $a/f$  is the area per counting frame (at the level of the tissue),
- $h$  is the section separation,
- $Q$  is the number of particles counted over all disector frames and
- $P$  is the number of disector counting frames falling on the tissue.

In practice, one will find that fields sometimes fall only partially on the reference space. This is dealt with using what is usually called “associated points”. This means that one point, somewhere within the counting frame is defined as the associated point. If this falls on the reference space, the area of the field is included in the denominator of equation (2). If it falls outside the reference space, it is disregarded. The particles ( $Q$ ) observed are counted in both cases. Thus, in practical use of the disector, the  $P$  in the formula will be the number of such “associated points”, rather than the total number of fields used. For further explanation, see Howard & Reed, 1998.

The number of particles or cells in the tissue is then estimated as:

$$estN = estV_{ref} * estN_v, \text{ where} \tag{3}$$

- $estN$  is the number weighted estimate of total particle number and
- $estV_{ref}$  is the estimated reference volume (e.g. volume of the organ or cell type. Not to be confused with volume fraction,  $estV_{(a,ref)}$ ).

It is clear from equation (2) that the estimation of number is not independent of tissue shrinkage and section distortion. As a result it is necessary to keep track of any dimensional changes after the stage when the reference volume was

estimated. E.g. if the volume of the organ was estimated using the fresh, unfixed organ, then any changes during fixation, embedding and sectioning need to be estimated. If, on the other hand, the reference volume was estimated after embedding, only changes due to the sectioning and staining need to be addressed. It is important to remember that the dimensions of the tissue and sections by no means are constant during/after embedding (Hanstede & Gerrits 1983).

## DISCUSSION

The application of stereological tools is becoming more and more common in all fields of microscopy. This is due to their simple nature and the accuracy (unbiasedness) of the estimates. Stereological estimates are in many cases the only way to obtain unbiased quantitative microscopical data. In other cases they are simply the most efficient estimates.

Generally, estimates of volume and volume fractions are easily obtained, as only point counting is required. Few "hits", generally less than 200 on the entire tissue (Gundersen et al. 1988b, Howard & Reed 1998), are necessary to obtain good estimates, making the method very efficient. There is also no need for sophisticated image analysis computers, as simple grids on overhead film or as a graticule in the ocular of the microscope is usually equally or more efficient. Estimates of area fraction (which is a direct estimator of volume fraction (Delesse, 1847)) using image analysis software require, to my knowledge, practically without exception that profiles are drawn manually

around the structures of interest. This not only considerably increases the workload, but may also bias the result due to the way the edge of the structure is interpreted (the line you draw has a width!). When I asked a group of students to make volume fraction estimates from the same (simple, colour coded, "synthetic") images, image analysis software gave estimates about 20% lower than (unbiased!) point counting. The bias in "real" situations may of course vary, but care should be taken if such software has to be used in quantitative work.

In fisheries biology volume fractions may prove very useful in obtaining ungraded staging criteria for male fish. In some situations, it may also be useful for grading the maturity of females, particularly in the early stages of development. In both these situations, the volume of cells in the different stages will be a sensitive measure for the developmental status of the individual. As the development proceeds, the volume fractions of the most advanced cell types will increase. If the development includes volume changes (as for oocytes), the volume fraction of the leading stages will tend to increase exponentially, producing a very sensitive tool for assessing e.g. the onset of a process. A further advantage in using volume fractions for grading is getting a continuous scale. In many situations, this will be preferable to a grading based on more or less well defined stages imposed on nature by the scientist. When quantitative data are needed, most natural processes (ontogenetic or maturational development being no exception) are best described as continua, rather than in stages.

**Table 1.** Summary of symbols used.

SYMBOL	LEGEND
$estV_{ref}$	estimated reference volume
$estV_{(a,ref)}$	volume fraction of structure <i>a</i> in the reference space
$P_{(a)i}$	number of points "hitting" structure <i>a</i> in section <i>i</i>
$P_{(ref)i}$	number of points "hitting" the reference space in section <i>i</i>
$estN_v$	number of particles per unit volume
$estN$	number weighted estimate of total particle number
$a/f$	area per counting frame (at the level of the tissue)
$h$	section separation
$Q$	number of particles counted in the disector counting frame
$P$	number of disector counting frames (in practice "associated points") falling on the tissue



For number estimation, the major part of the work is preparing and aligning the sections needed in the physical disector. This makes number estimates labour intensive. Still, a rather low number of particles need to be counted, and if an optical disector can be used, the workload is significantly reduced. It is my strong belief that the benefits of these methods far outweigh the increased workload. If a large amount of stereological counting is expected, it will probably be wise to look into semi-automated equipment to aid in the rather troublesome alignment of the sections.

Stereological estimates tend to produce more noisy (imprecise) estimates than non-random methods. The main explanation for this is the requirement of random sampling. When random samples are collected (at all stages in the sampling sequence), the chance of observing an event (i.e. counting a point or a profile) is more variable than would be the case if the counting frames were placed deliberately onto interesting areas of the section. However, the variability introduced by the method is easily reduced to an acceptable level by adapting the number of points of profiles counted. It should be remembered that in most biological studies, the major relative contribution to the overall variability is the inter-individual (i.e. biological) and intra-individual (i.e. between blocks) variation, whereas the variation between fields usually accounts for far less, and the counting error for only a tiny fraction of the variability in the estimate (Gundersen & Østerby 1981; Howard & Reed 1998). The biological variation is usually by far the greatest, and as a result, it is more often than not, better to increase the number of animals, using a less precise estimate for each animal. It should also be emphasised that the unbiased nature of most stereological estimates usually make them highly preferable to model-based (often biased) approaches.

The current discussion on quantitative microscopy in fisheries science is very similar to the one in the medical sciences some time ago (Howard 1986, discussion following Howards presentation; Coggeshall & Lekan 1996; Saper 1996). A reasonable assumption is that the benefits of design-based (unbiased) methods over the older assumption-based (and usually biased) methods will cause more fisheries biologists to convert to these methods in the future. In medical studies in need of quantitative microscopy, the unbiased stereological methods are more or less imperative. As an increasing fraction of microscopical examinations in fisheries biology requires quantitative data, stereology will, or at least clearly should, increase in importance also in this field. At least one journal in the medical sciences is now rejecting publications presenting unvalidated profile counts (Coggeshall & Lekan 1996; Saper 1996 (editorial)). It is time for the fisheries sciences

to take similar measures to ensure that the best available methods are being used. The increase in work required to obtain stereological estimates should be viewed against the background of the significant number of invalid estimates published today. In that context, it is likely a simple choice to spend the extra time to obtain unbiased estimates.

#### ACKNOWLEDGEMENTS

This presentation draws heavily on a number of published papers. Nothing “new” is presented, but it is nevertheless difficult to point to the source of everything. Most of the text and figures are modified from or inspired by papers in the reference list. Hopefully the present paper will inspire the reader to read the source material.

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# Progress towards a common gonad grading key for estimating the maturity of North Sea plaice

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## ABSTRACT

Dutch and English scientists have built up extensive time series of data on the sexual maturity of North Sea plaice. These are based on visual assessment of the stage of sexual maturation of the gonads. Most English plaice maturity studies have been conducted in the central and northern North Sea, whilst the Dutch studies have concentrated in the south. There are obvious benefits to be gained by combining the two datasets to provide a comprehensive temporal and spatial picture of the maturation of North Sea plaice. These include provision to investigate the timing of sexual maturation, to study the impact of environmental change and fluctuating fishing pressures on sexual maturation, and to facilitate the construction of maturity ogives applicable to the entire North Sea plaice population. The main problem in combining the North Sea plaice maturity data stems from the maturity keys used by the two nations, which are different. An attempt is made to salvage a degree of compatibility between the datasets based on “the lowest common denominator principle”, but with limited success. Progress towards developing a standard protocol for sampling plaice maturity is discussed, in order that in future maturity data derived from a variety of sources can be pooled into a common database.

Key words - sexual maturation, maturity ogive, North Sea, plaice, *Pleuronectes platessa*, sampling protocol

## INTRODUCTION

Maturity keys aim to partition gonad maturation into a series of distinct stages. Ideally, these should be readily identifiable and should preferably reflect histologically distinct aspects of gonad development. However, all schemes for staging maturity tend to be somewhat subjective and different workers have often adopted different matu-

rity keys (Wallace, 1909, 1916; Wimpenny, 1953; Simpson, 1959; Rijnsdorp, 1989), which creates difficulties and can even thwart meaningful comparisons between data derived using different sampling protocols. Dutch and English scientists have built up extensive time series of data on the sexual maturity of North Sea plaice (Rijnsdorp and Vethaak, 1997; Bromley, 2000). These are based on visual assessment of the stage of sexual maturation of the gonads. In general, little attempt has been made to introduce quality control measures to assess the reliability of visual staging and only occasionally have there been attempts to verify the findings against histological examination of the gonads (Morrison, 1990; Ramsay and Witthames, 1996). Indeed, without such verification, maturity studies based on visual staging alone are considered unreliable. However, historical time series are too valuable to be dismissed solely on these grounds, and as long as the sampling methodology has not changed significantly during the course of the study, such time series should provide useful indices of sexual maturity for monitoring trends through time.

Most English plaice maturity studies have been conducted in the central and northern North Sea, whilst the Dutch studies have concentrated in the south. There are obvious benefits to be gained by combining the two datasets to provide a comprehensive temporal and spatial picture of the maturation of North Sea plaice. These include provision to investigate the timing of sexual maturation, to study the impact of environmental change and fluctuation in fishing pressures on sexual maturation, and to facilitate the construction of maturity ogives for assessing the spawning stock biomass of the entire North Sea plaice population. Current stock assessments treat North Sea plaice as a single

stock (Anon., 1996), and it is desirable that the maturity ogives are representative of the whole area.

The main problem in combining the North Sea plaice maturity data stems from the maturity keys used by the two nations, which are different. An attempt is made here to salvage a degree of compatibility between the datasets based on “the lowest common denominator principle”, but with limited success. Progress towards developing a standard protocol for sampling plaice maturity is discussed, in order that in future maturity data derived from a variety of sources can be pooled into a common database.

#### MATERIALS AND METHODS

The maturity keys used by Dutch and English scientists (Rijnsdorp and Vethaak, 1997; Bromley, 2000) to assess the maturity stage of plaice caught by the commercial fishing fleets are shown in Table 1. The sampling was undertaken at the respective North Sea ports where the catches were landed. Both countries have extensive databases, going back many years and have supplemented the market sampling with data derived on fishing surveys. Most of the Dutch maturity data are from the southern North Sea, whilst the English data are from the central and Northern North Sea (Rijnsdorp and Vethaak, 1997; Bromley, 2000), though there is some spatial overlap between the two datasets.

## RESULTS

### a) Female plaice

Both the Dutch and English (Rijnsdorp and Vethaak, 1997; Bromley, 2000) use a 7-stage key (Table 1) for female plaice. The number of stages is one of the few similarities between the two keys and there is plenty of opportunity for confusion. Sometimes, similar stages are given a different number, or different stages are given the same number. For example, a stage 6 Dutch female is nearly spent but a stage 6 English fish is running. Both keys class immature ovaries as stage 1, but do not agree on the definition of what constitutes an immature plaice. The English definition of an immature ovary must be regarded as the extreme limit of the immature phase, since the yellowish orange colour probably marks the start of vitellogenesis and will therefore overlap into the Dutch maturity stage 2. It can therefore be expected that use of the English key will lead to a higher estimate of the proportion of fish classified as immature compared with the Dutch key (and by definition, therefore, a smaller proportion classed as maturing). When comparing maturity levels between the two keys, therefore, it is not possible to be certain as to what extent any differences are real or stem from an artefact of the sampling technique. The Dutch stage 2 is a broad category describing maturing fish and includes some of the English maturity stage 1 as well as all of stages 3 and 4. None of the other maturity

**Table 1.** Description of the Dutch and English sexual maturity stages of female North Sea plaice

	Dutch	English
1	Immature: lumen transparent grey	Immature: small ovaries <4cm in length, thin walled and internally yellowish orange in colour
2	Ripening: colour orange, vitellogenesis in progress	Spent, recovering: all eggs resorbed, little or no slime inside ovaries
3	Spawning: as 2 but with few ripe hyaline eggs	Half full: ovaries filling with eggs
4	Spawning: ovary completely filled with hyaline eggs	Full: ovaries full and usually distending body, no sign of hyaline eggs
5	Spawning: as 4 but partly shed	Hyaline eggs: ovaries containing from a few to many hyaline eggs, but ovaries will not run, even under heavy pressure.
6	Nearly spent: ovaries containing a small amount of hyaline eggs	Running: hyaline eggs can be extruded copiously under light pressure - fish cycling from stage 5 to 6 during the spawning season
7	Spent: ovary small, flabby and bloodshot back to stage 2.	Spent: few mainly opaque eggs in a state of resorption and much slime in ovaries

**Table 2.** Common maturity stages between the Dutch and English maturity key for female plaice

Common maturity stages	Dutch maturity stages	English maturity stages
A) Pre-hyaline egg phase	1 and 2	1, 3 and 4
B) Hyaline + phase	3, 4, 5, 6 and 7	5, 6, 7 and 2

stages exactly correspond either. The Dutch class spawning fish from the hyaline egg stage onwards into three stages - stages 3, 4 and 5, whereas the English class them into two stages - stages 5 and 6. The Dutch have one stage for spent ovaries, the English have two stages.

Pooling of maturity data can only be reliably achieved on the 'lowest common denominator principle' and if the maturity stages of one sampling regime overlap those of another sampling scheme then pooling data is not feasible. The final number of communal stages in the resulting combined database depends on how many of the maturity stages have common starting and end points (stage boundaries), which correspond across the various sampling schemes. If there are no matching common boundaries the data cannot be pooled.

In the case of the plaice data there was only one common stage boundary and that occurred at the start of the hyaline egg phase. For comparative purposes the various maturity

stages were aggregated into two classes, which are shown in Table 2. It is therefore justified to compare directly the proportion of pre- and post-hyaline ovaries between the Dutch and the English maturity keys. This should enable broad trends in the maturity of female plaice to be compared between the central and southern North Sea.

### b) Male plaice

The maturity keys applied to males are also different and can be dealt with using a similar approach to that used with the females. The male maturity stages are shown in Table 3. As in the case of females, none of the male maturity stages was identical in the two keys. The Dutch definition of an immature fish is not as precise as the English definition and it cannot be assumed that the two keys will give an identical estimate of the proportion of immature fish. In terms of combining the two datasets, the only common stage boundary occurred at the point where the males started running. As for females, therefore, it was only possible to merge the two datasets into two broad categories, pre- and post-run-

**Table 3.** The sexual maturity stages of male plaice according to Dutch and English protocols

Stage	Rijnsdorp and Vethaak, 1997	CEFAS studies
1.	Immature: testes very small	Immature: testes tight up against back of gut cavity and very small, usually not larger than 1.2 cm long by 0.25 cm in width
2.	Ripening: testes bigger, grey coloured	Spent, recovering: testes thin, redness lost, any sperm remaining in ducts can be extruded under moderate pressure
3.	Ripe: testes big and white, milt can be expelled under pressure	Half full: testes filling, roughly half full, no sperm in ducts
4.	Spawning: as 3, milt freely running or can be expressed under slight pressure	Full: testes fully swollen, but will not run, even with moderate pressure
5.	Nearly spent: milt brownish, can be expelled under strong pressure	No stage 5 in males
6.	Spent: small, form of half moon, brown	Running: sperm can be extruded under light pressure
7.	Spent: shrunken, often going back to stage 1	Spent: testes thin, flabby and often red in places. Any remaining sperm in the ducts can be extruded under fairly light pressure.

**Table 4.** Common maturity stages between the Dutch and English maturity keys for male plaice

Common maturity stages	Dutch maturity stages	English maturity stages
A) Pre-running phase	1 and 2	1, 3 and 4
B) Post-running phase	3, 4, 5, 6 and 7	6, 7 and 2

ning individuals, as shown in Table 4. Though not ideal, this does allow a direct comparison of broad trends in maturity to be made between the two datasets, and as a consequence, between the central and southern North Sea.

#### DISCUSSION

The broad maturity bands derived by aggregating the Dutch and English maturity data on a communal basis provide an opportunity to investigate gross regional and temporal trends in plaice maturity of North Sea plaice. However, the division between the bands is such that the aggregated data are of little value in ascertaining the spawning fraction of the population, which is often the prime purpose of maturity studies. Such information is needed in order to estimating the plaice spawning stock biomass. Rather than trying to aggregate the two datasets directly, a alternative option might be to conduct comparative trials where the two maturity keys are applied to the same fish samples, thereby allowing a degree of inter-calibration between the datasets.

In terms of estimating the spawning fraction, the simplest approach is to assume that all non-immature fish will spawn, in which case the English maturity key will probably underestimate the proportion of mature fish compared with the Dutch key. However, there is increasing evidence (De Veen, 1970, 1976; Bromley, 2000; Bromley and Casey, 2003) that some virgin fish approaching spawning for the first time might be adolescents that fail to reach spawning condition or only spawn infrequently, and their contribution to the spawning stock biomass should be down-weighted. In terms of identifying this fraction, the English key probably has the advantage, since it includes

ovaries that are classed as 'half-full' (English stage 3). This is certainly a very subjective stage, however, if fish with only partially developed ovaries are found towards the end of the spawning season it can reasonably be assumed that they will not participate in spawning, either because the vitellogenic oocytes are too poorly developed (Ramsay and Witthames, 1996), or they may possibly be subject to atretic degeneration (Hunter and Macewicz, 1985; Witthames and Walker, 1995; Bromley *et al.*, 2000). It is becoming increasingly important to be able to identify the proportion of potentially non-spawning adolescents in stocks such as North Sea plaice that are heavily fished and the spawning fraction is becoming increasingly dominated by young fish. Failure to do so could lead to overestimation of the spawning stock biomass and the belief that the stock is in a healthier condition than it really is. Usage of the English key, or an allied key with the facility to identify adolescents, offers the potential for improving estimates of the spawning fraction.

In order to standardise the collection of fish maturity data a 'Universal' four-stage gonad grading key (three-stages for males) has been recommended for use in maturity studies (Table 5). This was chosen because it was felt that it was generally not feasible to reliably separate out more than four maturity stages by eye, without resorting to histological screening. In practical terms, the 'Universal' grading key has close similarities with existing maturity keys, though there are fewer stage boundaries to contend with. Effectively, the total sampling effort is similar regardless of which maturity key is used; the only difference is in the detail and the combination of the information collected. In

**Table 5.** Proposed 'Universal' gonad grading key

Stage		Females	Males
I	Immature	Tube like, transparent	Blade like, transparent
M	Maturing	Vitellogenic oocytes visible to naked eye	
S	Spawning	Hydrated / running	Running milt
O	Early maturing / spent	Flaccid – no discernible yolk eggs	Flaccid – some development

the 'Universal' grading key, immature ovaries are classed stage 'I'. The 'Universal' stage 'M' is the same as stage 2 in the Dutch key and approximates to a combination of stages 3 and 4 in the English key. The 'Universal' stage 'S' corresponds to a combination of stages 3, 4, 5 and 6 in the Dutch key and a combination of stages 5 and 6 in the English key. The 'Universal' stage 'O' is equivalent to stage 7 in the Dutch key and a combination of stages 7, 2 and some stage 3's in the English key. The timing of the gonad sampling is also important since it is only around the time of spawning when it is possible to ascertain most accurately the spawning fraction of the population. If sampling is undertaken too early before the start of spawning, particularly in the case of young fish, it might not be apparent which individuals are likely to reach full maturity status. Too late after the end of spawning and there is the possibility of confusing resting with immature ovaries.

Changing the existing sampling procedures for North Sea plaice maturity to the new 'Universal' scheme could cause problems similar to those encountered when trying to merge the Dutch and English databases and might disrupt the integrity of both the existing North Sea plaice maturity time series. To avoid this there would appear to be little option but to continue sampling using the existing methodologies. However, it might be feasible to run the two sampling schemes alongside each other for a while to inter-calibrate the new maturity key against the old ones. For new investigations, there are clear advantages to be had from implementing a unified gonad grading system from the onset, thereby providing scope for merging databases from different sources and even for inter-species comparisons. The 4-stage key of the 'Universal' scheme advocated at the Bergen meeting is certainly the simplest to apply. It should yield consistent results and will greatly reduce the obvious confusion that arises when different maturity keys are used, and will facilitate the analysis of temporal and regional variability in fish maturation across a range of investigations. In the 'Universal' key, early maturing fish are classed in the same stage as spent fish (stage 'O'). Though it may not be possible in all cases to distinguish between these two sub-stages by eye alone, unless some attempt is made to separate the two groups, the proportion of spawning and non-spawning individuals could become confounded. In such circumstances it might not be possible to get a reliable estimate of the spawning fraction, which is often the chief objective of the investigation.

If there is a need to incorporate greater detail into the maturity key, it should be done in such a way that the maturity data can subsequently be aggregated down into a condensed key where the maturity stages map to those of the 'Universal' key. In effect, the more complex key should

retain common stage boundaries with the Universal key, but with the option of splitting the main 'Universal' stages into sub-stages as required. In this way the facility for pooling maturity data from different sources into a common database for comparative studies is retained. For example, it might be feasible to split the 'Universal' stage O into spent and early maturing phases. It might also be desirable to split the 'Universal' stage M into early and late vitellogenic stage ovaries, though this might require additional histological screening to be achieved successfully.

For most studies, the 'Universal' approach for grading gonads, as recommended by the Bergen meeting, should be satisfactory. In terms of implementing a standard gonad grading system, of paramount importance is the need to decide on a common definition of what constitutes an immature fish. It is also important at the start of any new study to rigorously define each maturity stage and particularly the stage boundaries. It is likely that such definitions will vary depending on the biology of the sexual maturation process of the species under investigation. In fish such as male Dover sole, *Solea solea*, which do not run freely when stripped, it may be necessary to resort to dissection of the gonad to ascertain if the fish are mature or not. If the maturity key requires expansion, as might be necessary with commercially important fish species in order to ascertain more precisely the spawning fraction, the basic integrity of the 'Universal' key should be maintained by retaining the common maturity stage boundaries. Whether it is possible to expand the maturity key to more than the 4-stages of the 'Universal' key without recourse to histological screening should be ascertained at the onset of any new investigations.

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# An attempt to ascertain the spawning fraction of female North Sea whiting based on visual staging of maturity

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## ABSTRACT

This paper is based on the analysis of female whiting maturity data collected during North Sea International Bottom Trawl Surveys from 1991-1995. This provided an opportunity to investigate the sexual maturation of whiting throughout the North Sea on a seasonal basis. A standard protocol based on a 4-stage maturity key was used to sample the maturity stage of the whiting and the data were pooled into a common database. There was evidence that 1-year old female whiting were predominantly non-spawning adolescents, or else spawned only occasionally. The older whiting exhibited a protracted spawning season during the first half of the year. Because of this, and the relatively simple gonad grading key used to assess maturity, it proved difficult to obtain a reliable estimate of the spawning fraction of the population as a whole. A more sophisticated gonad grading system appears to be required to achieve that objective.

Key words - maturity, maturity ogive, spawning fraction, North Sea, whiting, *Merlangius merlangus*

## INTRODUCTION

Whiting (*Merlangius merlangus*) is one of the North Sea gadoid species, the abundance of which is annually assessed by ICES (ICES, 2001) to provide the EU with advice for managing the commercial fishery and the setting of annual Total Allowable Catch (TAC) quotas. Estimates of the total number of whiting in the North Sea stock are assessed annually using Virtual Population Analysis (VPA) and maturity ogives (the proportion of mature fish by age group) are applied to estimate the size of the spawning stock. Over a five year period during the early 1990's, whiting maturity data were collected during the International Bottom Trawl Surveys (IBTS) of the

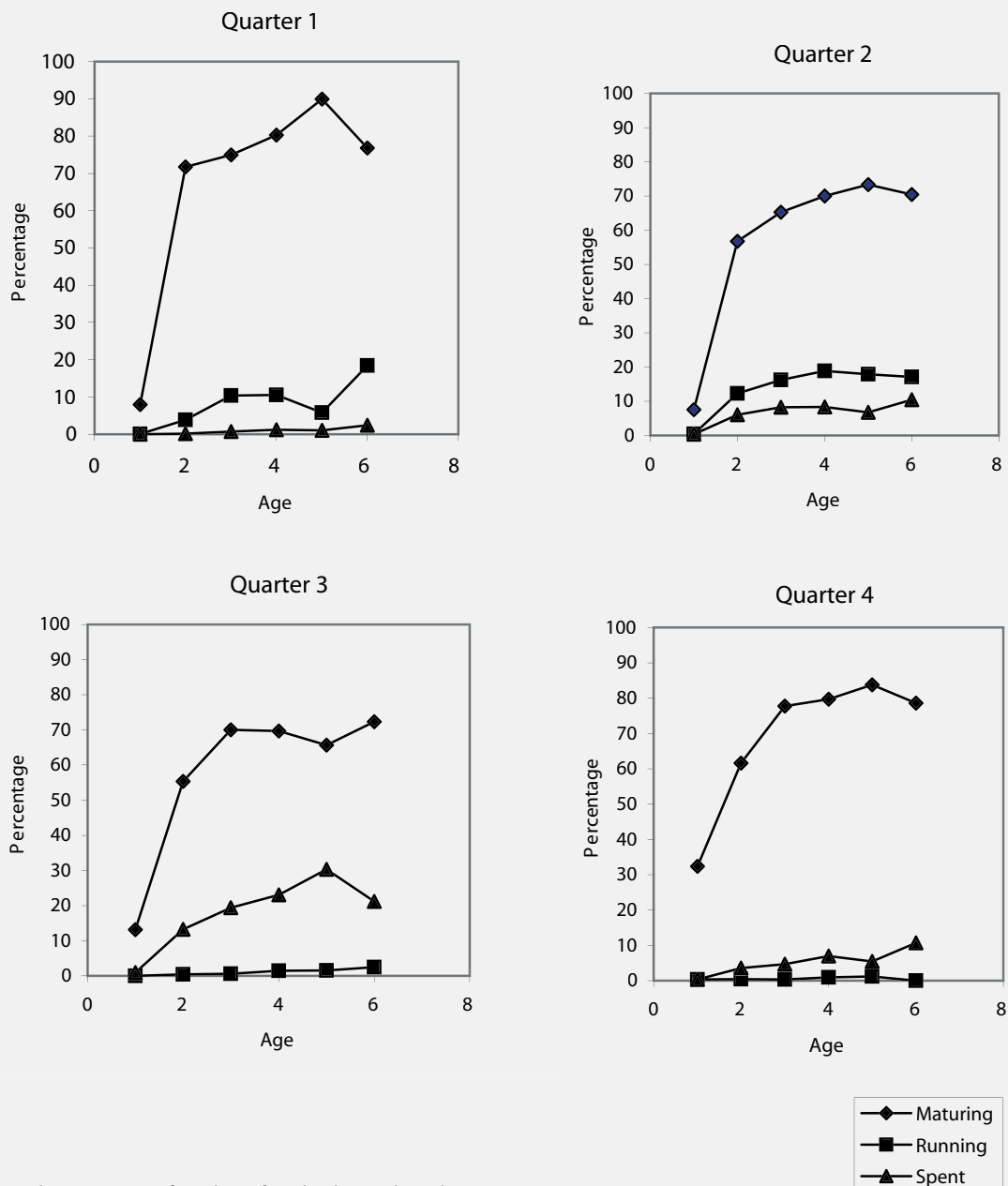
North Sea during all four quarters of each year using a standard sampling protocol. This provided an opportunity to investigate the sexual maturation of whiting throughout the North Sea on an annual and seasonal basis, with a view to providing estimates of the spawning fraction.

## MATERIALS AND METHODS

The whiting maturity data were collected during the IBTS of the North Sea from 1991-1995. The detailed materials and methods are described in the IBTS manual (ICES, 1992) and are only summarised here. The trawl surveys were conducted during all four quarters of each year by a number of nations within ICES. These included Denmark, England, Germany, Netherlands, Norway, Scotland and Sweden. The fish were caught mainly using a Grande Ouvriture Verticale (GOV) trawl with a cod end liner of 20 mm stretched mesh, in order to retain smaller fish than taken in the commercial fishery. Fishing was at pre-selected survey sites throughout the whole North Sea and the fish were sampled on a length-stratified basis. Typically, on each cruise, sampling was at the rate of 8 whiting per 1 cm length group in each of the seven ICES standard North Sea Roundfish Areas. Otoliths were taken for ageing. The whiting were sexed and the maturity stage of the ovaries was ascertained using a 4-stage key. This was chosen on the basis of its simplicity and ease of use, even by relatively inexperienced operators. The four maturity stages identified were immature (IM), maturing (MI), running with eggs (MA) and spent (SP). All participants used the same maturity key. Overall, the sampling regime was intended to provide a reasonably unbiased estimate of the proportions of immature, maturing, mature and spent

**Table 1.** Summary of the total numbers of female whiting sampled, the percentage of non-immature females and the ratio of running to maturing females (expressed as a %)

age	Quarter 1			Quarter 2		
	Number sampled	% non-immature	% running: maturing	Number sampled	% non-immature	% running: maturing
1	1611	8.1	1.6	1991	8.4	6.0
2	1463	75.7	5.3	1447	75.1	21.7
3	1142	86.1	13.9	1195	89.8	24.9
4	815	92.0	13.1	614	97.2	27.0
5	378	96.8	6.5	296	98.0	24.4



**Figure 1.** The percentage of North Sea female whiting classed a maturing (MI), running (MA) and spent (SP) on a quarter year basis using IBTS data from 1991-95.

whiting maturity for the North Sea as a whole. The aim was to attempt to assess the spawning fraction of North Sea whiting.

## RESULTS

Maturity data from 22000 female whiting aged 0-10 years were analysed. In the first half of the year, running females were distributed throughout the North Sea, except in the vicinity of the Dogger Bank, where there appeared to be an absence of spawning activity.

The maturity data for the female are summarised for each age group on a quarter year basis in Figure 1. Based on the proportion of running females, moderate spawning activity took place during the 1<sup>st</sup> quarter of the year, with spawning peaking in the 2<sup>nd</sup> quarter. The proportion of spent females peaked in the 3<sup>rd</sup> quarter, at which time the incidence of running females became sporadic and limited to the area west of 3° east. In the 4<sup>th</sup> quarter, running females were only occasionally found in the western North Sea, south of 58°N.

The percentage of females classed as non-immature (i.e. those fish which were in the process of maturing or else fully mature: MA, MI or SP), along with the ratio of running (MA) to maturing (MI) females during the first half of the year are shown in Table 1. For all age groups, the ratio of running:maturing fish (Table 1) was higher in the 2<sup>nd</sup> than the 1<sup>st</sup> quarter of the year, corresponding with the peak in spawning activity during April-June. In the 2<sup>nd</sup> quarter, the proportions of non-immature fish ranged from 8.4% in 1-year old females to 98% in 5-year olds. The ratio of running:maturing 1-year old females (Table 1) was 6%, which was significantly lower than for older fish (range 21.7-27%), suggesting that of the 1-year old females that initiated ovarian recrudescence, a substantial number were adolescents that either failed to reach spawning condition or produced fewer or smaller egg batches.

In older females during the 2<sup>nd</sup> quarter, up to 20% were running and 10% were spent, with the rest classified as immature or maturing (Figure 1). Based on the proportion of running and spent individuals in the population, therefore, it was only possible to be certain that 30% of females spawned. This is consistent with the data from the 3<sup>rd</sup> quarter, where the proportion of spent fish reached a maximum of 30%, apparently confirming the proportion of fish that could be definitely identified as having spawned. The majority of the females were classed as maturing, but, because of the limitations of the 4-stage key used to assess whiting maturity, it was not possible to ascertain with any degree of certainty what proportion of these fish would have reached spawning condition.

## DISCUSSION

Whiting spawning activity appears to be concentrated during the first half of the year, with peak activity in April-June. From July onwards, there may be sporadic localised spawning activity in the western North Sea, the contribution of which to the annual egg production of whiting is almost certainly negligible. Some information is known about the spawning biology of whiting (Hislop and Hall, 1974), but it is uncertain how many egg batches are produced in the wild and over what period. It is unclear, therefore, whether the protracted spawning season of whiting is due to individuals producing repeat batches of eggs over an extended time period, or whether there is variation in the time when individual females come into spawning condition.

The ICES Working Group currently assumes that 11% of 1-year old whiting spawn (sexes combined). In the present study, at best, only 8% of 1-year old females were classed as having initiated ovarian recrudescence. The scarcity of running 1-year old female whiting and the low ratio of spawning:maturing 1-year olds compared with the ratio for older whiting (Table 1) suggests that of those 1-year old females that were classed as maturing, a high proportion were probably adolescents that did not reach spawning condition or else only spawned occasionally or released only a few eggs per spawning, thereby making only a limited contribution to the egg production capacity of the stock. Similar conclusions have been drawn from market sampling of the commercial landings of 3-year old female North Sea plaice (Bromley, 2000) and 2-year old female sole (De Veen, 1970; 1976; Bromley, 2003). Bearing in mind that the 1-year olds make up about 60% of the whiting stock in the North Sea, even small errors in the spawning fraction are likely to have a big impact on the perceived size of the spawning stock biomass. During the 2<sup>nd</sup> quarter, 75.1% of 2-year old females whiting were classed as non-immature, which is lower than the 92% of 2-year olds currently assumed to spawn in the North Sea for assessment purposes (ICES, 2001). The ratio of running:maturing 2-year old females (21.7%) was also somewhat lower than for older fish (Table 1), suggesting the possibility that some 2-year old females might also be non- or reduced-spawning adolescents. Since the 2-year olds make up around a further 20% of the stock by numbers, again, errors in estimating the spawning fraction of these fish could also have a substantial impact on the reliability of the spawning stock biomass estimates. In the case of older whiting, the discrepancy between the estimated spawning fraction used for stock assessment by ICES and the proportion of non-immature fish identified from the IBTS data was less substantial.

The IBTS second quarter whiting maturity data only provides unequivocal confirmation that 30% of adult whiting

actually spawn, based on the proportion of fish sampled that were running or spent (Figure 1). This is almost certainly an underestimate of the actual spawning fraction. This is likely to be due to a combination of factors including the protracted spawning season of whiting, the lengthy quarter-year sampling interval, the simplicity of IBTS maturity key, and uncertainty over the duration of the spawning phase and what proportion of the time individuals remain in running condition. Only running individuals were classed as mature (MA), and individuals with well-developed ovaries containing advanced vitellogenic oocytes and hyaline eggs, and which were almost certain to spawn, were classified as maturing (MI). Such fish were lumped together with those that might have only just progressed beyond the immature phase and be unlikely to spawn. At the other end of the scale, any spent fish, having progressed to the spent-recovering phase, would also be liable to be classified as maturing. With such a simple gonad grading scheme it is not possible, therefore, to get a precise estimate of the spawning fraction. The alternative assumption, that all non-immature fish will spawn, is likely to overestimate the spawning fraction since there is evidence that amongst the younger age groups in particular, a substantial number of females might be non-spawning adolescents or only produce small or occasional egg batches. Based on the available information from the IBTS survey, therefore, it is only possible to conclude that in the case of adult whiting, the spawning fraction lies somewhere between 30-98%, probably nearer the latter than the former. The lack of precision is unfortunate since the wrong choice of maturity ogive might contribute to an inappropriate stock and recruitment relationship, which could adversely affect stock predictions and future management strategy (Rochet, 2000).

In recent years, there has been considerable effort devoted towards sampling the maturity of North Sea fish. At the same time, there has been a tendency to simplify the maturity keys used on fishing surveys to just a few maturity stages (ICES, 1992) that can be easily recognised by eye, even by relatively inexperienced operators. The present findings question the validity of this approach; particularly if the objective is to estimate the spawning fraction of the population. If the grading keys are too simple it becomes increasingly difficult both to quantify the spawning fraction and to arrive at meaningful confidence limits - and hence to reliably quantify spawning stock biomass. The situation could be improved by the introduction of a more detailed maturity key, preferably with at least some additional histological screening to provide a quality control check of the reliability of visual staging. In essence, if fewer fish were examined but the maturity stage was ascertained with more precision, the findings would be of greater utility for stock assessment purposes.

## ACKNOWLEDGEMENTS

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# Reproduction studies of Peruvian anchovy and hake – the 1997-1998 El Niño effect

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## INTRODUCTION

Fisheries are one of the main economic activities in Peru. During 2001 the total catch of fish was 6'284 793 metric tons, of which 88% and 1,42% corresponds to anchovy and hake, respectively, placing Peru as one of the main fishery countries of the world.

Species such as anchovy and hake are heavily exploited and have management plans that specify, among others things, the minimum size of capture, as well as the periods when it is necessary to close the fishing season in order to protect the spawning stock biomass.

To support these management plans it is important to consider how the reproductive cycle of hake and anchovy responds to changes in the environment. Of particular importance in this regard are direct measurements of reproductive rates, based on the fraction of females spawning per day (estimated from examination of histological sections of the ovary) and indirect measurements of the reproductive cycle, such as gonadosomatic index and fat content. These measurements provide a scientific basis for advising managers regarding the reproductive state of the stock. The present work describes the reproductive monitoring that is routinely carried out and presents some results on the effect of the 1997-1998 El Niño on anchovy and hake reproduction.

## MATERIAL AND METHODS

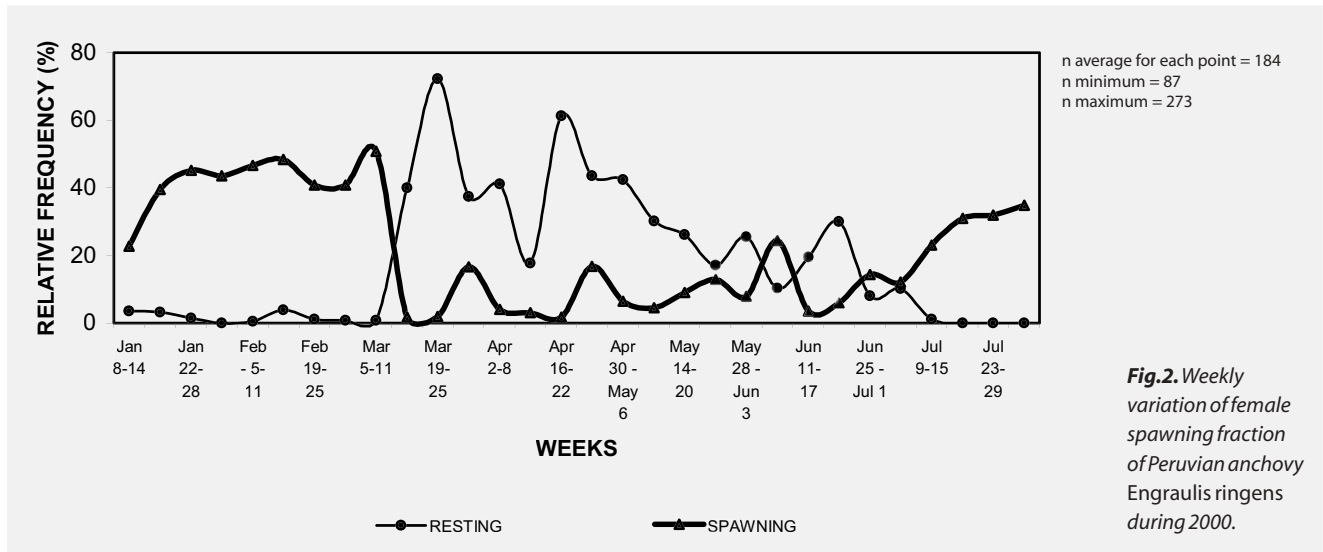
The samples of anchovy and hake were taken randomly and stratified by length from either research cruises or commercial fishery landings. Ovaries were processed either by infiltration in paraffin for wax histology or by freezing at

-29° C in a cryostat (Fig. 1) to prepare sections that were fixed and stained by haematoxyline and eosin. Both methods were used to produce slides that were scored for the presence of post ovulatory follicles to calculate spawning frequency, but when information is needed urgently, for an assessment, the faster cryostat approach has a major advantage. Both techniques were carried out in the laboratory as well as in the research vessel. The percentage of each maturity stage was calculated weekly (Fig. 2) or monthly and was weighted by the catch, giving the fraction of spawning (percentage of ovaries with post-ovulatory follicles and/or hydrated oocytes) and regressing females (ovaries with 50% or more of  $\alpha$ -atretic oocytes).

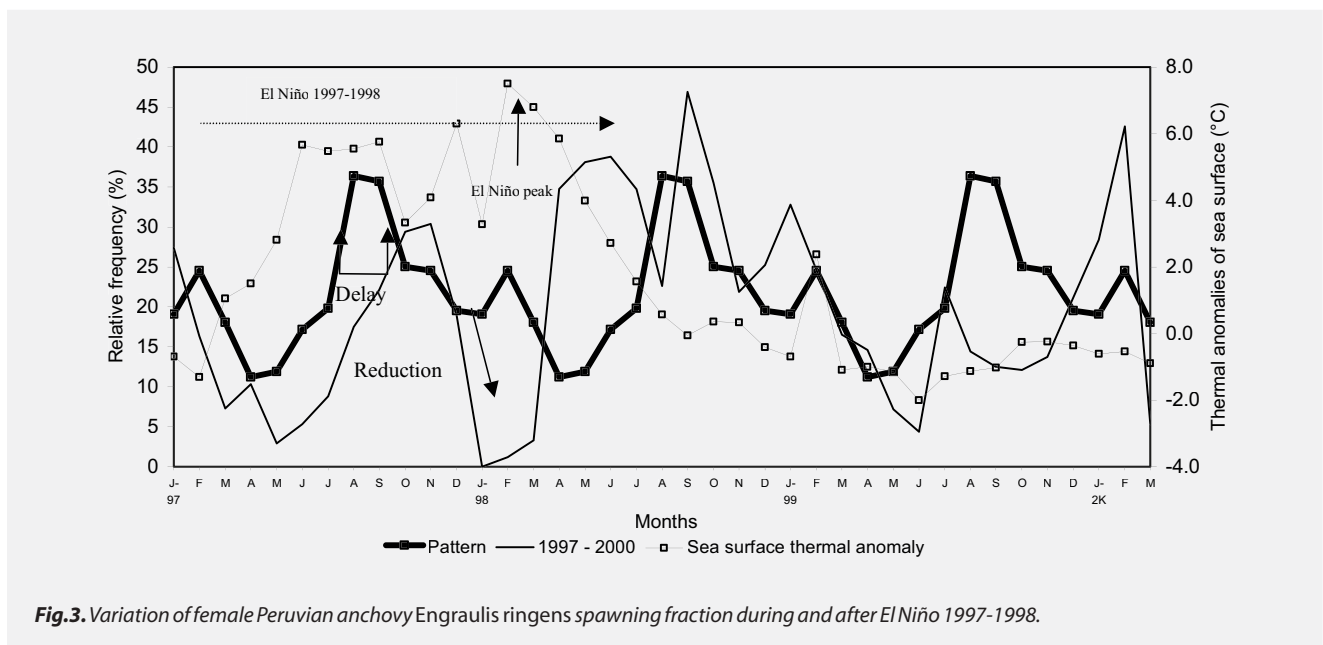
The annual cycle of lipid content in pelagic fish shows a decline during the spawning season due to the transfer of energy reserves for gonad development (Matthews 1960; Schulein 1971; Tsukayama 1989). We used this cycle as an indirect predictive tool to assess the duration of the annual spawning. The lipid extraction was done using isopropilic hexane (Krivobok and Tarkovskaya, 1964). The analysis considers two groups of individuals depending on their length and reproductive capacity: group 1 longer



**Fig.1.** Histological technique based on freezing, using cryostat.



**Fig.2.** Weekly variation of female spawning fraction of Peruvian anchovy *Engraulis ringens* during 2000.



**Fig.3.** Variation of female Peruvian anchovy *Engraulis ringens* spawning fraction during and after El Niño 1997-1998.

than 14,0 and group 2 shorter than 14,0 cm total length. Unfortunately, the fat analysis was suspended from 1987 until 1999, so no information exists for the 1997-1998 El Niño, which was catalogued as intense and characterized by sea-surface temperature anomalies from 2°C to 4°C.

Batch fecundity was estimated in anchovy using the hydrated oocyte method (Hunter et al., 1985) using hydrated females sampled on research cruisers carried out during each spawning season.

To calculate the size at first maturity of hake we used 70 individuals taken on the research cruise BIC *José Olaya Balandra* 9806-07. We estimated maturity as a function of length using microscopic criteria and expressed maturity as a cumulated frequency of mature females: mature ovaries were those with oocytes undergoing full vitellogenesis. The curve was fitted using the logistic model of Somerton (1980).

**RESULTS**

The ovaries of adult anchovy and hake were classified into 5 stages: resting (I), maturing (II), mature (III), spawning (IV) and regressing (V) using histological criteria (Buitrón et al, 1997; Perea et al, 1997). We consider stages III and IV as reproductively active females.

**Anchovy**

The reproductive cycle of anchovy shows a typical pattern of two spawning seasons, one in winter-spring (August-September) and another in the southern summer (February), with a characteristic resting period between April and May, as was described previously by Chirinos and Alegre (1969). However, during the 1997-1998 El Niño, Peruvian anchovy responded to the unfavorable environmental changes in three ways:

1. A reduction in the female spawning fraction: In Fig. 3 the monthly spawning fraction for 1992-1995 is com-

pared to the El Niño period and the recovery period from 1997 to 2000. In the winter-spring spawning period of 1997, the fraction of spawning females was lower for both the maximum value of 30,4% and the minimum (0%) compared to 36,4% and 12%, respectively, observed as a pattern from 1992-1995.

2. A displacement of the spawning period: Comparison of the fraction of spawning females observed during 1997 and 1998 to the pattern from 1992 to 1995, indicates a delay in the period of major spawning of 3 months from July-August until October and November in 1997 (Fig. 3). The shift in spawning coincides with the high sea surface temperatures, which had the greater positive thermal anomalies between June and September 1997 and between February and April 1998 (Vásquez and Tello 1998).
3. Batch fecundity declined markedly: Anchovy batch fecundity decreased by almost 22,0% compared to the previous years. Batch fecundity on average for a female with a weight of 21,7 g went from 16 634 oocytes per spawning batch in August-September 1996 to 12 976 oocytes per spawning batch in 1997 (Table 1).

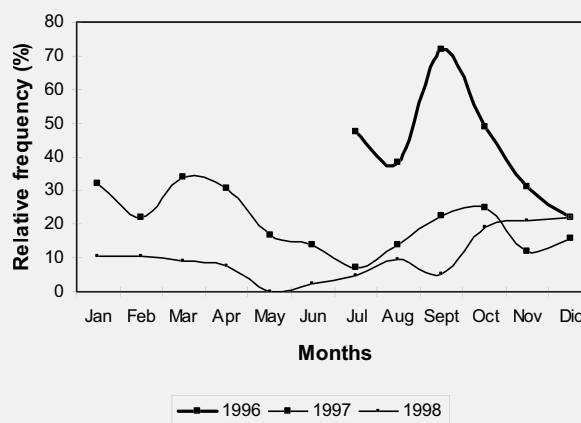
At the end of the 1997-1998 El Niño, the reproductive cycle of anchovy gradually returned to normal, with fecundity increasing to 15 792 oocytes per spawning batch (Table 1). Grouping the frequency of spawning females into two length classes indicates another possible effect of El Niño (Fig. 4). Spawning was dominated by the larger females (females equal or greater than 14.5 cm) during the 1997 El Niño year, while in the 1998 recovery period, the fractions for large and small females were similar.

**Hake**

The microscopic analysis of ovaries of hake shows that their reproductive cycle has two spawning seasons, a main season between July and August and a secondary one in January, as was already described by Canal (1989). The

**Table 1.** Batch fecundity by years for an average anchovy female of 26,74 g.

Year	Batch fecundity adjusted	Number of samples	SD
1981	15914	105	657
1994	13987	70	241
1996	16634	407	171
1997	12976	38	212
1999	15792	97	593
2000	15576	288	222

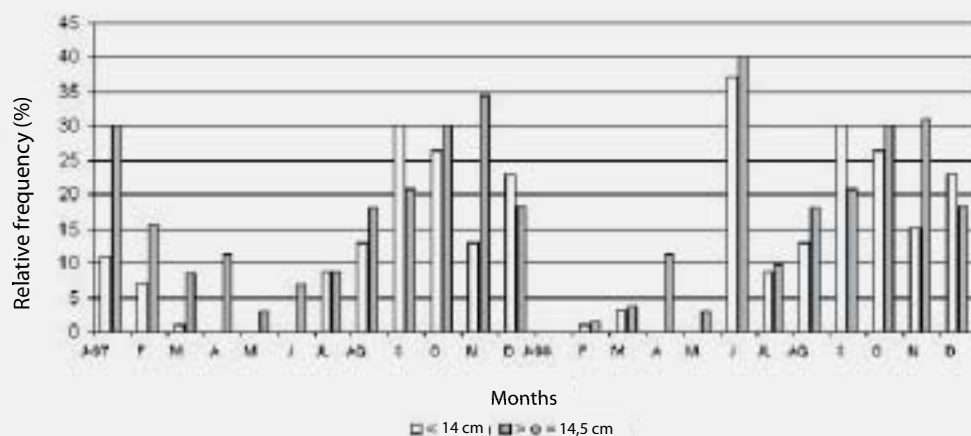


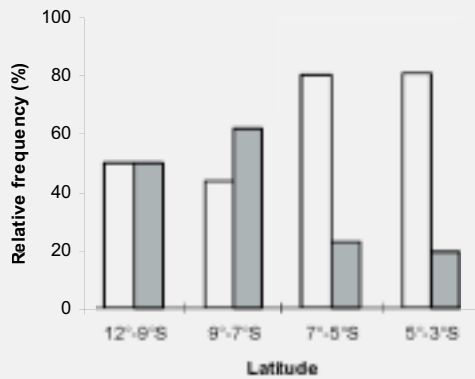
**Fig.5.** Reproductive activity of females of Peruvian hake, *Merluccius gayi peruanus*, from 03°S to 06°S.

1997-1998 El Niño effected the reproduction of Peruvian hake in three ways as follows:

1. Lower reproductive activity: During the 1997 El Niño the peak level of reproductive activity was lower than the previous year, but the spawning extended over a much longer period (Fig. 5).
2. Latitudinal variation of the reproductive state: Results

**Fig.4.** Relative frequency of spawning females of Peruvian anchovy *Engraulis ringens* between January 1997 and December 1998.

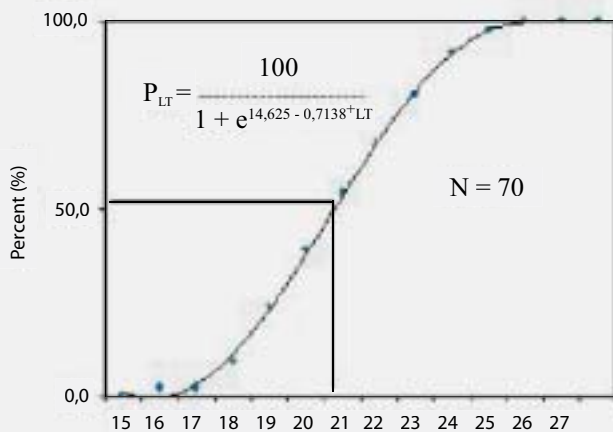




**Fig. 6.** Relative frequency of maturity stages of Peruvian hake *Merluccius gayi peruanus* females during May and June 1997.

obtained from a research cruise carried out in May and June 1997, during the 1997-1998 El Niño (Perea et al., 1997), showed two latitudinally different groups based on their spawning activity: One group located between 03°59'S and 06°59'S, was mainly reproductively inactive; whilst a second group situated between 07°00'S and 11°59'S had more advanced ovaries, with considerable higher frequency of mature spawning females (Fig. 6).

3. Early ripening: Between 09°S and 12° S smaller hake, ranging in size from 16 to 24 cm (mean length of 20,6 cm) total length, had a greater reproductive activity than those found to the north of 09°S. (Fig. 7).



**Fig. 7.** Length at first maturity of Peruvian hake *Merluccius gayi peruanus* females between 09°S and 12°S during May and June 1997.

## DISCUSSION

The 1997 to 1998 El Niño greatly perturbed the environment experienced by Peruvian anchovy. Ñiquen and Gutierrez (1998) recorded a drop in the quality and quantity of food available, which caused a reduction in individual fish weight of about 30%. Probably, the reproductive strategy adopted by anchovy during the 1997-1998 El Niño was to invest the scarce energetic resources in survival rather than spawning. A smaller percentage of adult females were reproductively active outside the usual periods, when environmental conditions were less unfavorable. Although at low levels, the productivity concurrently favored both spawning activity and improved larval survival as noted previously (Wootton, 1990). This also demonstrates the high plasticity of anchovy, which was strongly influenced by environmental changes (Alheit 1989). The decrease of batch fecundity caused by the 1982-83 El Niño has already been described by Arntz and Fahrbach (1996) and by Picquelle and Stauffer (1985) for *Engraulis mordax*.

The consequences of the 1997-1998 El Niño on the reproduction of anchovy, was that fewer adult females had the capacity to spawn, because of their poor condition. Some fish did spawn, but the timing of the reproductive cycle was shifted, so they spawned when the conditions of the environment were less unfavorable. Those fishes that were able to reproduce did so with a lower batch fecundity.

The fact that spawning was sustained by the larger females, raises some interesting energetic questions concerning anchovy reproduction in responses to short and long term environmental change. Laboratory experiments with captive individuals taken from the sea would help us to elucidate many of these aspects.

In the case of hake, only one previous study has been published on their length at first maturity, which was estimated at 27,3 cm (Canal, 1989) using histological examination during the spawning season. Compared with this, we have shown a reduction of the length at first maturity to 20,6 cm for individuals inside a specific area. Wosnitz-Mendo and Guevara-Carrasco (2000) indicate that early gonadal maturation in hake can increase natural mortality, because it diverts energy from growth and smaller fish have a reduced probability of survival. Potts and Wootton (1984) noted that an organism facing a stress it cannot avoid tends to have slower growth and switches to reproductive investment and lowers its length at first maturity. The latter authors note that change of age and length of maturity could be genetic or environmentally determined. The genotype controls the amount the organism can accommodate environmental change whilst the environmental regime elicits a response in the balance allocation in reproduc-



tion and growth. Trippel (1995) argues that shifts in length at maturity in many species in response to high levels of exploitation, are most likely to be a consequence of a mix of factors having both compensatory and genetic origins.

The observation of older individuals of Peruvian hake, located to the north of 6° 59' S had lower reproductive activity than individuals from the south was corroborated by Ayón and Aronés (1997), who observed larvae of hake in the samples of ichthyoplankton taken only southern to 07° S. Besides, Alamo and Espinoza (1997) differentiated two groups of hake with a distinct feeding spectrum in the north and to the south of 07° S. These observations support the hypothesis of the existence of two stocks of hake (Guevara-Carrasco and Wosnitza-Mendo 1997) that were discovered thanks to the unfavorable conditions of the environment. Genetics studies in the mitochondrial DNA level in the future will be able to test this hypothesis (Guevara-Carrasco and Wosnitza-Mendo 1997).

In all cases, the monitoring of the reproductive process and of the changes in the reproductive pattern of these species can be possible thanks to the application of precise methodologies such as microscopic analyses of ovaries.

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# Applications of Generalized Additive Models (GAMs) on recruitment data: a review

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## ABSTRACT

Traditional linear models are often inadequate to detect and quantify complex, non-linear interactions of environmental variables with biological factors. Generalized Additive Models (GAMs) offer an attractive possibility to overcome statistical problems linked to normality and linearity assumptions. GAMs extend the power of any conventional regression techniques by fitting nonparametric functions to estimate relationships between the response and the predictors. Therefore, GAMs have been used with an increasing frequency in the last decade as an exploratory tool to define complex interaction between variables of different origin. Quantifying the reproductive potential of marine fish stocks is one of the crucial step for improving assessment and management of exploited fish populations. Unfortunately, the indeterminate relationship between spawning stock biomass and recruitment (SR) has historically puzzled population modellers and impeded fisheries management. Recent research has shown that specific biotic factors, such as maturing-at-age, age/size population structure and maternal effect, may contribute significantly to explain recruitment variability in fish stock. Nevertheless, SR relationship is often complicated by highly variable environmental conditions (i.e. salinity, temperature, oxygen level, wind-induced flux and others). In this context, GAMs have been successfully used to model how cod recruitment is affected by population size (density-dependent effect), age population structure (maternal effect), different oceanographic variables and their interactions. For several cod stocks, results indicate that stock structure constitutes a crucial factor in explaining recruitment variability. However, abiotic factors are also shown to influence recruitment. Changes in temperature, oxygen, salinity, wind variables and sea-level atmospheric pressure are known to contribute significantly to explain recruitment variability of several fish species.

## INTRODUCTION

Generalized Additive Models (GAMs) were launched for the first time in 1990 (Hastie and Tibshirani 1990) and are currently available for example in S-PLUS computer program (version 2000, 1999 Statistical Sciences, Seattle, WA). In GAMs, the range of the relationships can be extended to curves and non-linear surface. However, as stressed by Daskalov (1999), the price of this larger flexibility is the limited possibility for statistical inference and the large number of degrees of freedom (i.e. parameters) used by the smoothing terms. These statistical inconveniences can be reduced using more parsimonious models (i.e. the minimum residual deviance with the minimum number of predictors). However, it is important to point out, as stressed by Hastie & Tibshirani (1990), that GAMs are not superior to Generalised Linear Models (GLMs). Instead, the user should be aware of the strengths and the weaknesses of GAMs using this tool as well as linear modelling within the frame of his/her overall research and then making use of particular tools to resolve specific problems (Daskalov 1999). GAMs have been used with an increasing frequency in the last decade as an exploratory tool to define complex interaction between variables of different origin. In particular, GAMs have been successful in fishery ecology (Table 1) to model the effects of oceanographic features and food density on spatial aggregation and abundance of pelagic (Maravelias 1997, Maravelias and Reid 1997, Maravelias 1999, Maravelias et al. 2000a and b) and demersal fish species (Swartzman et al. 1992, 1995).

Quantifying the reproductive potential of marine fish stocks represents a pivotal step for improving assess-

**Table 1.** Applications of GAMS to fisheries data.

Authors	Year	Application
Swartzman et al.	1992	Fish distribution & abiotic factors
Swartzman et al.	1994	Fish distribution & abiotic factors
Swartzman et al.	1995	Fish abundance & abiotic factors
Welch et al.	1995	Fish abundance & abiotic factors
Jacobson & MacCall	1995	Fish recruitment & biotic and abiotic factors
Borchers et al.	1997	Fish egg production
Stefansson & Palsson	1997	Fish feeding ecology
Maravelias	1997	Fish abundance and distribution & abiotic factors
Maravelias & Reid	1997	Fish abundance & biotic and abiotic factors
Augustin et al.	1998	Fish egg production
Stratoudakis et al.	1998	Fish egg spatial distribution
Bigelow et al.	1999	Fish abundance & abiotic factors
Daskalov	1999	Fish recruitment & biotic and abiotic factors
Maravelias	1999	Fish distribution & abiotic factors
Adlerstein & Welleman	2000	Fish feeding ecology
Faure et al.	2000	Cephalopod recruitment & abiotic factors
Fox et al.	2000	Fish egg spatial distribution
Maravelias et al.	2000a	Fish distribution & abiotic factors
Maravelias et al.	2000b	Fish distribution & biotic and abiotic factors
Cardinale & Arrhenius	2000a	Fish recruitment & biotic factors
Cardinale & Arrhenius	2000b	Fish recruitment & biotic and abiotic factors
Cardinale	2001	Fish recruitment & biotic and abiotic factors
Maury et al.	2001	Fish abundance & abiotic factors
Stoner et al.	2001	Fish distribution & abiotic factors

ment and management of exploited fish populations. Unfortunately, the indeterminate relationship between spawning stock biomass and recruitment (SR) has historically puzzled population modellers and impeded fisheries management. Historically, two basic stock-recruitment models have been developed (Hilborn & Walters 1992). The Beverton & Holt model assumes that the number of recruits increases with spawning biomass up to an asymptotic level. The Ricker model incorporates larger density-dependent effects (i.e. cannibalism) and therefore the number of recruits decreases at high level of spawning biomass. A central assumption in SR models is that the spawning biomass is proportional to the reproductive potential of the stock (Trippel et al. 1997). However, different authors have recently challenged this paradigm (Lowerre-Barbieri et al. 1998, Marteinsdottir & Thorarinsson 1998, Marshall et al. 1998, 1999, Scott et al. 1999). The depletion of large individuals may not only affect the quantitative reproductive potential of the population but, if poorer gamete quality is exhibited by younger fish as compared to older fish, the qualitative reproductive output of the stock may also be seriously depleted (Trippel et al. 1997).

Environmental factors (e.g., salinity, temperature, oxygen, wind-induced flux etc.) have long been recognised as important (Cushing 1982) and have recently been included in several models to explain recruitment variability of fish stocks (e.g. Daskalov 1999, Marshall et al. 2000, Jarre-Tiechmann et al. 2000; Sundby 2000). Specifically, GAMS have been recently applied to model recruitment of four Black Sea species (i.e. sprat, whiting, anchovy, horse mackerel) (Daskalov 1999), in particular how recruitment is affected by population size (density-dependent effect) and several abiotic variables. Moreover, GAMS have been used to model the contribution of first and repeated spawners along with several abiotic variables to recruitment in several cod stocks (Cardinale & Arrhenius 2000a). For Baltic cod, GAMS have been used to quantify the effect of age structure and its interaction with variable environmental conditions on recruitment (Cardinale & Arrhenius, 2000b, 2001). Here, I review the results from several GAMS modeling of recruitment data with special emphasis on my own results derived from applications on cod stocks.

## MATERIALS AND METHODS

### Fisheries data

I analysed fishery-dependent and -independent data of the Baltic, North Sea, Kattegat and North East Arctic cod stocks from the ICES (International Council for the Exploration of the Sea) annual stock assessment reports calculated with a Virtual Population Analysis (VPA) (ICES 1998, 1999a,b). Catch-at-age data from commercial landings were tuned with survey index of abundance estimated from trawl surveys. Natural mortality is set to 0.2 for all the age classes. VPA calculates past stock abundances for each year based on past catches. The stock size estimates, which include recruitment estimates for each year, can be used for stock and recruitment analysis. VPA, known also as cohort analysis, is one of the most powerful techniques available for the analysis of fisheries data and forms the heart of many current stock assessment methods where catch-at-age data are available (Hilborn & Walters 1992). For further details on mathematical calculations of a VPA-type model, see Hilborn & Walters (1992).

### Reproductive potential of cod stocks

To assess the contribution to cod recruitment of different age classes of the stock, I calculated their potential egg production. The total potential egg production for each age class was based on yearly estimates of stock numbers, proportion of mature individuals, sex ratios and weight-at-age in the stocks (ICES 1998, 1999a,b). Relative fecundity estimates (number of eggs/weight of fish ( $g^{-1}$ )) for North Atlantic and Baltic cod stocks were available from Kjesbu et al. (1996) and Kraus et al. (2000).

### Reproductive volume for the Baltic cod stock

Time-series of reproductive volume (the volume of water suitable for successful spawning) used in this study were from MacKenzie et al. (2000). The hydrographic data set consists of measurements from 36 different standard stations in the Baltic Sea (see MacKenzie et al. 2000 for details). The survey data were used to calculate the thickness of the reproductive layer of Baltic cod. Horizontal fields of the thickness of the reproductive layer were constructed by objective analysis (Bretherton et al. 1976), which is based on a standard statistical approach, the Gauss-Markov Theorem giving an expression for the least square error linear estimate of the variables. Thus, at every single point an estimate of the environmental conditions can be given, which depends linearly on the total number of measurements, i.e., a weighed sum of all observations (Bretherton et al. 1976). Therefore, the reproductive volume is calculated by a simple integration between two horizontal planes, whereby the upper is usually given by the 11 ‰ isohaline and the lower one by the bottom of the layer below which the oxygen content declines down to 2

$ml^{-1}$ . For each of the deep basin in the Baltic, data are available for February, March, April, May, August and October, except for the central Gotland basins, where estimates are available as the mean for the period February-May, May and August.

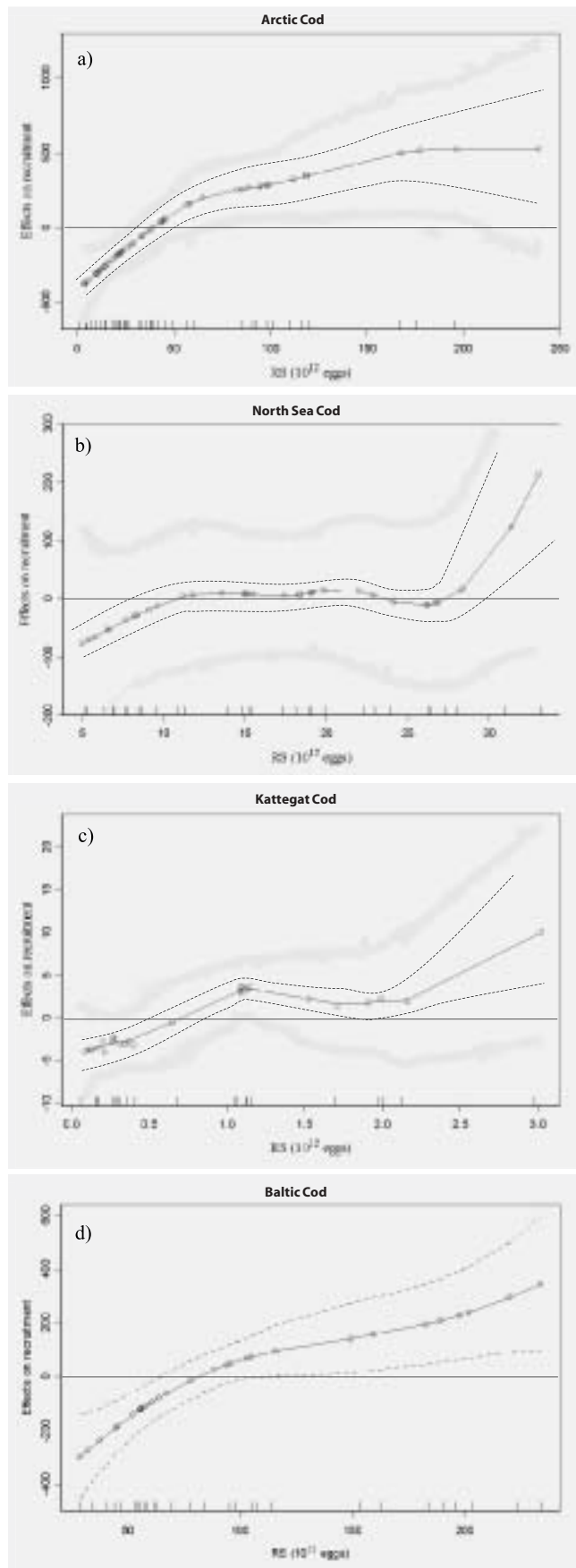
### Statistical analysis

#### *Generalized additive models (GAMs)*

Generalized Additive Models (GAMs) is a useful tool for exploratory analysis able to identify functional relationships suggested by the data alone, in cases where conventional linear methods have failed (Daskalov 1999). GAMs extend the power of any conventional regression techniques by fitting nonparametric functions to estimate relationships between the response and the predictors. The underlying probability distribution for the data can be any distribution from the exponential family, including the normal, Poisson, Gamma and binomial distributions (Swartzman et al. 1992). Here I used the normal distribution for North Sea, Kattegat and North-East Arctic cod stocks and the gamma distribution for Baltic cod. Gamma distribution was chosen for Baltic cod since when testing different distributions, we found out that the gamma gave better fit with the data and the minimum residual deviance in the models (Cardinale & Arrhenius 2000b).

In GAMs, the predictors, through additive, unspecified smooth functions affect dependent variable. Recruitment (dependent variable) was expressed as a sum of smooth functions of the predictors. The hypothesised predictors were: number of eggs produced by first spawners (FS), number of eggs produced by second spawners (SS), number of eggs produced by repeated spawners (RS), reproductive volume (RV) and their first-order interactions term. The age classes used in the analysis to distinguish between first, second and repeat spawners were depending on the age-at-maturity of different stocks. Recruitment was the number of age 1 or 2 individuals depending on the stock as defined by ICES (1998). Cubic *B*-spline algorithms were used to estimate the smooth functions (Hastie & Tibshirani 1990, Swartzman et al. 1992). The following two-step procedure was applied in analysing the data. First the functional relationship between the response and the predictors was explored using non-parametric GAM. In this way the form of the function was found empirically according to data without prior assumptions. Secondly, a more parsimonious model was tested and the final models were selected based on the following criteria:

- parsimonius principle (the largest amount of variance explained with the minimum number of predictors)
- analysis of residuals (non-violation of the normality and homogeneity assumption)
- biologically meaningful predictions by the model



**Figure 1.** Effects of repeated spawners (RS) egg potential production on recruitment of cod stocks: a) North East Arctic cod (Arctic cod); b) North Sea cod; c) Kattegat cod and d) Baltic cod. From Cardinale and Arrhenius (2000a and b).

Parsimony was evaluated using Akaike Information Criteria (AIC, Chambers & Hastie 1992). The AIC statistic accounts simultaneously for the degrees of freedom used and the goodness of the fit. More parsimonious models have a lower AIC. Confidence intervals (95%) and significance levels for the predictors were estimated using permutation test and bootstrap resampling (1000 samples) (percentile method) (the techniques are described in detail in Swartzman et al. (1992)).

GAM fits are illustrated using partial regression graphs showing the shape of the estimated relationship between the response variable (i.e. recruitment) and each of the significant covariates together with its approximate 95% confidence intervals. In our case, the 0-line indicates the mean recruitment estimated by the model while the y-axis is a relative scale where the effect of different values of the predictors on the response variable (i.e. recruitment) is showed. Thus, negative values on the y-axis indicate that, at those levels of the predictor (x-axis), the model estimates a recruitment that is lower than the mean value while the opposite holds at positive values on the y-axis. Spikes on the x-axis indicate the observed values of the response variable. Fewer points usually lead to larger confidence interval bands.

Residuals were analysed to test for departure from the model assumptions or other anomalies in the data or in the model fit using both analytical ( $Q_1$  statistic) (Kitanidis 1997) and graphical methods (Cleveland 1993). The residuals were tested for normality and for auto-correlation using the Durbin and Watson and the Shapiro and Wilk's test, respectively. Residuals were also plotted against the predicted values to test for their homogeneity.

Statistical analysis was performed with the S-PLUS software (version 2000, 1999 Statistical Sciences, Seattle, WA). The level of significance was set at 5% for the statistical tests used in this study.

## RESULTS

### Effect of age of spawners on recruitment of cod stocks

Results from the GAMs analysis for different cod stocks revealed a significant effect of age of spawners on recruitment for North-East Arctic, North Sea, Kattegat and Baltic cod. Noticeably, the effect of first spawners (FS) on recruitment was not significant in all the cod stocks. Instead, the second (SS) but mostly repeat spawners (RS) have a significant effect on recruitment (Fig. 1a-d). Importantly, when the number of eggs produced by repeat spawners is low, the recruitment is strongly depleted (negative effect on y-axis) while the effect on recruitment is positive at large values of

RS eggs in all the cod stocks. Therefore, it appears that the number of recruits are dependent on the population age structure, with second but mostly, repeat spawners providing both the largest amounts of recruits and thus likely the highest offspring survival rates.

#### Interaction between biotic and abiotic factors influencing recruitment of Baltic cod

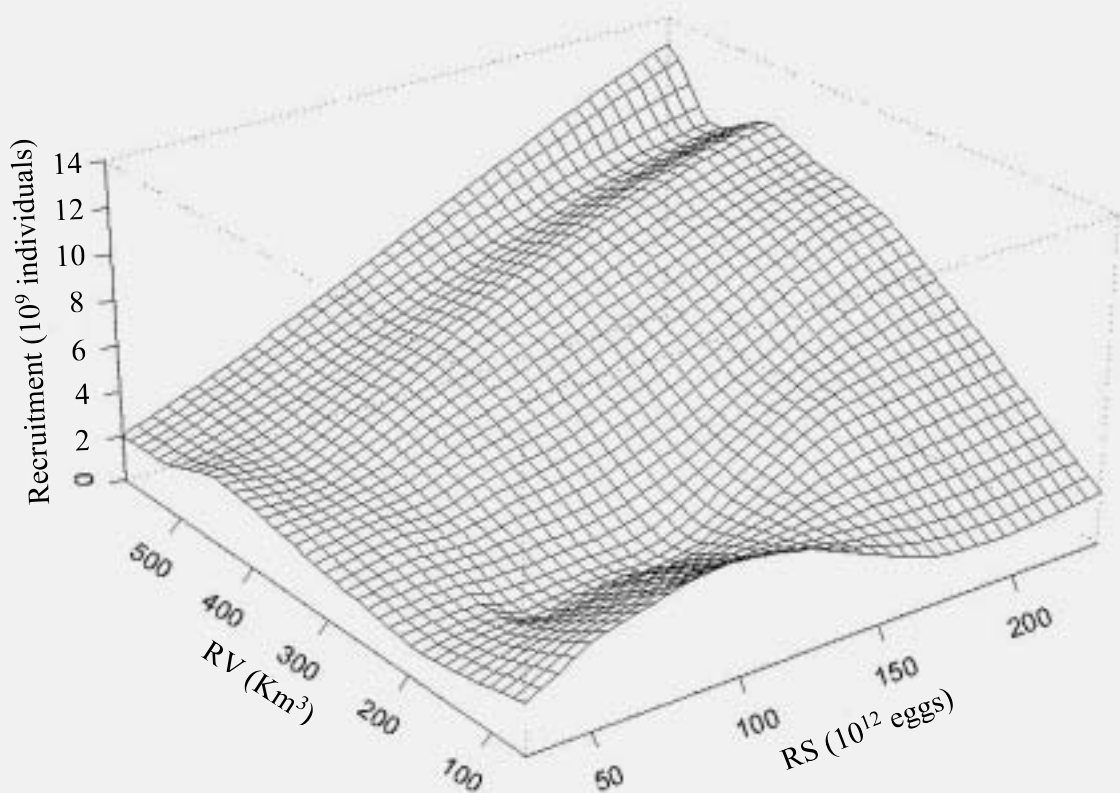
The repeat spawners (RS) and the interaction between RS and reproductive volume (RV) were the most significant explanatory variables, explaining around 70% of recruitment variability. The effect of first spawners (FS) was not significant and therefore excluded from the final model. As for North-East Arctic, North Sea and Kattegat cod, when the reproductive contribution from RS is at the lowest level, recruitment is strongly depleted, while recruitment of Baltic cod was largest at highest RS levels (Fig. 1d). Increase of the variation at larger values of RS was probably due to sparse data (Fig. 1d).

The selected GAM model for Baltic cod recruitment was used to make predictions at different combinations of RS and RV (i.e. the most important explanatory variables)

values using a matrix with 1600 simulated points from the models. Differently from GAMs plots, the values predicted here were estimated on the scale of the original response (i.e. recruitment of 1-year cod individuals). The largest recruitment was predicted at the combination of large values of both RS and RV (Fig. 2). However, at relatively low values of RV (i.e. sub-optimal environmental conditions for successful spawning of Baltic cod) the recruitment was moderately good when RS potential eggs production was high.

#### DISCUSSION

Results from GAMs modelling have stressed the importance of stock structure for the recruitment of several cod stocks (Cardinale & Arrhenius, 2000a,b) highlighting the presence of maternal effect (e.g. Marshall et al. 1998, Scott et al. 1999) on recruitment of cod. Moreover, the advantage of using GAM was that it was able to identify the quantitative effect of both biotic and abiotic factors and their complex, non-linear interactions on Baltic cod recruitment. It is well known that in the Baltic, in absence of water mass inflow from the North Sea, oxygen below the halocline progressively decreases to  $< 2 \text{ ml}\cdot\text{l}^{-1}$ , i.e., to concentra-



**Figure 2.** Recruitment predicted of Baltic cod at different values of repeat spawners (RS) potential egg production and reproductive volume (RV).

tions at which few Baltic cod eggs are unable to develop and hatch successfully (Nissling & Vallin 1996). In particular for Baltic cod, Vallin & Nissling (2000) have recently shown that egg buoyancy (i.e. the ability of eggs to float and thus avoid deep anoxic layers) is positively related to female size, stressing the importance of larger and older females on recruitment of this stock. Data from our study strongly support their hypothesis. In years with relative large anoxic layers (i.e. low values of RV), high buoyancy eggs from the older individuals give the largest contribution to recruitment. This implies that the impact of stock structure on Baltic cod recruitment would be even larger at very unfavourable environmental conditions. Those results were also in agreement with Jarre-Tiechmann et al. (2000).

Similar type of results were obtained by Daskalov (1999) analysing, using GAMS, the effect of several abiotic variables on recruitment of four fish species of the Black Sea. Significant correlation appeared between fish recruitment, stock biomass and physical environment. Patterns of the recruitment response to wind variables and sea level atmospheric pressure were found to be similar in all species. Again, GAMS were a suitable tool for fisheries and environmental data modeling, providing a flexible and powerful way to explore non-linear relationships (Daskalov 1999).

Several authors have recently stressed the fact that biomass-based Stock-Recruitment (SR) theory is poorly supported by empirical data (Marshall et al. 1998, Daskalov 1999, Cardinale & Arrhenius, 2000a,b). In particular, conventional approaches overestimate the reproductive potential of age-truncated populations assuming proportionality between spawning biomass and recruitment (Trippel 1998). This paradigm has been challenged (Gilbert 1997) and rejected for many cod stocks (Cardinale & Arrhenius, 2000a,b). It is now evident that the presence of a rich variety of age classes in the spawning population, increases the probability of successful cod recruitment (i.e. Marteinsdottir & Thorarinsson 1998, Cardinale & Arrhenius, 2000a,b). The contribution of older and larger individuals on recruitment is important due to both the production of larger larvae and the combination of more batches over an extended spawning period (Kjesbu et al. 1996, Trippel 1998). The larger size of those larvae is likely to increase their chance to survive (Kjesbu et al. 1996) while their presence along an extended period may increase the chance of matching favourable growing conditions (i.e. optimal feeding and environmental conditions) (Cushing 1982, Kjesbu et al. 1996). Therefore, current stock assessment increases, rather than decreases, the risk of commercial extinction of exploited marine fish by overestimating the reproductive potential of the stocks. This leads to a

dangerously optimistic view of the future status of the populations. Overestimation happens especially when populations are currently "truncated" by fishing mortality and the number of "high quality" individuals (i.e. repeat spawners) is below a certain limit. Thus, the subsistence of exploited fish stocks depends also on the maintenance of an adequate age population structure. Nevertheless, abiotic factors also influence recruitment. Temperature, oxygen, salinity and wind-induced fluxes together with other biotic factors as food availability and egg predation contribute significantly to recruitment variability of fish stocks (Kjesbu et al. 1991, Daskalov 1999, Jarre-Tiechmann et al. 2000, Vallin & Nissling 2000, Köster & Möllmann 2000; Sundby, 2000). In particular, it is now evident that the reproductive success of Black Sea fish stocks is highly dependent on the physical features of the marine environment (Daskalov 1999) and similar results have been obtained for Atlantic cod stocks (Sundby 2000). In Baltic cod, the combined impact of maternal effect and physical features of the environment on recruitment is likely to be even larger than in other oceanic areas. The peculiar environmental conditions (i.e. brackish waters) of the Baltic Sea strongly affect cod recruitment mainly via their effect on the buoyancy of the eggs (Vallin & Nissling 2000, Cardinale 2001).

The above results hint that to assure the persistence of exploited fish populations it is crucial to view the assessment of the stocks from a holistic perspective. For several decades fisheries biologists focused their attention on the taxonomy, life history and population dynamics of single species of fish. There have been attempts to bring together the species relationships into some integrated general picture of the ecosystem, but this incentive has never been strong enough (Beamish & Mahnken 1999). Several recent events have now provided this incentive. An important recognition is that climate impacts on population productivity must be understood for fisheries management (Cushing 1982, 1995). The lessons from recent fisheries management issues such as Northern cod (Cook et al. 1997) have highlighted that there are serious problems with previous concepts (single species management) and that it is cost effective to study marine ecosystems and manage and protect them as a whole (Beamish & Mahnken 1999). On the other hand, we should also be aware that fishing mortality is often, if not always, the main reason why fish stocks collapse (Hutchings & Myers 1994; Jonzén et al. 2000). Spectacular stock collapses and recent commercial extinction should provide policy-makers the core evidence that the collapse of marine resources is not due to environmental causes (Hutchings & Myers 1994). Nevertheless, the management of exploited fish stocks in the future cannot disregard the incorporation of the interaction between biotic and abiotic factors in the assessment of fisheries resources.



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# Estimating daily spawning fraction using the gonadosomatic index: application to three stocks of small pelagic fish from Chile

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## ABSTRACT

The spawning fraction of fish with multiple spawnings and indeterminate fecundity is usually estimated through time-consuming histological procedures, e.g. the proportion of females with post-ovulatory follicles of 1 day of age. Alternative methods based on macroscopic observations of the gonads are desirable. In previous works we showed theoretical and statistical arguments, which supported the use of the gonadosomatic index as a proxy of spawning fraction, when this fraction is conceptualized as an area under a normal distribution of females classified according to oocytes diameters in the more advanced batch. We applied our methods to *Sardinops sagax* from northern Chile, using a time series of gonadosomatic index that spanned from 1980 to 1996. In this work we combined our previous results with estimates of total abundance to produce a time series of total egg production during the period. This information has interesting ramifications into the area of basic populations dynamics, namely the study of the relation between stock and recruitment. Furthermore, here we apply our methods to two stocks of pelagic fish from central Chile, *Engraulis ringens* and *Strangomera bentincki*. The new approach yielded results which were in agreement with those from histological procedures. The use of our indirect approach seems to be an interesting low-cost alternative to estimate spawning fraction in fish with multiple spawnings and indeterminate fecundity.

## INTRODUCTION

How many eggs do stocks of fish with multiple spawnings and indeterminate fecundity produce on a given period, say a year? This question is highly relevant for population dynamics and fisheries biology since many fish stocks of commercial importance show indeterminate fecundity (Hunter *et al.*, 1985). For example, one of the major issues

in the field is the existence and nature of a relation between the abundance of spawners and recruitment (Hilborn and Walters, 1992). This issue can be clarified by considering total egg production, instead of spawning biomass, as best representing the true reproductive potential of a stock, and then search for its relationship with recruitment. This is not just a change of focus since spawning biomass and total egg production in fish with indeterminate fecundity are not expected to be proportional (Parrish *et al.*, 1986). The same case can also be presented for some determinate spawners. For instance, Marshall *et al.* (1998) observed that in the Northeast Arctic cod stock, *Gadus morhua*, total egg production is a better predictor of recruitment variation than spawning biomass.

So how can total annual egg production be measured in fishes with indeterminate fecundity? A direct method may include histological analyses of some daily samples of fish gonads to produce a sampling estimate of the proportion of females with post-ovulatory follicles of 1 day of age. This proportion can then be expanded to the female stock and the full period under consideration. The main problems with this approach are related to the effort involved in the histological procedures and the consequently low sample size for each daily proportion and number of days sampled in the period. Alternative methods based on indirect procedures and large sample sizes are therefore desirable. Claramunt and Roa (2001) developed theoretical and statistical arguments for a new method, previously outlined by Claramunt and Herrera (1994), which utilizes the gonadosomatic index to estimate annual spawning fraction, and applied it to *Sardinops sagax* from northern Chile. In this work we extend our previous results by com-

binning annual spawning fractions from *Sardinops sagax* with stock-assessment based estimates of population abundance to generate a time series of total egg production. Furthermore, aiming to show the potential of the approach to other fish stocks, we apply it in a tentative way to two additional stocks of pelagic fish from central Chile, *Engraulis ringens* and *Strangomera bentincki*.

**MATERIALS AND METHODS**

**Source of Information**

*Sardinops sagax*: The information used for developing and corroborate the method is outlined in Claramunt and Roa (2001). The historical database of gonadosomatic index (1974 to 1996) was provided by the Instituto de Fomento Pesquero, Valparaíso, Chile. Abundance of sardine came from results of sequential population analysis (SPA) (GTE: IFOP – IMARPE, 1998).

*Strangomera bentincki* and *Engraulis ringens*: Data came from sampling the catch of vessels from the Talcahuano fleet operating off central Chile (see Cubillos *et al.*, 1999 for details). There was no complete annual series of histological analyses available for any of the two species, so we pooled data from 1993 to 1997 to obtain monthly estimates of spawning fraction through the proportion of hydrated females per month.

**The Method and its Approximations**

Our view of the problem of determining the spawning fraction in fish with multiple spawning and indeterminate fecundity has several components, which are listed below (see Fig. 1):

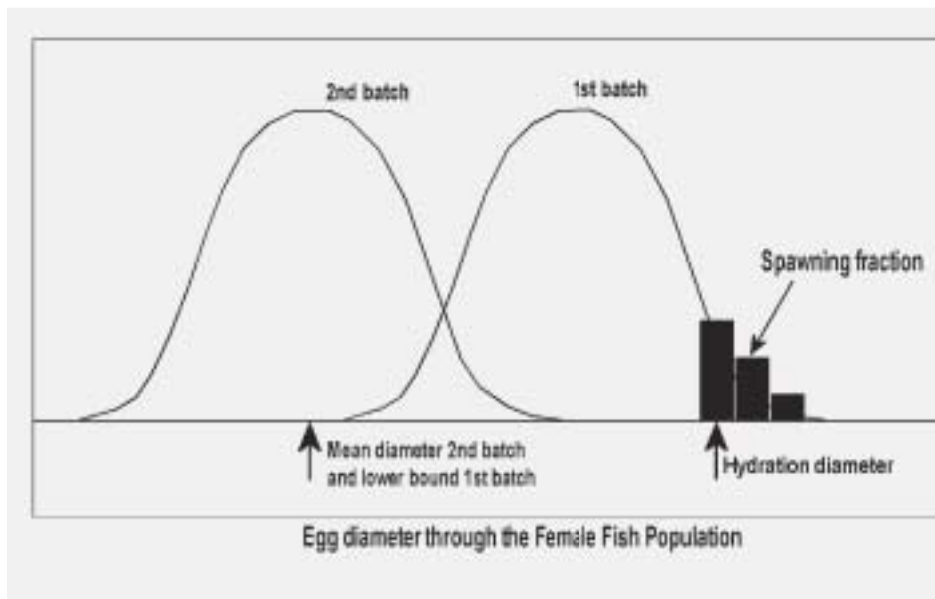
- 1) we conceptualize the daily spawning fraction as the daily proportion of females whose oocytes in the most

- advanced batch (hereafter 1<sup>st</sup> batch) present a diameter equal or higher than the hydration diameter;
- 2) the distribution of females classified according to mean oocyte diameter in the 1st batch forms a normal curve (see Claramunt and Herrera, 1994);
- 3) as a consequence of 1) and 2) we consider the daily spawning fraction to be an area to the right of the hydration parameter in a normal curve, an area whose size is determined by two unknown parameters, the mean and the standard deviation, and one known parameter, the hydration diameter;
- 4) next, by assuming certain known cutoff values for quantiles in the normal curve, we reduce the problem to one unknown parameter: the standard deviation;
- 5) subsequently, we fix the standard deviation parameter by calibrating the resulting proportion with that obtained from the same sample by the histological method;
- 6) finally, we expand to larger samples and for periods in which there are no histological analyses by using a linear relation between the standard deviation and the gonadosomatic index (defined as the ratio of ovary weight to body weight).

Explicitly, the daily spawning fraction  $F_t$  is considered to be

$$F_t = \frac{1}{\sqrt{2\pi\sigma_t^2}} \int_{X_t=H_t}^{\infty} \exp\left[-\frac{(X_t - \mu_t)^2}{2\sigma_t^2}\right] dX \quad (1)$$

where  $H_t$  is the hydration diameter ( $X_t = H_t$ ), and  $\mu_t$  and  $\sigma_t$  are the mean of the oocyte diameter distribution in the 1st batch across the female fish population and its standard



**Fig. 1.** Conceptualization of the daily spawning fraction as an area under a normal curve. Each female fish contributes one observation (oocyte diameter in her most advanced batch) to the frequency distribution. 1st batch: More advanced batch in the ovary, that at the end of maturation is hydrated and then spawned. 2nd batch: Oocytes that remains in the ovary after spawn.

deviation, and  $t$  indexes day. Thus, the integral is taken over the female fish population and it includes all those females whose eggs in the 1<sup>st</sup> batch are equal or higher in diameter than  $H_t$ . The integral operator is valid here because we are summing up though a continuous variable (oocyte diameter) though actual instances of the variable come in the form of discrete events (individual female fish). To reduce the number of unknowns from two to one, we can use some known quantiles along the normal distribution. In the whole population of females there is a hydration diameter of oocytes in the 1<sup>st</sup> batch  $H_t$ , and a mean diameter of oocytes in the 2<sup>nd</sup> batch (lower batch: oocytes that remain in the ovary after spawn and will conform the next batch). Assuming that the latter mean provides a minimum diameter for the oocytes in the 1<sup>st</sup> batch, a lower bound called  $l_t$ , and assuming additionally that the distance ( $d$ ) between  $H_t$  and  $l_t$  remains constant through the whole year (see Tascheri and Claramunt, 1996 and Plaza *et al.*, 2002 for *S. sagax*), we can drop the  $t$  subindex and write

$$l = H - d \quad (2)$$

We also know that 99% of the area of any normal curve is within  $3\sigma$  units to the left and right of the mean, so

$$\begin{aligned} l &= \mu - 3\sigma \\ \mu &= H - d + 3\sigma \end{aligned} \quad (3)$$

where the mean is now expressed as a function of the standard deviation given than the hydration parameter and the distance between it and the mean oocyte diameter in the 2<sup>nd</sup> batch are known. The proportion of hydrated females in the population in Eq. (1) becomes

$$F_t = \frac{1}{\sqrt{2\pi\sigma_t^2}} \int_{X_t=H_t}^{H_t-d+6\sigma_t} \exp\left[-\frac{(X_t - H_t + d - 3\sigma_t)^2}{2\sigma_t^2}\right] dX \quad (4)$$

The upper limit of integration is the mean  $\mu$  (Eq. 3) plus  $3\sigma$  units to the right. Under this formulation, fluctuations of the population spawning fractions remain solely dependent on the standard deviation of the oocyte diameter distribution in the 1<sup>st</sup> batch across the female fish population. In practical terms, this formulations is simplified by using  $d = 400 \mu\text{m}$  (Claramunt and Roa, 2001) and calculating the spawning fraction in a standardized form:

$$F_t = \frac{1}{\sqrt{2\pi\sigma_t^2}} \int_{400}^{\infty} \exp\left[-\frac{(400 - 3\sigma_t)^2}{2\sigma_t^2}\right] dX \quad (5)$$

in which the hydration diameter is not present, but its difference with the mean diameter of the lower (2<sup>nd</sup>) batch. This is an important simplification, because the hydration diameter may vary to some extent (e.g. Atlantic cod: Kjesbu *et al.*, 1996) while the difference may not (Fig. 1). An additional approximation that we found useful is to increase the window of observation from daily to monthly. This reduces resolution but increases sample size by pooling daily observations. In the next step the determination of the standard deviation is carried out by calibrating its value against histological observations by using numerical optimization methods (e.g. Newton-Raphson). Finally, it is observed that the monthly standard deviation parameters are linearly related to the corresponding gonadosomatic index observations from the same samples (see Claramunt and Roa, 2001, for details in *S. sagax*).

Applied to the historical database of *S. sagax* (1974 – 1996), the monthly averages of the gonadosomatic index were transformed into spawning fractions and then into number of spawning events per year. These results combined with female abundance (from age-structured sequential population analysis) and partial fecundity, allowed computation of total egg production per year.

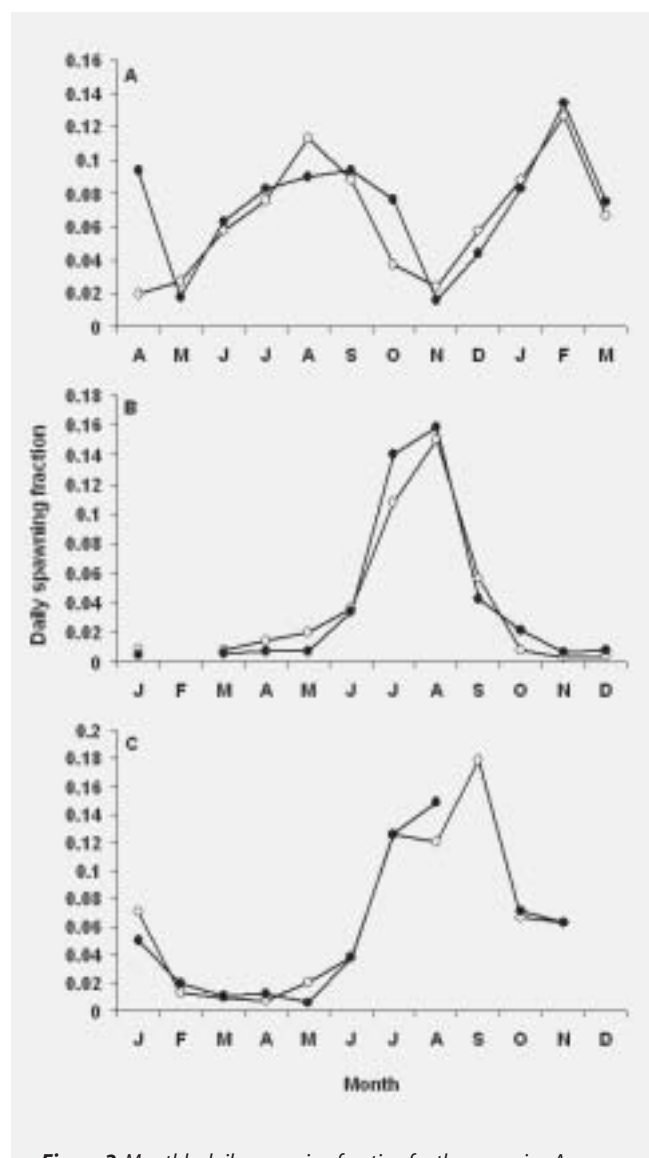
## RESULTS

The relationship between calibrated standard deviation (Table 1) and the gonadosomatic index show a clear positive linear relationship in the three species:

<i>S. sagax</i>	:	$\sigma = 64.257 + 4.806 \text{ GSI}$	$r^2 = 0.78$	$n = 11$
<i>S. bentincki</i>	:	$\sigma = 64.402 + 4.339 \text{ GSI}$	$r^2 = 0.88$	$n = 11$
<i>E. ringens</i>	:	$\sigma = 52.939 + 9.127 \text{ GSI}$	$r^2 = 0.88$	$n = 10$

The standard deviation obtained through these equations predict monthly spawning fraction values, which are very closed to the values obtained from direct methods (hydrated females proportion in *S. bentincki* and *E. ringens* and postovulatory follicles in *S. sagax*) (Table 1; Fig. 2). In addition, when regressing the monthly spawning fractions from both methods the three regressions have an intercept not significantly different from 0 and a slope not significantly different from 1.

Annual total egg production in *S. sagax* (Fig. 3) follows the same trend as female abundance at low stock levels (e.g.: 1988 – 1994), but the relation tends to break down at higher abundances (1977 - 1986).



**Figure 2.** Monthly daily spawning fraction for three species: A: *Sardinops sagax*. B: *Strangomera bentincki*. C: *Engraulis ringens*. Solid circles: Spawning fraction from direct methods, in *S. sagax* from postovulatory follicles, and in *S. bentincki* and *E. ringens* from proportion of hydrated females. Empty circles: Spawning fraction estimated through monthly averages of GSI. (Data of *S. sagax* from Claramunt and Roa, 2001)

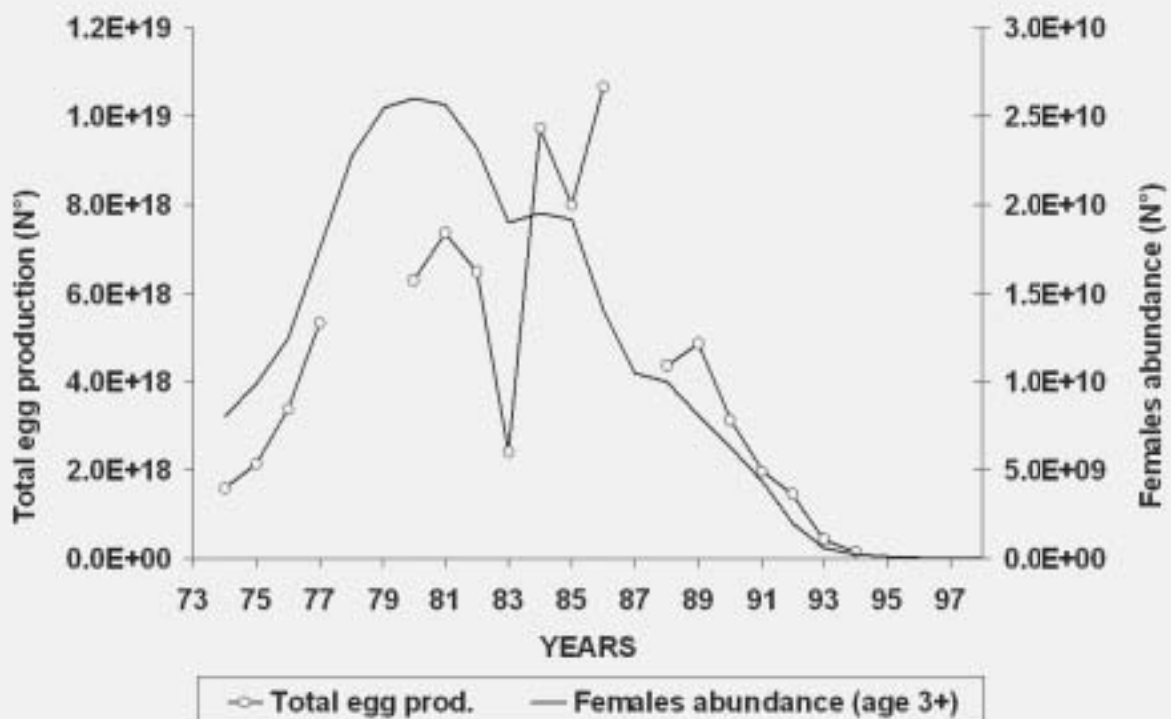
## DISCUSSION

The conceptual core of our approach to calculate spawning fraction is the view of the population of females, characterized by oocyte diameter in the first batch, as a normal distribution. On what conditions could this view represents what is going on in any particular stock of fish? Firstly, oocyte development and thus spawning need to be synchronized or otherwise there would be multiple modes in (first-batch) oocyte diameter distribution at any given time. Supporting evidence for *S. sagax* comes from the spawning frequency estimated by histology and atresia index (Herrera *et al.*, 1994): it shows the same temporal trend in fish from different size classes. This is consistent with the idea that the frequency of spawning among mature females is governed by a common biorhythm (Hunter and Lo, 1997) and/or environmental clues experienced by most mature females. Secondly, first-batch oocyte diameter must be sufficiently independent of fish body size or otherwise its distribution at any given moment would mimic that of the fish themselves, which most likely is non normal. Wallace and Selman (1981) have shown that the main determinant of oocyte diameter in teleost fish is the amount of yolk and the degree of hydration, and Claramunt *et al.* (1994) have shown that the amount of yolk is independent of body weight in *S. sagax* from northern Chile. This same argument may not apply for other fish species, including the *Strangomera bentincki* and *Engraulis ringens* stocks tentatively analyzed here. For example, as the length range increases there might be more room for variation in oocyte diameter, including a dependence on fish body size. We believe however that small departures from strict independence between (first-batch) oocyte diameter and fish body size shall not distort too much a supposedly normal (i.e. symmetric and unimodal) distribution as required by our approach. Nevertheless, we recommend that in using this method for other fish stocks authors present direct or indirect evidence supporting the condition of normality of oocyte diameter in the first batch across the female population.

A further important feature of our approach is the use of the gonadosomatic index as a proxy variable representing size-standardized gonad development (DeVlaming *et al.*, 1982; Hunter and Macewicz, 1985; West, 1990; Claramunt and Roa, 2001). Long time series of the index are available for many exploited fish stocks, like *S. bentincki* and *E. ringens* from central Chile as used in this work. Thus, the existence of a relation between the calibrated standard deviation of the normal distribution and the gonadosomatic index could prove useful in the construction of time series of annual egg production for fish with multiple spawning and indeterminate fecundity. It is necessary to assume a temporal constancy in the relevant parameters and to check that the index truly represents size-stand-

**Table 1.** Calculation of monthly spawning fraction for 3 small pelagic fishes from Chile. For *S. bentincki* and *E. ringens* the monthly values are averages from the period 1993 to 1997. GSI: gonadosomatic index;  $F_{POF}$ : spawning fraction of females with post-ovulatory follicles of 1 day of age as determined from histological analyses;  $F_{HYD}$ : spawning fraction of females estimated through the proportion of hydrated females;  $\sigma$ : standard deviation of the normal distribution of oocyte sizes in the most advanced batch from individual females as calibrated from the histological analyses;  $F_{GSI}$ : spawning fraction of females as determined from the linear relation between  $\sigma$  and GSI.

Month	<i>Sardinops sagax</i>					<i>Strangomera bentincki</i>					<i>Engraulis ringens</i>				
	<i>n</i>	GSI (%)	$F_{POF}$	$\sigma$ ( $\mu\text{m}$ )	$F_{GSI}$	<i>n</i>	GSI (%)	$F_{HYD}$	$\sigma$ ( $\mu\text{m}$ )	$F_{GSI}$	<i>n</i>	GSI (%)	$F_{HYD}$	$\sigma$ ( $\mu\text{m}$ )	$F_{GSI}$
April	1078	3.063	0.094	92.667	0.014	588	2.31	0.005	71.828	0.009	412	4.01	0.051	86.241	0.071
May	1072	3.527	0.018	78.478	0.021	421	1.74				443	2.58	0.020	79.121	0.013
June	1211	4.823	0.063	88.299	0.052	668	2.29	0.006	72.557	0.009	628	2.38	0.011	75.623	0.009
July	1134	5.388	0.083	91.216	0.072	517	2.96	0.008	73.786	0.015	547	2.27	0.013	76.375	0.008
August	1323	6.413	0.090	92.150	0.115	532	3.40	0.008	73.645	0.020	528	2.89	0.006	72.290	0.020
September	948	5.759	0.094	92.666	0.086	521	4.38	0.035	83.026	0.036	327	3.40	0.038	83.831	0.039
October	1133	4.029	0.076	90.242	0.031	756	6.90	0.140	98.054	0.108	394	4.77	0.126	96.533	0.126
November	656	3.337	0.016	77.756	0.018	586	8.00	0.159	100.005	0.150	268	4.70	0.149	99.014	0.120
December	659	4.818	0.044	84.997	0.052	468	5.26	0.043	84.749	0.056	368	5.38			0.179
January	214	5.754	0.083	91.217	0.086	510	2.26	0.022	79.643	0.008	439	3.93	0.072	89.647	0.066
February	805	6.718	0.134	97.378	0.130	434	1.35	0.007	73.234	0.004	255	3.87	0.063	88.220	0.062
March	868	5.132	0.075	90.099	0.063	350	1.53	0.009	74.301	0.004	128	4.91			0.137



**Figure 3.** Total egg production per year and females abundance of *Sardinops sagax* from northern Chile.

ardized gonad development. The type of reasoning and statistical results reported in Claramunt and Roa (2001) may serve as a proper justification of the use of the index. This method may also work for multiple, determinate batch spawners if there is synchronization in spawning and oocyte size does not depend strongly on fish body size, though it might be easier to apply traditional methods based on counting vitellogenic oocytes at the start of the reproductive season.

A final noteworthy feature in the approach showed here is the use of the second oocyte batch as a benchmark indicating a minimum bound in the normal distribution. Does the difference of this lower threshold with hydration diameter, which in the case of our application is 400  $\mu\text{m}$ , remain constant throughout the year as new spawning events take place? There is some evidence backing this assumption. Firstly, yolk incorporation into the oocytes begins at most at 200 - 250  $\mu\text{m}$  of diameter (Herrera and Claramunt, 1990; Macewicz *et al.*, 1996; Hay *et al.*, 1987; Tyler and Sumpter, 1996). Secondly, the immediately lower batch of oocytes is in the range of 200 to 450  $\mu\text{m}$  of diameter, which according to Claramunt and Herrera (1994) corresponds to the size range with a greater rate of growth. This indicates that intra-annual changes in oocyte size would result in parallel trends between lower and more advanced batches. Thus, the diameter difference between both batches would remain constant, in agreement with Tascheri and Claramunt (1996) and Plaza *et al.* (2002), whom found that the dry weight and average diameter of the lower batch follows the same seasonal trend as the more advanced batch in *S. sagax*.

In summary, the approach to estimate spawning fraction that has been developed through Claramunt and Herrera (1994) and Claramunt and Roa (2001), and which has been extended here and tentatively applied to two new fish stocks, could be used more generally because it conveniently utilizes a minimum of laboratory work. It also conveniently utilizes a maximum of information normally taken on a routine basis for many stocks of exploited fish, such as the gonadosomatic index. The principal applications that we foresee are to estimate annual egg production in fish with indeterminate fecundity in dynamic population studies. It must however be used with caution. Care must be taken to check the validity of its main assumptions and approximation for any particular application.

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# Atresia in Icelandic cod (*Gadus morhua* L.) prior to and during spawning

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## ABSTRACT

Using the stereological Disector method to quantify relative number of different types of oocytes in prespawning and spawning fish, this study presents new insight in vitellogenic oocyte recruitment and atresia regulation in relation to fish length and condition in Icelandic cod. It is demonstrated that the Atlantic cod is a determinate spawner and that atresia is a complex function of several factors including presently oocyte size, spawning period, and condition factor in the analysis.

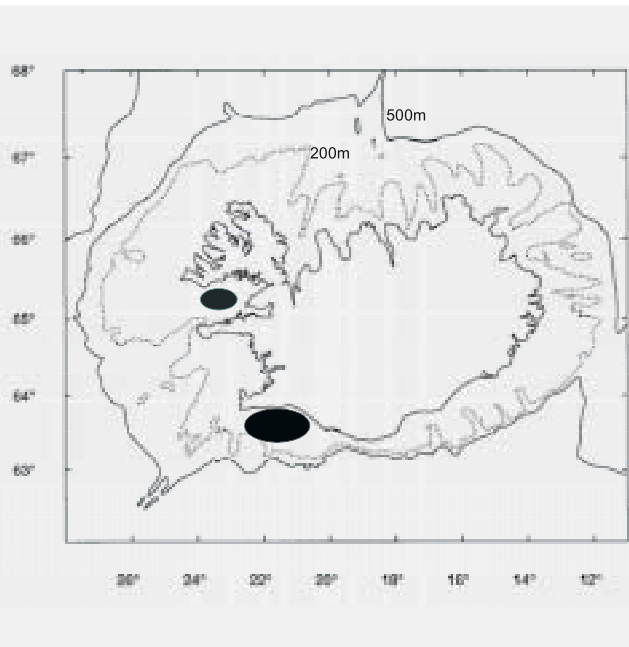
## INTRODUCTION

Atlantic cod (*Gadus morhua*) is a determinate spawner with a high potential fecundity (Kjesbu *et al.*, 1990). When fecundity is determinate, a definite number of previtellogenic (immature) oocytes start vitellogenesis (yolk uptake) for a period of time prior to spawning whereas no previtellogenic oocytes are assumed to enter vitellogenesis during the spawning season (for further information and discussion on this topic, see Hunter & Macewicz, this volume, and Kjesbu *et al.*, 1990; Greer Walker *et al.*, 1994; Witthames & Greer Walker, 1995; Kjesbu *et al.*, 1996a).

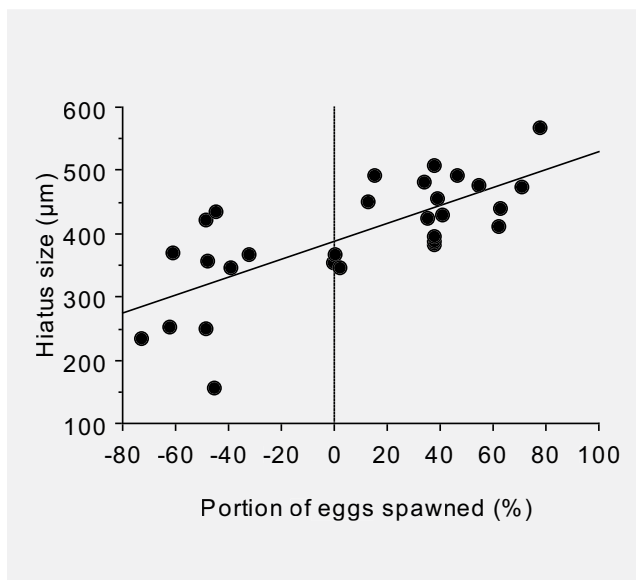
Fecundity in cod generally increases with maternal size, as larger females are more fecund per body weight, compared with smaller females (Hutchings & Myers, 1993; Kjesbu *et al.*, 1996b; Kraus *et al.*, 2000; Lambert *et al.*, 2000; Marteinsdóttir *et al.*, 2000; Marteinsdóttir & Begg, 2002). Though this is the general rule, size-specific fecundity can be highly variable from one year to another (Kjesbu *et al.*, 1998; Marshall *et al.*, 1998; Kraus *et al.*, 2000; Marteinsdóttir & Begg, 2002).

Under ideal circumstances potential fecundity may be approximately equivalent to the number of eggs spawned during the spawning season (i.e., realized fecundity). When environmental conditions are unfavorable some or all, developing oocytes included in the potential fecundity estimate may be lost through degeneration from the ovary, a process known as atresia (Kjesbu *et al.*, 1991; Tybjerg & Tomkiewicz, 1999). Atresia is a well-known process, seen in captivity and under natural conditions, both in fresh-water and marine fish (Htun-Han, 1978; Hunter & Macewicz, 1985; Trippel & Harvey, 1990; Kjesbu *et al.*, 1991; Greer Walker *et al.*, 1994; Karlou-Riga & Economidis, 1996; Ma *et al.*, 1998; Webb *et al.*, 1999; Witthames *et al.*, 2000).

To estimate the effects of fish size and condition on potential and realized fecundity in Icelandic cod, samples were collected on the spawning grounds southwest of Iceland and west of Iceland (Figure 1) prior to and during the spawning season. The proportion of maturing (vitellogenic) oocytes in relation to immature (previtellogenic) oocytes and intensity of vitellogenic oocyte undergoing atresia were estimated histologically and correlated with fish size and condition. The size frequency distribution of cod vitellogenic oocytes (VO) is known to change during the spawning season. As maturation advances the mean VO diameter increases, approaching the mean size of fully mature hydrated oocytes. At the same time, the corresponding standard deviation of mean VO diameter, decreases as the portion of total eggs spawned per season (PES) increases (Kjesbu *et al.*, 1990). This knowledge was used to calculate PES as well as relative time to onset of spawning (based on PES).



**Figure 1.** Main sampling areas (in black), southwest and west of Iceland



**Figure 2.** Relationship between portion of eggs spawned (PES) and hiatus size, prior to and throughout the spawning season (PES ≤ 0 and PES > 0, respectively).

Our first task was to affirm that Icelandic cod is a determinate spawner as has been earlier indicated for other Atlantic cod stocks (Kjesbu *et al.*, 1990). For this purpose two criteria were established. Our first criterion was that prior to spawning, we expect to see a gap (hiatus) between the size frequency distributions of the most advanced previtellogenic oocytes (PVO) and the size frequency distributions of the least advanced VO (Witthames & Greer Walker, 1995; Ma *et al.*, 1998). During the oocyte development we might also expect to see an increase in this hiatus as the VO increase in size during the spawning season, whereas the size of PVO should remain approximately constant. Our second criterion was that the relative number of VO in some way is linked to female condition prior to spawning, as condition might be a factor determining the total number of oocytes that start maturation each season (potential fecundity). After onset of spawning we expected to see a decline in the number of VO throughout the spawning season, as fully mature oocytes are gradually ovulated and no PVO are expected to enter vitellogenesis during spawning.

**MATERIAL AND METHODS**

We studied Histoiresin-sectioned oocytes (4 µm thickness, nucleus in section plane) stained with toluidine blue, measured by image-analysis (area transformed to diameter) and using the stereological Disector principle to estimate the relative number of the various types of cells (for more details on the Disector method, see Andersen, this volume).

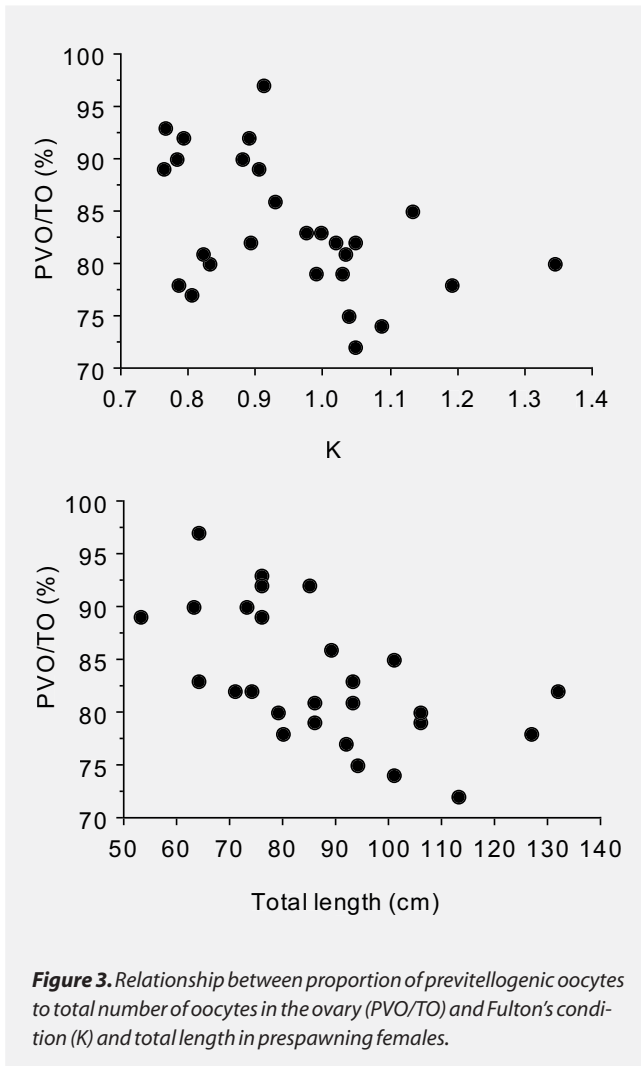
Number of females studied and their maturity status is given in Table 1.

**RESULTS AND DISCUSSION**

A notable hiatus (gap) between the size frequency distribution of PVO and VO was seen prior to spawning and increased as the spawning season advanced (Figure 2). This shows, based on the outlined definitions, that Icelandic cod is a determinate spawner, as has been indicated for the other cod stocks reported above.

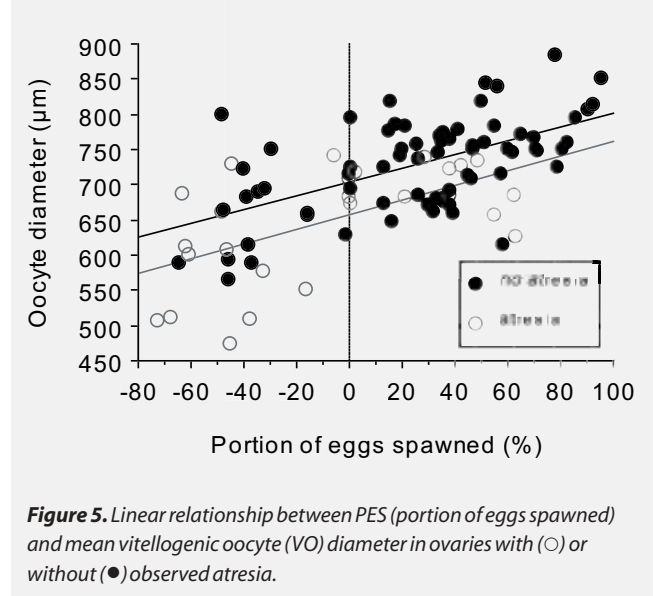
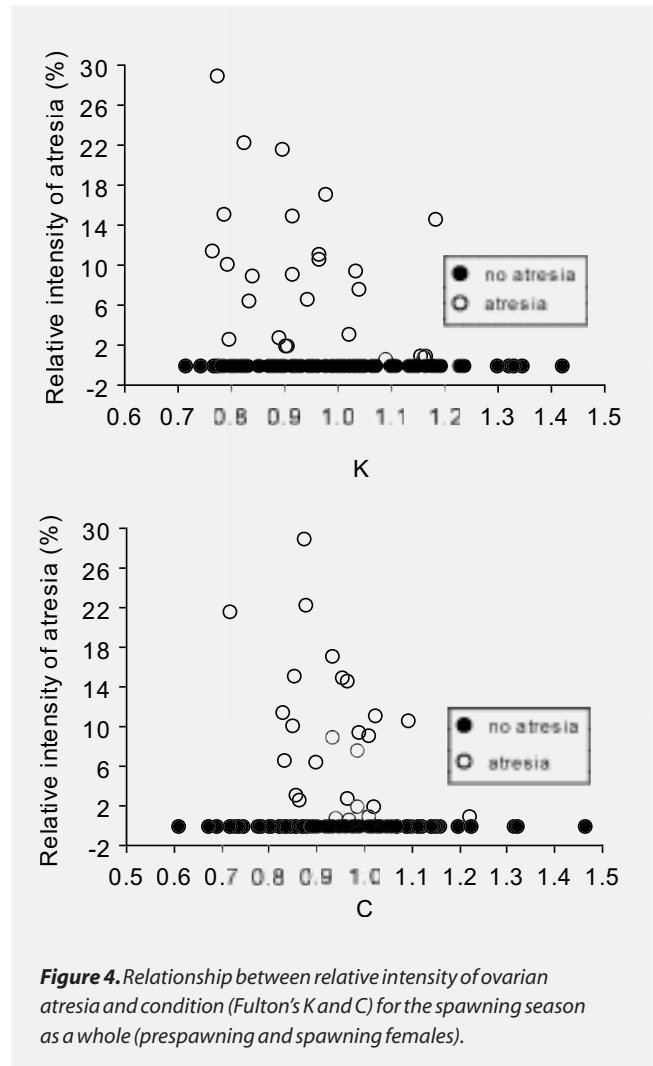
**Table 1.** Mean and standard deviation (in parentheses) of prevalence (fraction of the population) and relative intensity (number of atretic vitellogenic oocytes in relation to total number of atretic and normal vitellogenic oocytes, in percentage) of ovarian atresia in Icelandic cod females with observed atresia, in prespawning and spawning females.

Spawning status	No. of females	Prevalence	Relative intensity (± SD)
Prespawning	36	0.42	11 (8.0)
Spawning	70	0.16	8 (6.7)

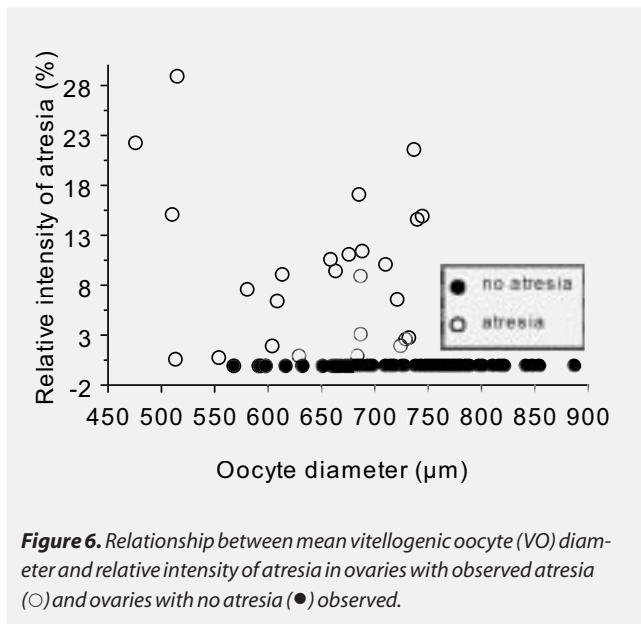


Fulton's condition factor (K), total length and whole body weight of females were found to be significant factors influencing the number of oocytes that start maturation prior to spawning (potential fecundity) (Figure 3). After onset of spawning a significant decrease was seen in number of VO as spawning proceeded, further implying that cod is a determinate spawner. Larger females in good condition showed a larger proportion of VO prior to and throughout the spawning season, indicating that these females are able to invest more in potential fecundity than smaller females.

To measure relative intensity of VO reabsorbed through atresia, 106 females were analyzed. Ovarian atresia was mainly detected prior to spawning, as 42% of the observed females contained atretic oocytes, while only 16% of the spawning females (Table 1). In prespawning females ovarian atresia was accelerated by low K, or relative condition factor, C (C = observed weight/expected weight given from a regression) (Scott *et al.*, unpubl.), and low liver index (Figure 4). No significant correlation was noted between K or C and liver index, and atresia after onset of spawning.



Atresia seems to be oocyte-size dependent as smaller and less mature oocytes were being reabsorbed from the ovary (Figure 5). In other words, being involved in hiatus enlargement. It might be advantageous to reabsorb energy from oocytes where less energy has been invested and from oocytes that might never reach the final maturation stage



**Figure 6.** Relationship between mean vitellogenic oocyte (VO) diameter and relative intensity of atresia in ovaries with observed atresia (○) and ovaries with no atresia (●) observed.

due to slow growth rate (Kjesbu *et al.*, 1991). The final maturation phase seems to be oocyte size related with a minimum of ca. 800 µm (Kjesbu *et al.*, 1996a). Atresia was not detected in ovaries where the mean diameter of vitellogenic oocytes exceeded about 750 µm (Figure 6) and in ovaries where estimated portion of eggs spawned (PES) exceeded about 65% (Figure 5).

As seen from above, fecundity of Atlantic cod is not a fixed pre-programmed process, but rather a flexible process adjusted to internal and external condition factors, that females are experiencing prior to and during spawning. Larger females and females in good condition, produce higher number of vitellogenic oocytes and a larger fraction of oocytes are developing to final maturation during vitellogenesis, leading to ovulation. This points out the importance of sustaining larger females in the spawning stock to maximize the spawning output of the stock as a whole. Even though larger females are producing larger fraction of vitellogenic oocytes, the condition seems to be an important factor determining the realized fecundity. Atresia seems mainly to be restricted to prespawning fish and in lesser extend to the first 2/3 of the spawning period. However, more work is obviously needed to better understand this temporal dynamics.

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# Improving the accuracy and precision of reproductive information used in fisheries

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## INTRODUCTION

Estimates of the onset of sexual maturity and annual or lifetime fecundity play a vital role in fishery science because they are closely linked to stock productivity. In addition, reproductive rates make possible the conversion of egg and larval abundance to adult biomass using egg or larval production methods. In this paper we discuss some of the potential biases and uncertainties in common methods used to estimate such reproductive rates and consider how these subjects could be treated in a manual on measurement of reproductive rates for fisheries.

## ANATOMICAL GRADING SYSTEMS

Anatomical grading systems are used in fisheries to measure size and age at first maturity, the duration of the spawning season, diel timing of reproduction, and under some circumstances spawning rates (Table 1). Many more precise methods exist to classify ovaries (histology, microscopic appearance, diameter of whole oocytes, and length specific gonad weight (West 1990)) than using a gross anatomical grading system. Although these more precise methods are preferred, gross anatomical grading systems will continue to be used in fisheries, regardless of their imprecision and biases, because they are an inexpensive way to routinely monitor the reproductive state of the catch.

Gross anatomical grading systems could be improved by reducing the number of classes, and focusing on the most reliable characters. The most reliable characters to describe an ovary are yolked oocytes present or absent, and hydrated (hyaline) oocytes present or absent. When yolked oocytes are first visible to the unaided eye they are opaque with the yolk filling at least half of the volume of the oocyte. The size

of the oocyte at which yolking is first visible varies depending upon the size of egg produced by the species. In species with small eggs, yolking may be visible in oocytes as small as 0.3 mm diameter (Nichol and Acuna 2001), and in teleosts with the typical 1 mm diameter egg, yolking is visible at about 0.4 mm diameter. Regardless of the differences in the onset of yolking among species, detection of yolked oocytes by the unaided eye, is a reliable marker for reproductively active females. Females, with yolked oocytes visible in their ovaries, are clearly capable of spawning within the current reproductive season or have already begun spawning. Postovulatory follicles often occur in ovaries in which only small but visibly yolked oocytes are seen. The presence of hydrated oocyte (hyaline or translucent oocyte) indicates imminent spawning and at temperate temperatures spawning may take place in less than 24 hours. Using the presence or absence of yolked oocytes, presence or absence of hydrated (hyaline) oocytes, one can accomplish all the primary functions of anatomical grading without the misleading complexities or ephemeral characters of the older systems. Certain reproductive states (such as spent, recovering, resting, partially spent, developing virgin,) can not be reliably detected using a cursory visual examination of a whole ovary so that adding such classifications provides no benefit. The "spent" stage is confusing because spent is undefined in most classification systems and could mean after one spawning or all spawning in a year. Spent characteristics (ovary flaccid, numerous blood vessels apparent, thick ovarian wall, and possibly remnant hydrated oocytes in lumen) may be useful for identification of the recent completion of the spawning season in cold water species ( $T \leq 10^\circ \text{C}$ ) where the condition persists for sufficient time to be reliably detected. If used to identify the end of spawning,

**Table 1.** Three classifications systems for maturity stages in fishes, with approximate correspondence between them.

From Kesteven (1960)	From Nikolsky (1963)	From Dickerson et al. (1992)
<p><b>I. Virgin</b> Very small sexual organs close under the vertebral column. Testes and ovaries transparent, colorless to grey. Eggs invisible to naked eye.</p>	<p><b>I. Immature</b> Young individuals which have not yet engaged in reproduction; gonads of very small size.</p>	<p><b>1</b> Virgin individuals. Very small sexual organs close under vertebral column. Females: often wine-colored, with torpedo-shaped ovaries. Eggs invisible to naked eye. Males: testes very small, knife-shaped, and quite thin. In chub mackerel testes can be longer than half the ventral cavity.</p>
<p><b>II. Maturing virgin</b> Testes and ovaries translucent, grey-red. Length half, or slightly more than half, the length of ventral cavity. Single eggs can be seen with magnifying glass.</p>	<p><b>II. Resting stage</b> Sexual products have not yet begun to develop; gonads of very small size; eggs not distinguishable to the naked eye.</p>	<p><b>2</b> Maturing virgins or recovering spents. Females: ovaries not longer than half the ventral cavity. Eggs may or may not be visible to naked eye. Males: tests easily identifiable, but still thin and knife-shaped.</p>
<p><b>III. Developing</b> Testes and ovaries opaque, reddish with blood capillaries. Occupy about half of ventral cavity. Eggs visible to the eye as whitish granular.</p>	<p><b>III. Maturation</b> Eggs distinguishable to the naked eye; a very rapid increase in weight of the gonad is in progress; testes change from transparent to a pale rose color.</p>	<p><b>3</b> Sexual organs swelling. Eggs definitely visible to naked eye. Ovaries and testes occupying about half the ventral cavity.</p>
<p><b>IV. Developing</b> Testes reddish-white. No milt-drops appear under pressure. Ovaries orange reddish. Eggs clearly discernible; opaque. Testes and ovaries occupy about two-thirds of ventral cavity.</p>		<p><b>4</b> Ovaries and testes filling nearly 2/3 of ventral cavity. Eggs still opaque. Testes swollen, milt whitish.</p>
<p><b>V. Gravid</b> Sexual organs filling ventral cavity. Testes white, drops of milt fall with pressure. Eggs completely round, some already translucent and ripe.</p>	<p><b>IV. Maturity</b> Sexual products ripe; gonad have achieved their maximum weight, but the sexual products are still not extruded when light pressure is applied.</p>	<p><b>5</b> Ovaries and testes filling ventral cavity. Ovaries often with some large transparent eggs.</p>
<p><b>VI. Spawning</b> Roe and milt run with slight pressure. Most eggs translucent with few opaque eggs left in ovary.</p>	<p><b>V. Reproduction</b> Sexual products are extruded in response to very light pressure on the belly; weight of the gonads decreases rapidly from the start of spawning to its completion.</p>	<p><b>6</b> Roe and milt running. Slight pressure on belly of fish exudes roe or milt.</p>
<p><b>VII. Spawning/spent</b> Not yet fully empty. No opaque eggs left in ovary.</p>		
<p><b>VIII. Spent</b> Testes and ovaries empty, red. A few eggs in the state of reabsorption.</p>	<p><b>VI. Spent condition</b> The sexual products have been discharged; genital aperture inflamed; gonads have the appearance of deflated sacs, the ovaries usually containing a few left-over eggs, and the testes some residual sperm.</p>	<p><b>2</b> Maturing virgins or recovering spents. Females: ovaries not longer than half the ventral cavity. Eggs may or may not be visible to naked eye. Males: tests easily identifiable, but still thin and knife-shaped.</p>
<p><b>II. Recovering spent</b> Testes and ovaries translucent, grey-red. Length half, or slightly more than half, the length of ventral cavity. Single eggs can be seen with magnifying glass.</p>	<p><b>II. Resting stage</b> Sexual products have been discharged; inflammation around the genital aperture has subsided; gonads of very small size, eggs not distinguishable to the naked eye.</p>	

investigators need to specify how the ovary differs from one taken after completion of a single spawning. In temperate and tropical species, the gross anatomical features used to characterize the “spent” condition are ephemeral, and can not be reliably detected. We advise the abandonment of this stage for temperate and tropical species.

The confounding of the first maturity (size or age when 50% are mature) of a species, and the annual spawning maturity that is attained each year by iteroparous species (Kock and Kellermann 1991), is a serious flaw in all classification systems (Table 2). No ovary classification system, including histological ones, can separate all females with regressed ovaries that have spawned in the current year (postovulatory follicles completely reabsorbed and no yolked oocytes) from females with inactive ovaries that have yet to attain their first maturity. Finding large individuals well past the size of first maturity with inactive or “immature” ovaries during the spawning season is not unusual. One may propose that such individuals have either refrained from spawning in the current year, or that the female matured, spawned, and regressed the ovary out of phase with most of the females in the stock. For example, in the Antarctic Neothenoïd fish (*Champscephalus gunnari*), large females with inactive ovaries were so numerous in the samples that they were believed to have refrained from spawning in the current year (Kock 1990). We used a detailed histological analysis of females, *Microstomus pacificus*, to determine the extent that histological characters could be used to improve the accuracy of first maturity estimates by detection of past spawning activities (Figure 1). The addition of criteria such as incidence of alpha and beta stages atresia improved the accuracy of the estimate, but the most striking feature of this analysis was, regardless

of the criteria, the size at 50% maturity was always larger if the measurement were made during the reproductive season rather than before it started (Table 3). This indicates that even with the best available histological criteria, the regressed ovaries of some postspawning females become indistinguishable from females defined as immature or inactive. The surest way to reduce this potential bias is to confine maturity samples to a period just before the onset of spawning for the stock, when postspawning females would be expected to be uncommon. This may be more easily discussed than done. A representative sample of a spawning population may be more difficult to obtain because of differential movements by the immature, spawning and post spawning components or their spatial separation. Thus it is necessary to understand the spatial and temporal characteristics of the reproductive season to develop an effective sampling strategy.

#### BATCH FECUNDITY METHOD

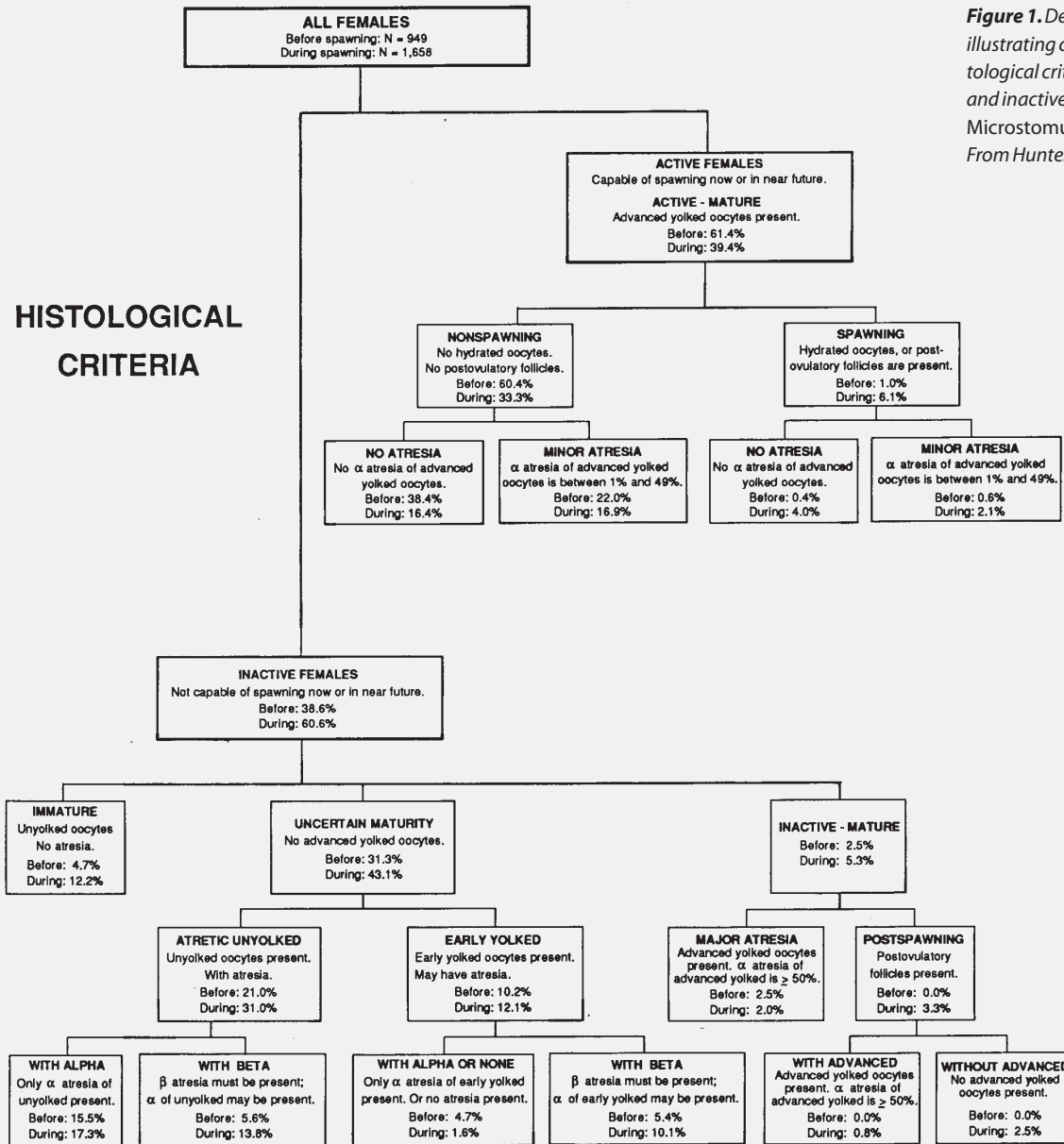
In the batch fecundity method, daily spawning rates are estimated by multiplying the batch fecundity (number of oocyte released in a single spawning) times the daily spawning fraction. Daily spawning fraction is the frequency of mature females spawning per day. The criteria or combination of criteria (advanced migratory-nucleus stage oocytes, or hydrated oocytes, or aged postovulatory follicles (POF)) selected to identify a spawning female depends on the peak time of daily spawning, the duration of the stage (possible temperature effects), and the hours of sampling. The batch fecundity method is usually used to estimate spawning biomass by applying the Daily Egg Production Method. Annual fecundity also may be estimated by measuring daily spawning rates throughout a season or by calibrating a proxy for spawning frequency based on oocyte size

**Table 2.** Comparison of California Department of Fish and Game anatomical maturity classification to histological classification of the ovaries of *S. japonicus* from Dickerson et al. 1992.

Number of Females	ANATOMICAL CLASSIFICATION		HISTOLOGICAL CLASSIFICATION			
	Stage Number	Fish Maturity	Immature %	Mature		
				Postspawn %	Nonspawn %	Spawn %
34	1	Immature	44	56	0	0
174	2	Mature	23	68	3	5
91	3	Mature	3	29	32	36
26	4	Mature		4	42	54
2	5	Mature		0	50	50
1	6	Mature		0	0	100
Fraction Mature:		294/328 = .90		270/328 = .82		

**Figure 1.** Dendrogram illustrating classes of histological criteria for active and inactive ovaries of *Microstomus pacificus*. From Hunter et al. 1992.

**HISTOLOGICAL CRITERIA**



**Table 3.** Estimated length at which 50% of Dover sole females are sexually mature, estimated using logistic model using six histological definitions of ovarian maturity before and during spawning.

Definition number	Histological criteria sets included in maturity definition <sup>a</sup>	Before spawning (854 females)	During spawning (1321 females)
		Length at 50% mature (mm)	Length at 50% mature (mm)
I	1	373	419
II	1, 2	361	396
III	1, 2, 3	348	391
IV	1, 2, 3, 4	332	348
V	1, 2, 3, 4, 5	258	255

<sup>a</sup> 1 Certain Maturity - Advanced yolked oocytes present  
 2 Maturity uncertain - Early yolked oocytes present with beta atresia  
 3 Maturity uncertain - Early yolked oocytes present with alpha atresia or none  
 4 Maturity uncertain - Only unyolked oocytes with beta atresia  
 5 Maturity uncertain - Only unyolked oocytes with atresia of unyolk  
 6 Certain immaturity - Only unyolked oocyte with no atresia

distribution, or gonad weight (Claramunt and Roa 2000). Bias in estimating spawning rates will occur if the ovarian criteria selected to identify a spawning event persist longer than 24 hours (any POF versus POF in a 24 hour age class such as 6-30 hrs old, or 18 to 41 hrs old, or 26-50 hrs old) or when the hydrated oocyte method is used and females with hydrated oocyte are more vulnerable to capture as seems to be the case for Peruvian anchovy (Alheit 1985). Care must also be taken, if one uses migratory-nucleus stage oocytes to measure batch fecundity, to be sure that all the oocytes that will become the batch have fully recruited into this stage (Macewicz and Hunter 1993). These are small but important details, but in the context of general fecundity issues, they seem minor. Overall if the standard procedures for estimating batch fecundity and spawning fraction (Hunter et al. 1985 and Hunter and Macewicz, 1985a) are followed, the chances for bias in using the batch fecundity method are quite low. It may be impractical to use the batch method in some applications because of the cost of obtaining a sufficient number of samples of spawning animals. Sometimes the batch fecundity method may be rejected because of the cost of placing observers on fishing boats to preserve ovaries of freshly caught specimens for histological analysis. This problem may be overcome if the catch is flash frozen at sea as it may be possible to use landed specimens for histological analysis under these conditions (Farley and Davis 1998). Normally landed specimens are not useful for histological analysis because of autolysis. The hydrated oocyte method for estimating spawning rates may be used under these circumstances.

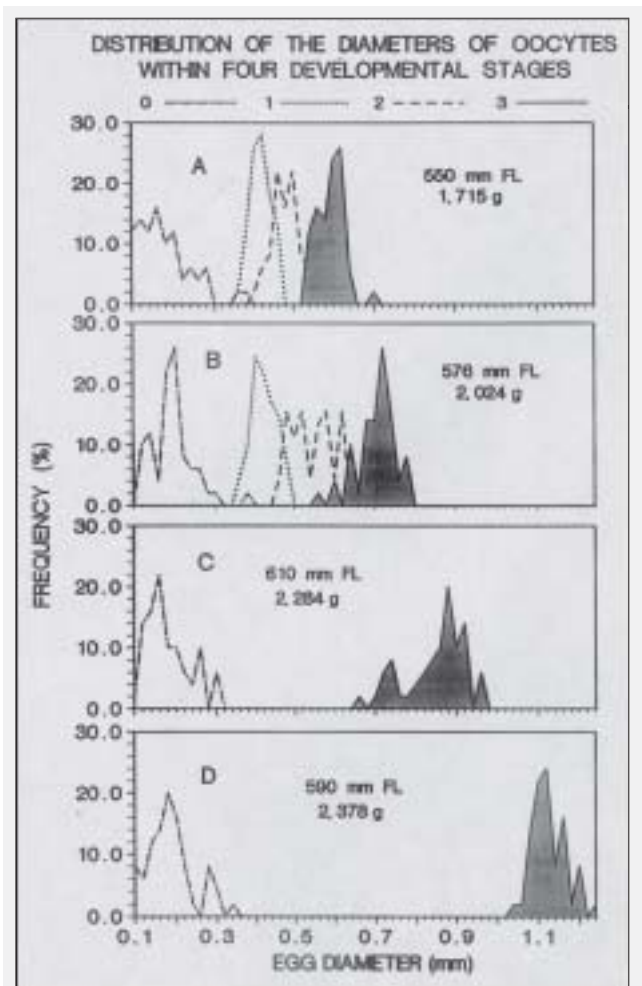
#### POTENTIAL ANNUAL FECUNDITY METHOD

Potential annual fecundity is used as a proxy for the actual fecundity realized by a female during an entire spawning season. In this method, a group of oocytes are identified that are believed to represent the maximum number of oocytes that could be spawned in a season. In semelparous animals such as market squid, all oocytes are included in the potential. This group of oocytes, or the potential annual fecundity, are counted at the beginning of the spawning season before there is a risk of oocyte losses due to spawning. Potential fecundity is used to estimate spawning biomass using the annual egg production, fecundity reduction, and egg deposition methods (Hunter and Lo 1993). Other applications include estimating escapement fecundity in squid (Macewicz et al. 2003), and annual or lifetime reproductive effort.

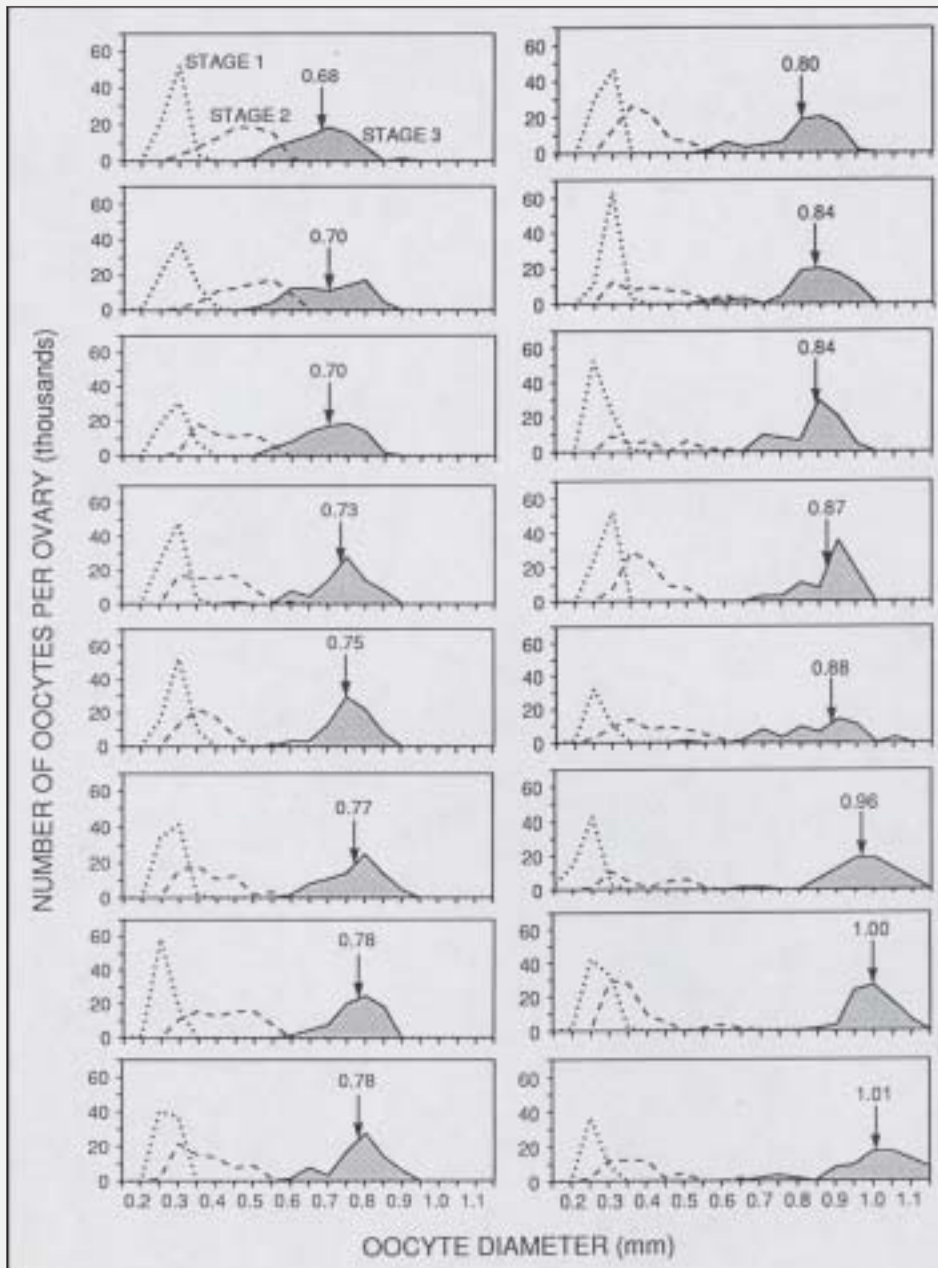
Potential fecundity is a valuable and widely used method but major uncertainties may exist. The method requires making three key assumptions which are infrequently validated: 1) one can identify a certain stock or size range of oocytes into which no new oocytes are recruited once spawning begins;

2) females used to estimate potential fecundity have not spawned; and 3) atretic losses are negligible. Clearly, for most fisheries applications potential fecundity is of value only to the extent that it is an accurate proxy for actual annual fecundity. We discuss the assumptions below.

*Identification of the stock:* In fishes that produce large eggs, a wide gap in the oocyte frequency distribution often occurs between the stock of advanced oocytes considered to be the potential fecundity and the rest of the smaller oocytes in the ovary (Figures 2 and 3). As the advanced stock continues to develop during the spawning season this gap typically widens and the average size of oocytes in the stock increases. As the gap may not be extensive at the onset of spawning, it is best to test for the recruitment of oocytes into the advanced stock of eggs considered to be the potential fecundity, in order to be sure that all oocytes have been fully recruited. We have done this by relating average oocyte diameter to



**Figure 2.** The frequency distribution of the diameter of oocytes within four oocyte stages based on the apparent yolk concentration under a dissection microscope for *Anoplopoma fimbria*. Stages are 0, no yolk; 1, initial layer of yolk along periphery of the oocyte; 2, yolk extending from periphery to the nucleus; 3 (shaded area) yolk so dense nucleus is indistinct or occluded (advanced yolked oocyte). Each panel represents one female. From Hunter et al. 1989.



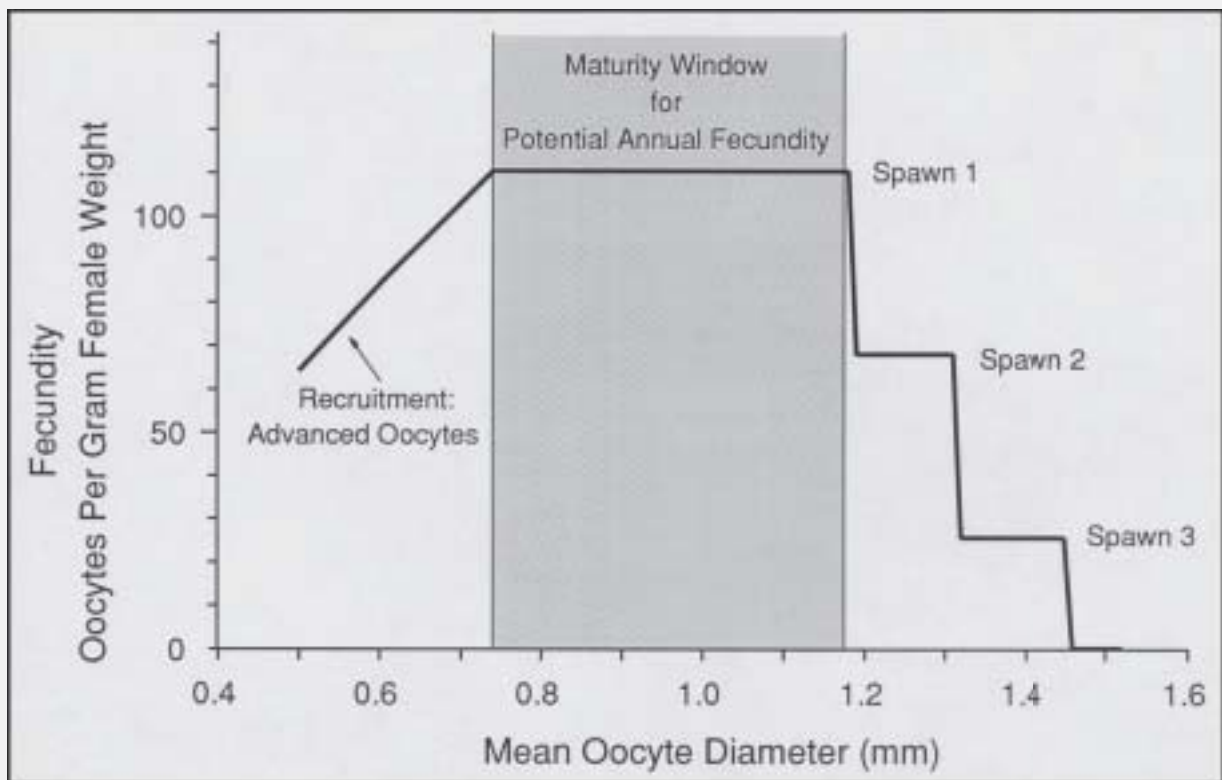
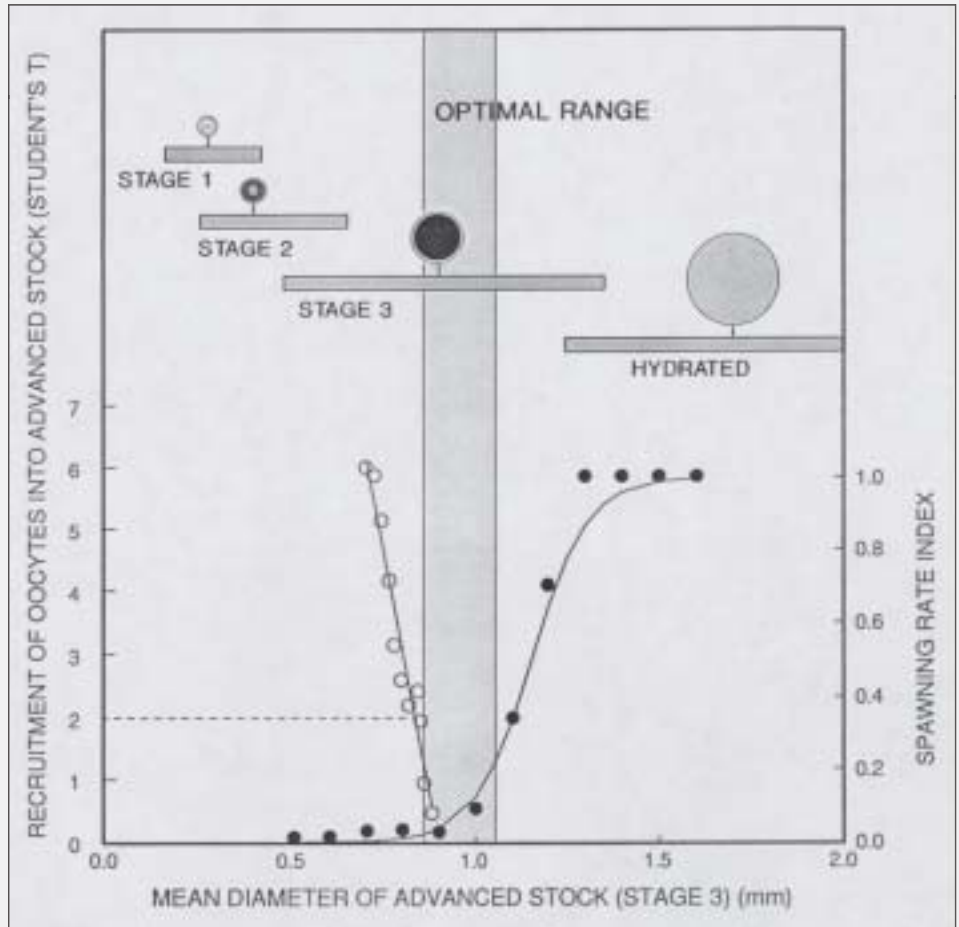
**Figure 3.** The frequency distribution of the diameter of oocytes within three maturity stages (based on the apparent yolk concentration under a dissection microscope) for *Microstomus pacificus*. Stage 1 is initial layer of yolk along periphery of oocyte; stage 2 is yolk visible from periphery to the nucleus but nucleus is distinctly visible; and stage 3 is advanced yolked oocytes where the nucleus is indistinct or occluded by yolk. Numbers are mean size of stage 3 oocytes. Each panel represents one female. From Hunter et al. 1992.

potential fecundity using multiple regression (Figure 4). When no significant positive correlation exists between the average size of oocytes in potential fecundity and the number of oocyte it contains, one can assume that the stock has been fully recruited. We are not entirely satisfied with this regression approach, but it is the best test we have been able to devise; this is an area needing further work. In sum, there is a window of opportunity for sampling females for a potential fecundity where risks due to incomplete recruitment and risks due to spawning are minimized (Figure 5).

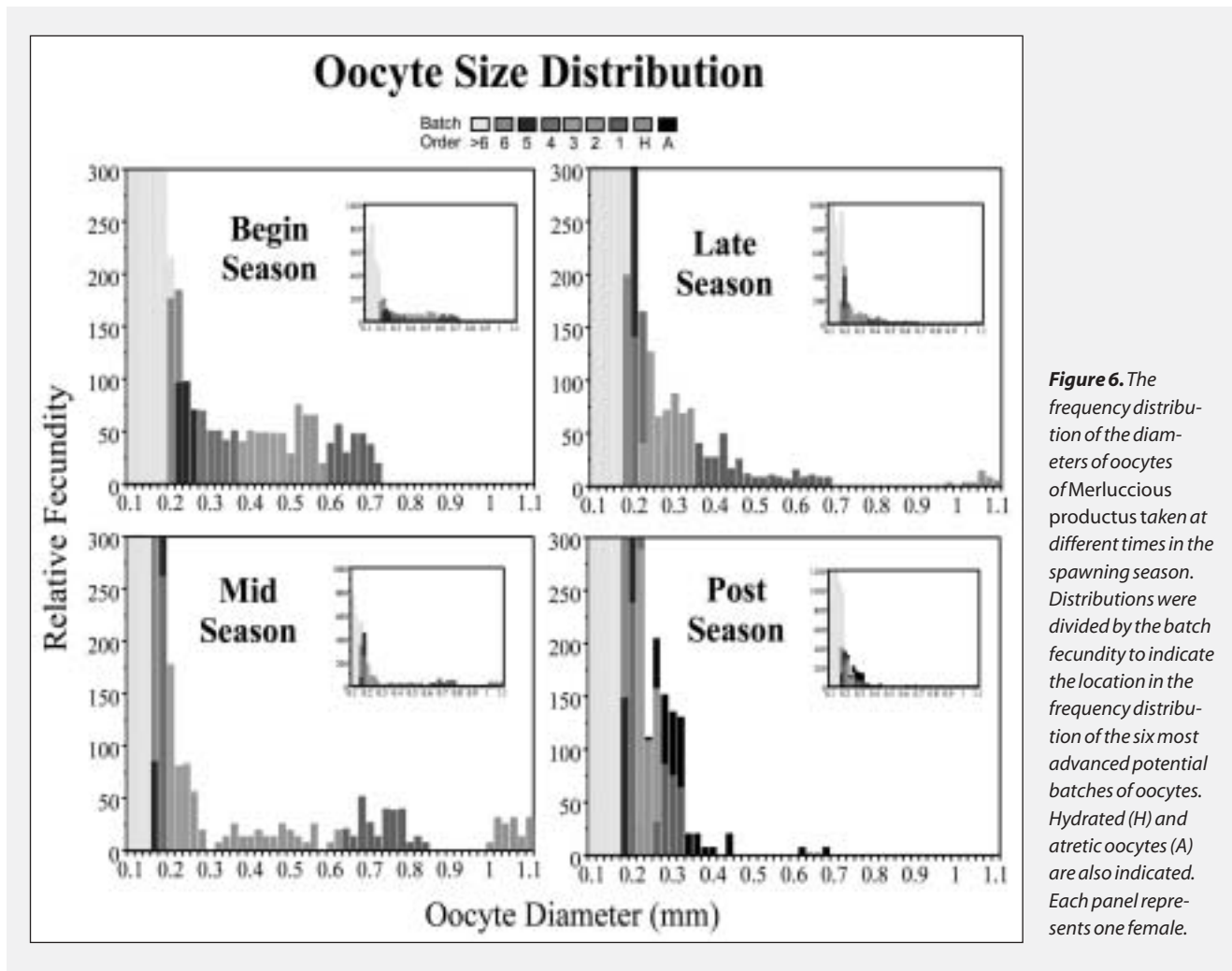
In many species no wide and obvious gap exists in the oocyte distribution in prespawning animals, consequently, different criteria are needed to separate the potential fecundity from the rest of the oocytes in the ovary. Most pelagic fish, which produce eggs of about 1 mm in diameter

fall into this category. In such animals smaller gaps may exist in the oocyte frequency distribution, (Greer Walker et al. 1994) or no consistent gap may exist at all (Figure 6). Typically in such animals, a small oocyte class between 0.1 mm and 0.2 mm diameter associated with the initial onset of seasonal oocyte maturation (cytoplasmic vacuole stage, a.k.a. cortical alveoli, yolk vesical stages) is used to separate the potential fecundity for the year from the rest of the oocytes in the ovary. It is unlikely that all such small oocytes will be recruited and spawned in a season. A major difficulty in using the cytoplasmic vacuole stage, or similar small oocyte stage, as a criterion for potential fecundity is that considerable atretic winnowing of oocytes may occur during the season, making the potential a rather inaccurate proxy for actual fecundity. Atretic winnowing of oocytes would be expected to vary from year to year depending

**Figure 4.** The optimal range of mean diameters of advanced yolked oocytes for determining potential annual fecundity of *Microstomus pacificus*. Open circles, Student's *t* as a function of the mean diameter of oocyte in the data set; solid circles, spawning rate index, proportion of females showing hydrated oocyte and/or post-ovulatory follicles. From Hunter et al. 1992.



**Figure 5.** Maturity window in *Anoplopoma fimbria* indicated by the range of advance yolked oocyte sizes over which estimates of the potential annual fecundity can be made without bias. From Macewicz and Hunter 1994.



**Figure 6.** The frequency distribution of the diameters of oocytes of *Merluccius productus* taken at different times in the spawning season. Distributions were divided by the batch fecundity to indicate the location in the frequency distribution of the six most advanced potential batches of oocytes. Hydrated (H) and atretic oocytes (A) are also indicated. Each panel represents one female.

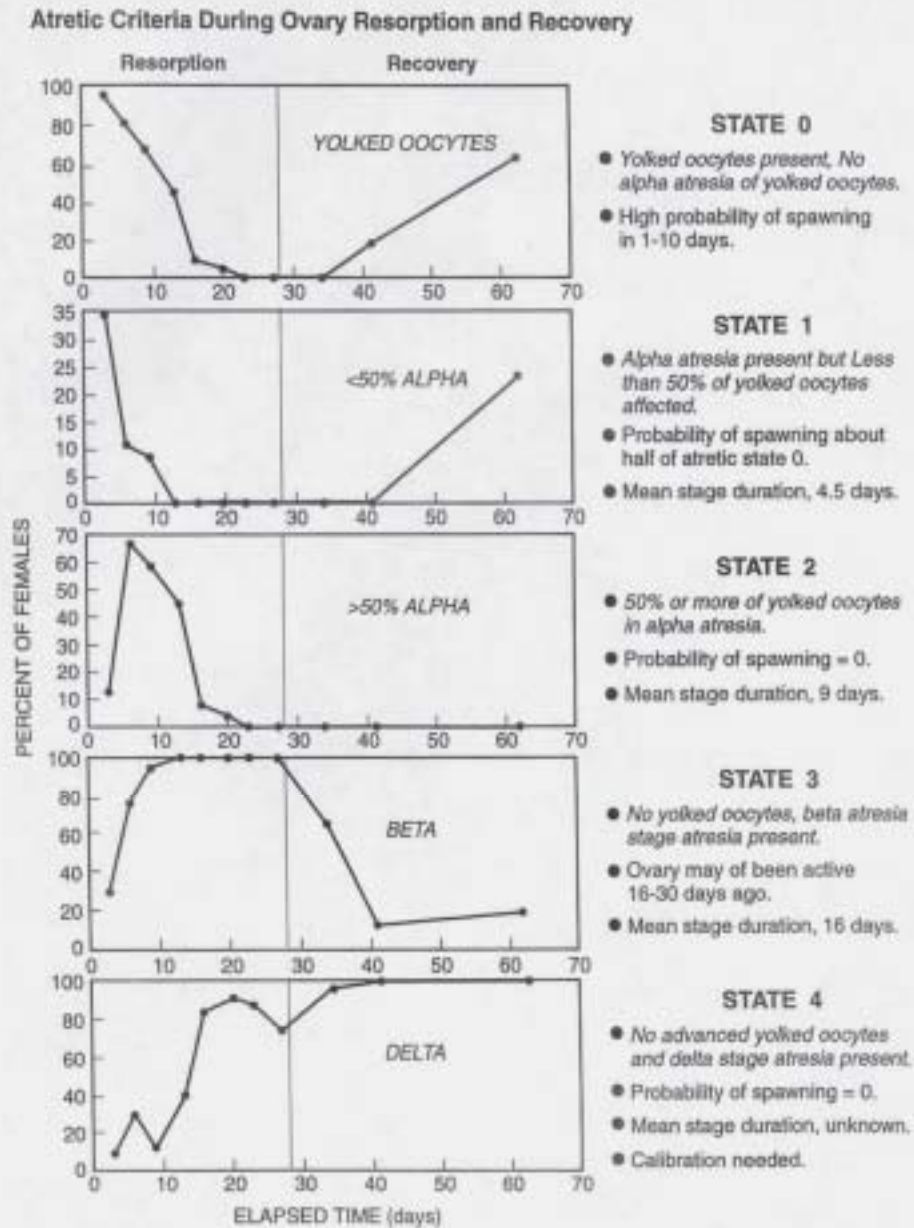
on food and energy reserves. This small oocyte approach to demarcation of the potential seems impractical when a spawning season extends throughout most of the year and spawning is nearly daily such as in the tropical tunas (Schaeffer 1998).

*Insuring against spawning losses:* We include insuring against spawning losses as a potential bias in potential fecundity estimations because up to this time this has not become a standard procedure as it has for batch fecundity estimates. Since the steps to be taken are quite straight forward, and the potential for bias considerable, we believe that potential fecundity estimates are unacceptable unless the necessary precautionary steps have been taken. One way to insure against major spawning losses in the potential stock is to examine histologically all females used to estimate potential fecundity, determine if postovulatory follicles or oocytes in beta atresia are present and discard all those that have them. Even if postovulatory and atretic follicles are absent, it is always possible that a female may have spawned because eventually these indicators of past spawning are completely resorbed. The risk of undetected spawning may increase if females are in elevated water temperatures because the rate of absorption would be acceler-

ated, or if the intervals between spawning are long enough for complete resorption to take place. Another approach is to relate average oocyte diameter to the probability of spawning using the prevalence of females with hydrated oocytes or postovulatory follicles (Nichol and Acuna 2001) as an indicator of spawning. Then use this information to establish a window of opportunity for sampling using the average oocyte diameter, or similar proxy, as the criteria (Figure 4). This method of validation of the potential fecundity is not possible when cytoplasmic vacuole stage is used as the demarcation point for potential fecundity because so many small oocytes exist in the potential fecundity that the mean diameter of the potential does not change as spawning takes place (Greer Walker et al. 1994). For such species histological analysis would have to be used to select prespawning females for the potential using the presence of migratory nucleus oocytes, hydrated oocytes, postovulatory follicles and beta atresia to exclude the spawners.

*Atretic losses are negligible:* A major weakness in the potential fecundity method is that the potential and actual fecundity may differ substantially depending upon the numbers of oocytes in the potential that are resorbed during the spawning season (Kjesbu et al. 1991; Tuene et al.





**Figure 7.** Duration of various atretic states during ovary resorption and recovery in *Engraulis mordax*. From Hunter and Macewicz 1985b.

2001). This is a key issue in the application of the potential fecundity method because its primary value in iteroparous animals is as a proxy for actual annual fecundity. Atretic losses to the potential stock may be the most extensive when many small oocytes are included in the potential fecundity. *Micostomus* and *Anoplopoma* have low prevalence of atresia, produce large eggs and have a wide gap separating the potential from other oocytes. On the other hand, *Engraulis*, *Sardinops*, *Trachurus*, *Scomber*, and *Merluccius* produce small eggs, have a more or less continuous distribution of oocytes, and may have extensive atresia affecting all stages of vitellogenic and previtellogenic oocytes. We suspect that extensive atresia that we have observed in these animals is a regular event occurring at the end of a females spawning season when the continu-

ous distribution of oocytes supplying the spawning batches are resorbed. Scombroid fishes may be the most difficult to analyze because atresia in scombroid seems patchy (Priede 1990; Dickerson et al. 1992) and groups of females with highly atretic ovaries, clearly in postspawning condition, may be captured most any time during the spawning season. Scombroid fishes might be able to mature and regress ovaries more than once during a spawning season depending on food availability.

If the assumption that atretic losses are negligible is unacceptable, then the potential fecundity lost to atresia must be estimated. This is a complex undertaking, that exceeds estimation of daily spawning rates in difficulty, and requires the following: 1) the incidence and density of atretic oocytes

**Table 4.** Some commonly used terms in fecundity studies.

FECUNDITY	SPAWNERS	MATURITY
absolute fecundity absolute individual fecundity advanced standing stock age specific fecundity annual fecundity batch fecundity clutch determinate fecundity fixed fecundity hydrated batch indeterminate fecundity potential fecundity potential annual fecundity relative fecundity standing stock total fecundity oocyte development: - asynchronous - synchronous - group synchronous	active fractional iteroparous multiple partial semelparous serial spawns once	active advanced maturation cycling developing virgin early maturation immature inactive massive atresia mature maturing ovulated partially spent post spawning prespawning proliferation recovering regressing resting ripe running ripe sexually active spawning spent total maturation virginal

must be quantified; 2) quantification must extend throughout the season with more intensive sampling near the end since the duration of alpha atresia is short, about 8 to 13 days (Hunter and Macewicz 1985b, Kjesbu et al. 1991); 3) the temperature specific time at stage for alpha and other atretic stages must be known so that standing stock estimates of atretic oocyte can be converted to a loss rate; 4) the variance associated estimation of atretic losses must be incorporated into the fecundity estimate, and ultimately that of the spawning biomass; and 5) the atresia estimate needs to be repeated each time spawning biomass is estimated since annual variability in reproductive output is tied to annual variability in atretic rates. Such a comprehensive approach has never been followed in its entirety, Priede (1990) study on *Scomber scomber* is one of the most thorough in this regard. One of the difficulties is that the direct measurements of the duration of the atretic stages (Figure 7) are often lacking.

#### GUIDELINES FOR A FECUNDITY STUDY

*Concepts and terminology:* Some of the terms used in fisheries to describe oocyte maturation, fecundity and spawning (e.g. multiple spawners, serial spawners, batch spawners, synchronous or asynchronous oocyte development, determinate and indeterminate fecundity, fixed fecundity) are confusing, and misleading (Table 4). We regret our role

in popularizing some of these terms and believe their use should be discouraged. In fact, most of the terms apply to nearly all species. Most species reported in the literature spawn in batches so making distinctions between multiple, or serial spawners, and the rare case of a one batch spawner, solves few problems. The distinction between fixed and indeterminate fecundity is also not useful because even in species that spawn daily, one might be able to pick a small enough oocyte class as a marker to separate the potential fecundity for a season or a year from smaller oocytes in the ovary. In this sense, any species may be considered to have a fixed or determinate fecundity, hence discussions on whether or not fecundity is fixed or indeterminate are unproductive. It is preferable to approach the problem of selecting a fecundity methodology by assessing how likely a method will provide an unbiased estimate of the desired parameter, and what the precision of the estimate is likely to be when all factors are considered. Of course one must also consider costs, practicality, and whether daily or annual fecundity is the desired parameter.

*Preliminary steps in a fecundity analysis:* The first step in the method assessment should include an examination of the oocyte size frequency distribution of some sexually mature females (do not pool the distributions because pooling different development states may lead to errone-

ous conclusions) and a determination of when and where specimens with hydrated oocyte may be obtained. The second step is to determine if the oocyte distribution is more or less continuous throughout spawning season, if females with hydrated oocyte are readily available from the field, and if it is practical to age postovulatory follicles through field or aquarium work. If these points are answered in the affirmative, the batch fecundity method will provide the most accurate estimate of fecundity because fewer assumptions are required. The easiest estimate to make under these conditions is the number of eggs spawned per day during the survey period. However, if annual fecundity were the desired outcome it may not be necessary to routinely measure spawning frequency throughout the season as a reasonably precise annual fecundity estimate may be possible using a calibrated proxy for spawning frequency such as the gonosomatic index (Claramunt and Roa 2000). On the other hand, if the oocyte distribution is discontinuous with the advanced oocyte class (containing more than one spawning batch) well separated from the rest of the smaller oocytes in the ovary (similar to the distributions illustrated in Figures 2 and 3), then the potential annual fecundity method could probably be used with a relative low risk of bias, although the three potential fecundity assumptions would need validation before going forward. Under these conditions, the annual or the fecundity reduction methods of egg production could be used. It should be remembered, however, that the extent of such a discontinuity in the oocyte frequency distribution depends upon when a sample is taken relative to the maturation and spawning cycle, with the gap widening as spawning progresses. If the gap appears late, when spawning is underway, adjusting the data for atretic losses may be necessary.

The most difficult situation is when prespawning females have no wide and consistent separation between the potential fecundity for the year and the less mature oocytes (Figure 6), females with hydrated oocytes are not available in sufficient numbers to measure spawning rates, and it is impractical to age postovulatory follicles. Under these circumstances the potential fecundity method may be the only way to obtain some measure of annual fecundity. Before deciding on a marker to separate the potential annual fecundity from the rest of the oocytes, such as the cytoplasmic vacuole stage, it may be worthwhile to add an additional step to the analysis. Make a rough estimate of the batch fecundity for the species, either from finding a few specimens in the hydrated or migratory nucleus stage, or using literature values. Then determine roughly how many spawning batches exist in the stock of oocytes presumed to be the potential fecundity for the year, in prespawning specimens. If the marker does not include the number of spawns that one might expect, given literature

values for spawning rates at the observed water temperature, one may want to select an earlier oocyte stage to mark off the potential. Once the demarcation for the potential becomes standardized, the effect of fish size on fecundity can be evaluated, and a crude measure of annual fecundity may be possible depending on the extent the three assumptions underlying potential fecundity estimates are validated.

After deciding if batch or potential fecundity will be estimated, the next step in an analysis of fecundity is to determine if the oocytes to be counted (hydrated, advanced yolked, or those greater than a marker size, etc.) are randomly distributed in the ovary. This is an important step if the gravimetric method for fecundity estimation is used because although most studies indicate there is no effect of the location of a tissue sample within the ovary on the counts of the targeted oocyte class (Hunter et al. 1985; Hunter et al. 1992) this is not always the case (Nichol and Acuna 2001). The homogeneity of the targeted oocyte class within the ovary can be adequately evaluated using as few as 10 females, three or more sampling locations within the ovary, and a tissue weight needed for an acceptable level of precision. The last preparatory step in fecundity work is to estimate the number of females and number of tissue samples needed for the desired level of precision (Hunter et al. 1985; Sanz and Uriarte 1989; Hunter et al. 1992). Generally, the within ovary variance is so low compared to the between fish variance, that seldom more than two gravimetric tissue samples are required per female but often more than 50 females are needed.

*Gilson's Fluid:* Gilson's Fluid (Bagenal 1971) used to free the oocytes from the tissues surrounding them, is a popular treatment commonly advocated in past fecundity manuals to enable total oocyte counting. We believe this fluid should not be used because it destroys the ovary for subsequent histological examination, shrinks the oocytes about 30% (Kjesbu et al. 1990), turns hyaline oocytes opaque, delays fecundity estimates (oocytes remain in fluid 3 to 12 months), and is very toxic. In most applications it is no more time consuming to count and measure oocytes without Gilson's treatment than with it. Loss of information related to the condition and stage of the oocyte being counted, as well as the structures being dissolved such as postovulatory follicles and atretic follicles, is a serious drawback. The subtle features and oocyte stages often hold the key to interpreting reproduction. A possible gain in precision by using Gilson's because larger numbers of oocytes are counted may be illusory since the controlling factor in fecundity determinations is the variance between females, not the within ovary variance (Hunter et al. 1985).

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# Comparative reproductive biology of some commercial marine fishes from Argentina

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## INTRODUCTION

The fishery activity constitutes one of the most important economical resources of Argentina, with an annual catch of around 850,000 t<sup>1</sup>. The fishery management requires knowledge about the reproductive biology of the species. To estimate fish biomass, most assessment models use information about reproductive parameters such as length at maturity, fecundity or spawning frequency. Annual changes of these variables could affect the stock fecundity and produce variability in the recruitment of fishes.

This review summarises some of the reproductive aspects of four commercial fishes that inhabit different environments in Argentine waters:

- *Micromesistius australis* (Southern blue whiting) is a mesopelagic species typical of the Cold Malvinas Current distributed mainly between 47° S and 55° S at depths ranging from 100 to 700 m. It is one of the most important resources in this area with an abundance estimated at around 700,000 t.
- *Engraulis anchoita* (Argentine anchovy) is a small pelagic species with a high biomass (3,000,000 t) distributed from 24° S to 48° S mainly at depths between 20 and 100 m.
- *Merluccius hubbsi* (Argentine hake), the main fishery resource of Argentina, is a demersal species distributed from 22° S to 55° S at depths between 50 and 500 m, with an abundance of around 1,000,000 t.
- *Micropogonias furnieri* (White croaker) is a demersal

coastal species, which inhabits marine and estuarine waters at depths less than 50 m. It is the most important coastal resource, but its abundance (40,000 t) is relatively low compared to the other species analysed.

The objectives of this paper are to describe spawning pattern and reproductive cycle of these species and to estimate reproductive variables such as fecundity, spawning frequency and length at maturity.

## MATERIALS AND METHODS

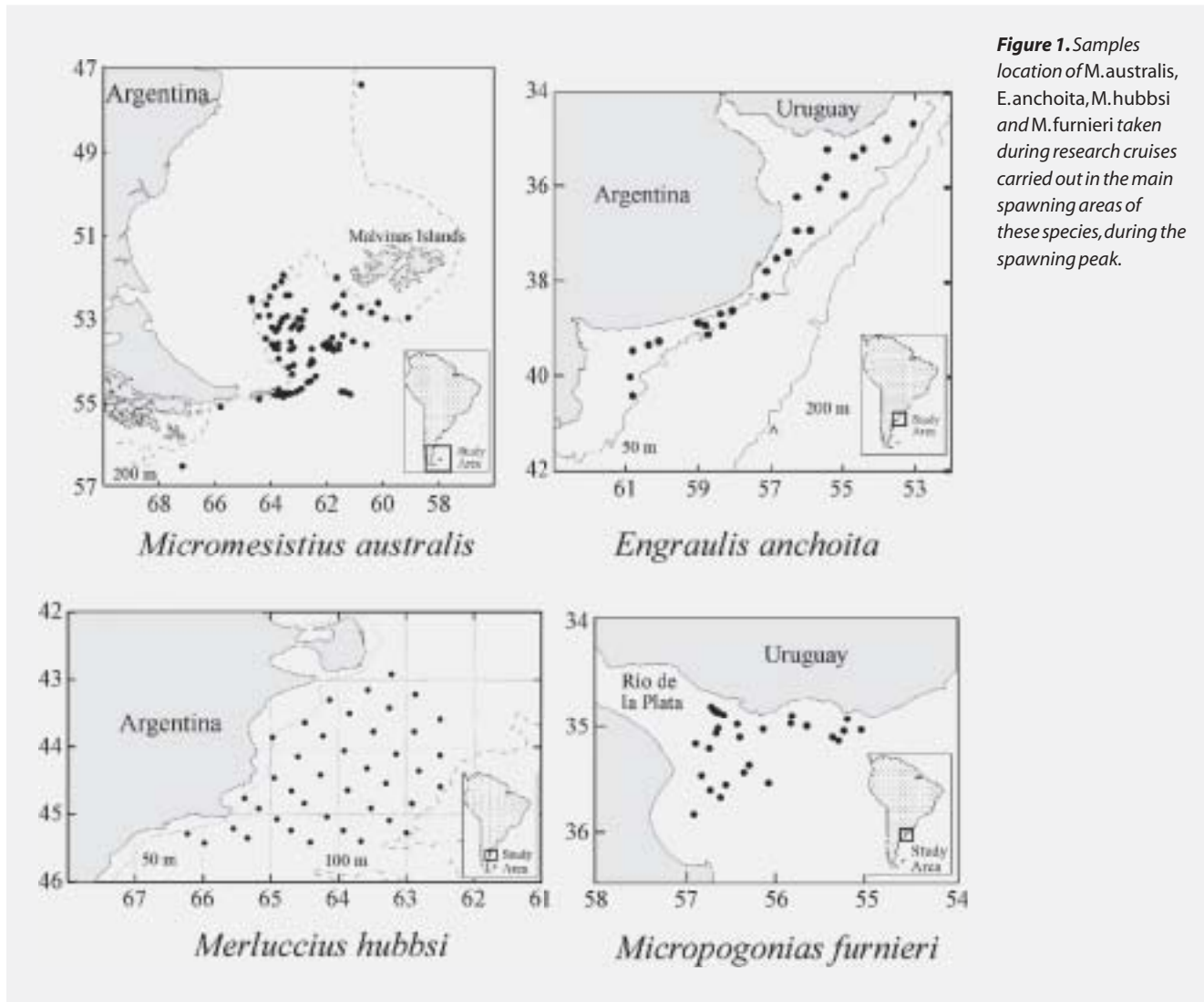
### Sampling

Information for reproductive studies was collected from research cruises carried out during the spawning season of the species. To determine reproductive cycle data collected from commercial catches were also analysed. During the research cruises fish samples and oceanographic data (temperature and salinity) were taken for each trawl station (Fig. 1). Total length (TL), total weight (TW) and macroscopic maturity stage were recorded for each fish sampled (about 200 individuals per trawl station). Macroscopic staging was determined for both sexes using a 5-stage maturity key (Table 1). Adult females were randomly selected from each trawl station ( $n \cong 30$ ) and the ovaries removed and fixed in 10% neutral-buffered formalin for about two weeks.

### Laboratory processing

Ovaries were weighed, and a portion of tissue (about 2.0 g) was removed from each gonad, dehydrated in ethanol, cleared in xylene and embedded in paraffin. Ovaries were cut into 5- $\mu$ m sections and stained with Harris's hematoxylin followed by eosin counterstain.

<sup>1</sup> Argentina. Secretaría de Agricultura, Ganadería, Pesca y Alimentación. 2001. Capturas Marítimas Totales 2000. Buenos Aires: SAGPyA. 8 p (MS)



**Table 1.** Macroscopic criteria used to describe gonadal reproductive stages for males and females.

	Females	Males
1 Immature (Juvenile)	Ovary small and translucent with a thin albuginea tunica. Oocytes can not be seen with the naked eye.	Testis small and translucent.
2 Developing <sup>1</sup>	Ovary becomes opaque and yellow. Individual oocytes can be seen.	Testis becomes larger and white in colour.
3 Spawning	Ovary fills most of the body cavity. Translucent (hydrated) as well as opaque (yolked) oocytes can be seen.	Testis white. Spermatozoa easily released from vent.
4 Spent	Ovary shrunken and flabby with few residual yolked oocytes.	Testis white with opaque areas. Residual spermatozoa.
5 Recovering	Ovary opaque with a thick albuginea tunica and without yolked oocytes.	Testis opaque without spermatozoa.

<sup>1</sup> This stage includes ovaries with evidence of spawning but with many yolked oocytes.

Histological staging of ovaries was based on the stage of oocyte development and on the occurrence of postovulatory follicles (POF) and atresia (Hunter and Goldberg, 1980; Hunter *et al.*, 1992). In view of this, the following classification was established:

- 1) Hydrated (spawning imminent): ovaries with many hydrated oocytes and no POFs
- 2a) Age-0 day (spawning females): ovaries with day-0 POFs and hydrated oocytes
- 2b) Age-0+ day (spawning < 24 h): ovaries with day-0 POFs and no hydrated oocytes
- 3) Age 1 day (spawning  $\geq$  24 h but < 48 h): ovaries with day-1 POFs and yolked oocytes
- 4) Non-spawning (mature) (spawning  $\geq$  48h): ovaries with many yolked oocytes; may contain POFs in advanced stage of degeneration and minor atresia ( $\alpha$  atresia of yolked oocytes < 50%)
- 5) Inactive (mature): ovaries with  $\alpha$  atresia of yolked oocytes  $\geq$  50%. May contain evidence of past spawning
- 6) Immature: ovaries with no yolked oocytes and no atresia of yolked oocytes

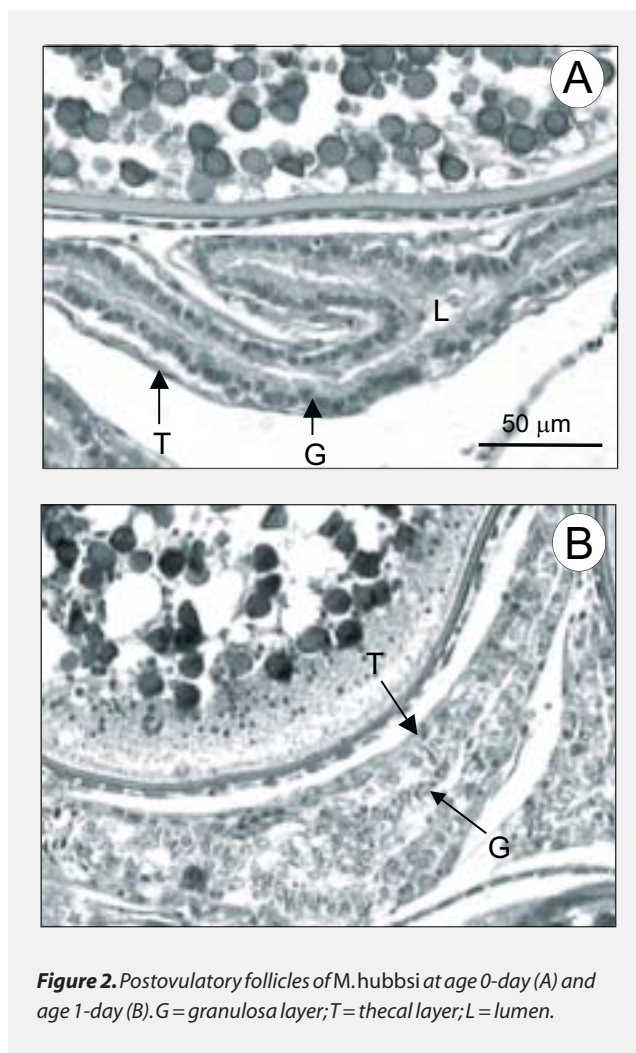
Females with ovaries in stages 1 to 4 were considered capable of spawning at the time of capture or in the near future (active females, Hunter *et al.* 1992).

The description of the POF stages was adapted from that given by Hunter and Macewicz (1985) for *Engraulis mordax* (northern anchovy):

- Day-0 POF has an irregular shape, the granulosa cells are aligned with the lumen clearly visible. The granulosa cells are columnar with a prominent nucleus and the cellular walls are well defined (Fig. 2A).
- Day-1 POF shows degenerative process, the linear appearance of the granulosa cells is not distinct and the lumen becomes reduced. The granulosa and theca cells cannot be clearly distinguished (Fig. 2B).

Although we could not validate the age of POFs in *E. anchoita* and *M. hubbsi*, we assigned to these species the duration reported for *E. mordax* (48 hours), because northern anchovy spawns at moderate temperatures (13° – 19° C, Hunter and Macewicz, 1985), similar to those recorded for Argentine anchovy (11° - 16° C, Sánchez, 1995) and Argentine hake (10° - 14° C, Pájaro and Macchi, 2001a) in their spawning sites.

In the case of *M. furnieri*, eight females were spawned in captivity during a research cruise and sampled at different hours after spawning (6h, 12h, 24h and 36h). The ovaries were preserved and processed for histological analysis.



**Figure 2.** Postovulatory follicles of *M. hubbsi* at age 0-day (A) and age 1-day (B). G = granulosa layer; T = thecal layer; L = lumen.

In *M. furnieri*, the degenerative process of the POFs was faster than that found in Argentine anchovy and hake; 24-h-old POF showed advanced signs of degeneration similar to those observed in *E. mordax* 48 h after spawning<sup>2</sup>. The highest speed of degradation could be associated with the higher water temperatures (20° - 25° C) in the spawning area of *M. furnieri*, which are significantly hotter than those recorded at the corresponding reproductive zones of *E. anchoita* and *M. hubbsi*.

#### Estimation of reproductive variables

The macroscopic maturity key was used to determine the seasonal reproductive cycle and the location of spawning areas. Moreover, macroscopic staging was employed to estimate length at maturity (L<sub>50%</sub>), regarding as mature those individuals that show gonads in stage 2 or higher. In females, it was necessary to complement macroscopic staging with histological examination, mainly in small females,

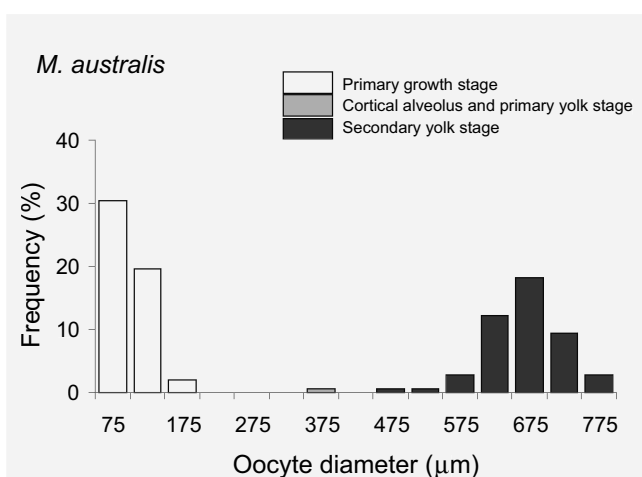
<sup>2</sup> Macchi, G.J., E.M. Acha and M.I. Militelli. 2003. Seasonal egg production pattern of whitemouth croaker (*Micropogonias furnieri*) in the Río de la Plata estuary, Argentina-Uruguay. Fish. Bull. (US) 101: 332-342.

which could have ovaries with uncertain appearance. To estimate this variable, the fraction of mature fish per length class was fitted to a logistic function applying the maximum likelihood approach.

Spawning frequency for *E. anchoita* and *M. hubbsi* was determined from the percentage of age-1 day females (Hunter and Goldberg, 1980). In *M. furnieri* incidence of spawning was estimated from the percentage of females with POFs in different stages of degeneration, because in this species POFs, as reported above, are degraded before 24 h after spawning. Mean and variance of this variable were calculated according to the equations developed by Picquelle and Stauffer (1985)

Samples of hydrated ovaries of *M. hubbsi*, *M. furnieri* and *E. anchoita* were used to show the oocyte size-frequency distribution. In the case of *M. australis*, ovaries with yolked oocytes and without signs of previous spawning were selected to analyse the pattern of oocyte development. Oocyte diameters of these females ( $n \cong 1000$  oocytes) were measured along the longest axes with an ocular micrometer after fixation.

Hydrated ovaries without evidence of spawning (no POFs) were used to estimate batch fecundity (number of oocytes released per spawning) by the hydrated oocyte method (Hunter *et al.*, 1985). Three pieces of ovary (about 0.1-0.2 g each) were removed from the anterior, middle and posterior parts of each gonad, weighed ( $\pm 0.1$  mg), and the hydrated oocytes counted. Batch fecundity for each female was the product of the mean number of hydrated oocytes per unit of weight and the total weight of the ovaries. Relative batch fecundity (number of hydrated oocytes per gram of body weight) was determined as the batch fecundity divided by



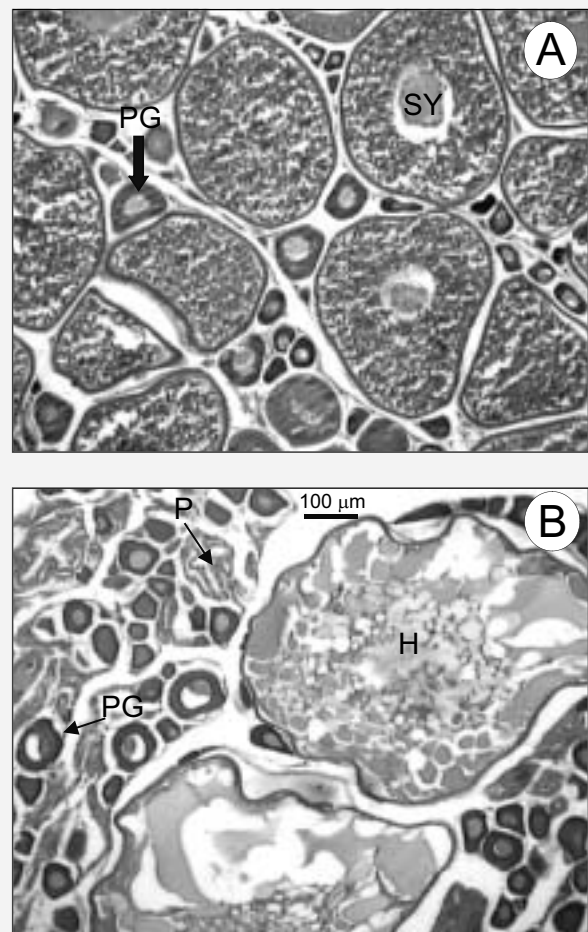
**Figure 3.** Oocyte diameter distribution for an ovary of *M. australis* in advanced maturity stage.

female weight (without ovary). The relationships of batch fecundity to total length and to total weight were described using standard regression analysis.

## RESULTS AND DISCUSSION

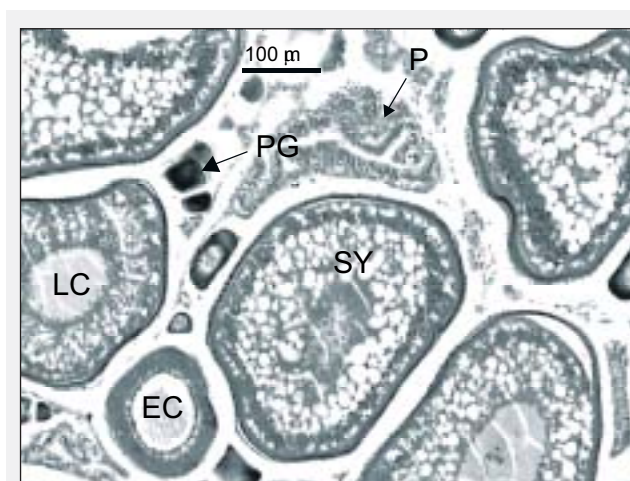
### Spawning pattern

Ovaries in advanced maturation (with yolked oocytes) of *M. australis* showed a pattern of oocyte development characteristic of fishes with determinate annual fecundity. Oocyte size frequency distribution (Fig 3) showed a hiatus between yolked oocytes (secondary yolk stage) and unyolked oocytes (primary growth stage), which indicates that the recruitment of unyolked oocytes into the advanced stock ceases during this phase (Hunter *et al.*, 1992). Besides this, histological analysis showed that the proportion of mature oocytes in pre spawning females (Fig. 4 A) was much higher than in females with evidence of spawning (Fig. 4 B). This observation could indicate that yolked oocytes decrease substantially over the spawning season.



**Figure 4.** A) Ovary of *M. australis* in advanced maturity stage showing a high proportion of yolked oocytes (SY). B) Ovary of *M. australis* with postovulatory follicles (P) and few oocytes in hydration (H). PG = primary growth stage (unyolked).





**Figure 5.** Ovary of *M. hubbsi* with postovulatory follicles (P) and oocytes in different stages of development. PG = primary growth stage; EC = early cortical alveolus stage; LC = late cortical alveolus stage; SY = secondary yolk stage.

The other species analysed (*M. hubbsi*, *M. furnieri* and *E. anchoita*) were multiple spawners according to observation of maturing ovaries with POFs and yolked oocytes (Fig. 5). Furthermore, in contrast to that observed for *M. australis*, the oocyte size frequency distribution of hydrated females showed a pattern with continuous batches of growing oocytes (Fig. 6), characteristic of species with indeterminate annual fecundity.

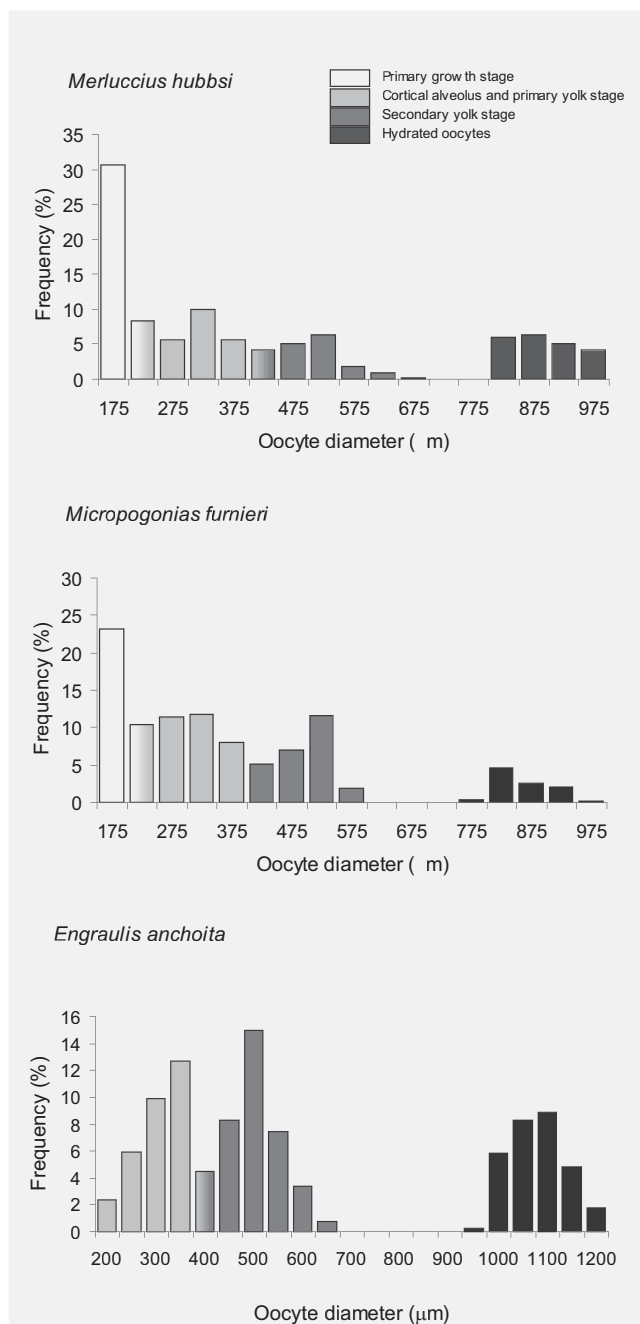
### Spawning season

Temporal variation of maturity stages showed that spawning females of *M. australis* can be observed in a short period between late winter and early spring with a main peak in August (Fig. 7 A). During these months the maximum of productivity in the cold waters south of Malvinas Islands, the main spawning area for southern blue whiting (Pájaro and Macchi, 2001b), occurs. The observed oocyte development pattern, with a fixed number of yolked oocytes at the onset of spawning, may be an adaptive strategy to the short period of good conditions for reproduction.

In contrast, *E. anchoita*, *M. hubbsi* and *M. furnieri*, which spawn in temperate waters, have a protracted reproductive season, which extends from spring to summer with a main spawning peak between December and January (Fig. 7 B and C). A long reproductive season is generally characteristic of species with multiple spawning and indeterminate annual fecundity (Brown-Peterson *et al.*, 1988).

### Length at maturity

Length at 50% maturity was estimated for males and females of *M. furnieri* and *M. hubbsi*. A good fit was

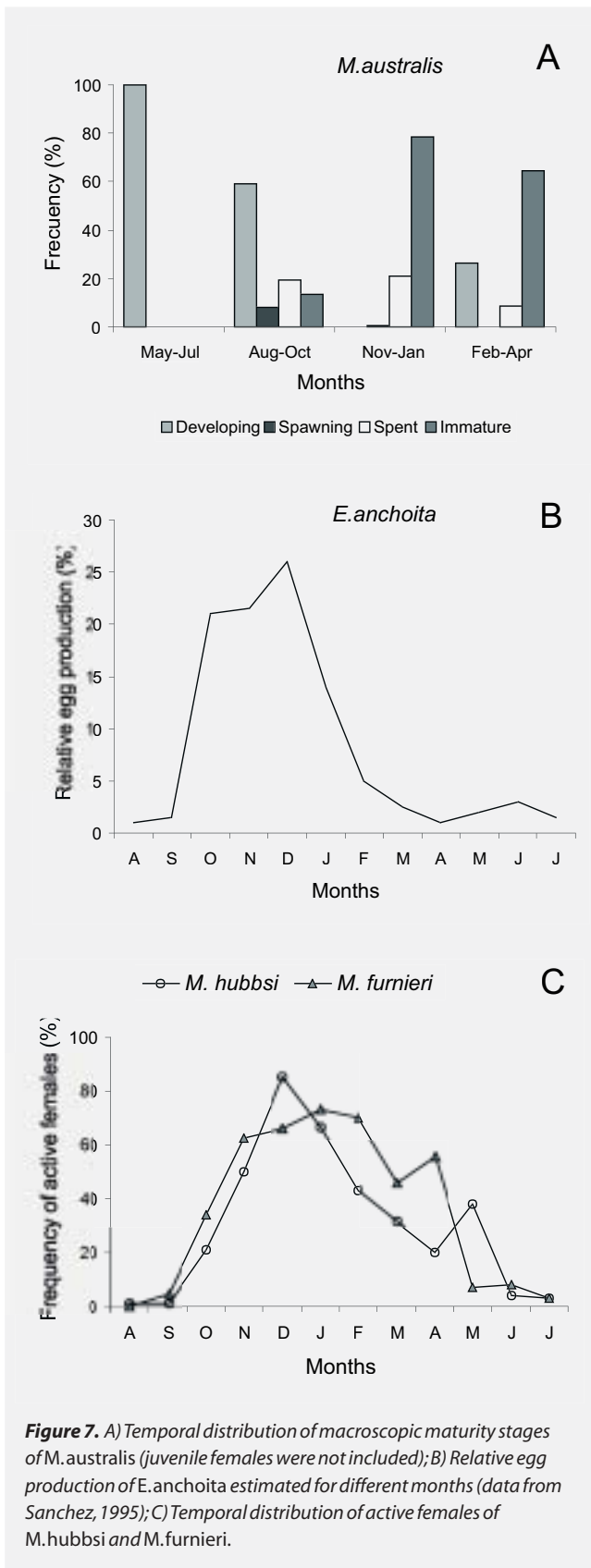


**Figure 6.** Oocyte diameter distribution for hydrated ovaries of *M. hubbsi*, *M. furnieri* and *E. anchoita*. Oocytes smaller than 150 μm were not included.

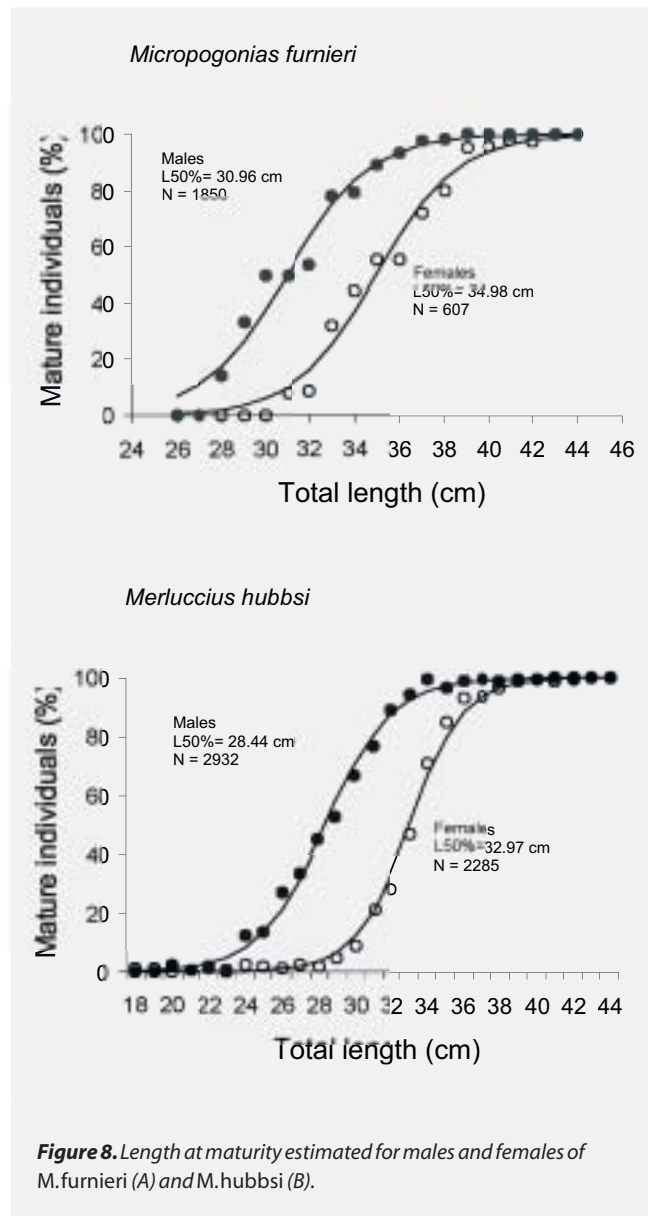
obtained between percentages of maturity and length in both cases (Fig. 8). Females of these species reached sexual maturity at a higher size than males. In the case of Argentine hake, which has been suffering over-exploitation during the last ten years, length at maturity showed a decreasing trend in comparison with previous estimates reported for this species (Pájaro and Macchi, 2001a).

### Spawning frequency and batch fecundity

Spawning frequency and batch fecundity were estimated for *E. anchoita*, *M. hubbsi* and *M. furnieri* with samples

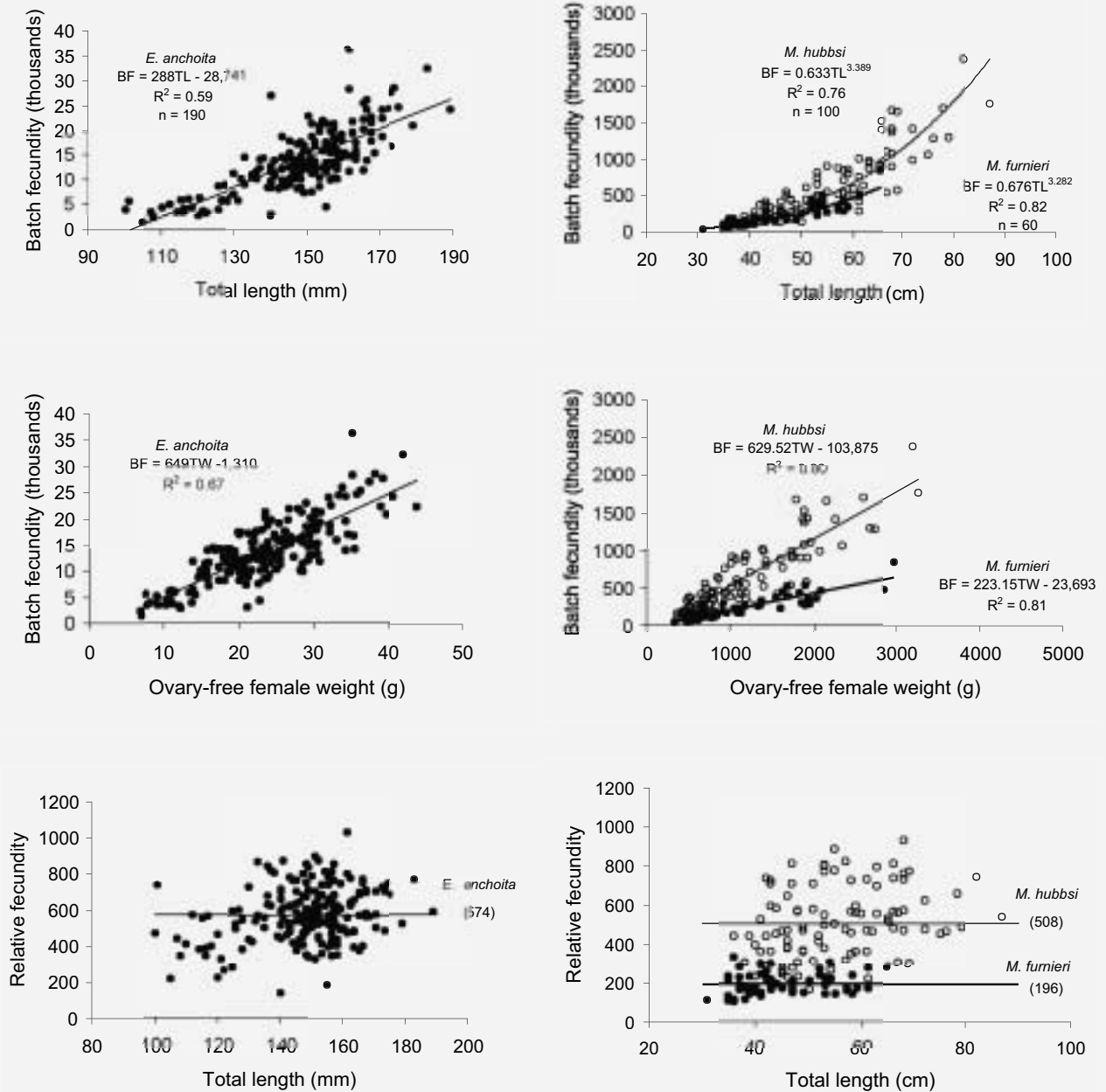


collected during the spawning peak of these species. Table 2 shows the number of mature females in different stages sampled during three cruises carried out for these species. Of all the specimens examined, about 12 % of *E. anchoita* and 15 % of *M. hubbsi* females had day-1 POFs, which indicated that these species spawn once every 8 days and 7



days, respectively. *M. furnieri* showed the highest spawning frequency with about 31 % of females with POFs, which indicated an average spawning interval of about 3 days during the main reproductive peak.

Batch fecundity estimates for *M. hubbsi* and *M. furnieri* were fitted to a power function of total length and a linear function of ovary-free body weight; in the case of *E. anchoita* both relationships were fitted to a linear model (Fig. 9). Batch fecundity of Argentine hake was higher than that estimated for white croaker in the same weight range. This difference was also evident for relative batch fecundity; the mean number of hydrated oocytes per female gram of *M. hubbsi* (508 oocytes, SD = 180) was more than twice that estimated for *M. furnieri* (196 oocytes, SD = 55). Batch fecundity of Argentine anchovy was much lower than that estimated for Argentine hake and white croaker (Fig. 9), but the mean relative batch fecundity was the highest estimated (574 oocytes/g ovary-free body weight, SD = 151).



**Figure 9.** Batch fecundity as a function of total length and total weight (without ovary) and relative batch fecundity values obtained for *E. anchoita*, *M. hubbsi* and *M. furnieri*. The horizontal line shows the mean relative batch fecundity estimated for each species.

**Table 2.** Number of females in reproductive activity of *E. anchoita*, *M. hubbsi* and *M. furnieri* that were histologically staged for estimation of spawning frequency. Data were collected from three cruises carried out during peak spawning for each species.

	Trawl stations (n)	Hydrated ovaries (n)	Day – 0 ovaries (n)	Day – 0+ ovaries (n)	Day – 1 ovaries (n)	Total mature females	Spawning frequency	Coefficient of variation
<i>E. anchoita</i> <sup>1</sup>	23	77	23	74	67	590	0.12	0.13
<i>M. hubbsi</i> <sup>1</sup>	25	70	16	50	112	739	0.15	0.19
<i>M. furnieri</i> <sup>2</sup>	20	39	33	85		342	0.31	0.18

1 Age of POFs by analogy to northern anchovy (Hunter and Macewicz, 1985).

2 Age of POFs by measurement of degradation time.

## REMARKS

- We consider that the macroscopic 5-stage maturity key is useful and easy to apply in fishery studies to determine spatial and temporal spawning.
- To estimate length at maturity it is necessary to complement macroscopic analysis with histological examination of ovaries, mainly in small females.
- Histological analysis is necessary to describe spawning pattern and to estimate incidence of atresia and reproductive variables such as spawning frequency and fecundity.
- Spawning pattern seems to be related to the environmental conditions in the spawning area. In general, species that spawn in temperate waters have a long reproductive season and show continuous recruitment of growing oocytes along the spawning period. In the case of *M. australis*, which spawns in colder waters with temperatures between 5° and 6° C (Perrota, 1982), it has a short spawning season and shows determinate annual fecundity. This characteristic could be associated to the narrow productivity period in the spawning area of this species. Furthermore, plankton data showed that the diameter of *M. australis* eggs (1,260 – 1,430 µm, Ciechomski and Booman, 1981) is larger than those reported for species of temperate waters with spherical eggs, such as Argentine hake (800 – 900 µm, Ehrlich, 1998) or white croaker (730 - 1053 µm, Weiss, 1981). The larger eggs may be advantageous during the first days of life, because hatchlings could have larger quantity of yolk reserves (Hinckley, 1990; Wootton, 1994).
- Spawning frequency, estimated as the percentage of females with postovulatory follicles, was the most problematic reproductive variable. In most cases it was considered to be a preliminary estimate, because we could not validate the age of these structures.
- Degradation of postovulatory follicles seems to be faster in *M. furnieri* spawning at higher temperatures than hake and anchovy. This observation confirms that increasing temperatures decrease the time that POF can be detected in fishes, as was reported for other species (Hunter *et al.* 1986; Fitzhugh and Hettler, 1995; Schaefer, 1996).

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# Lifetime fecundity of the market squid, *Loligo opalescens*, with application to monitoring escapement

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## ABSTRACT

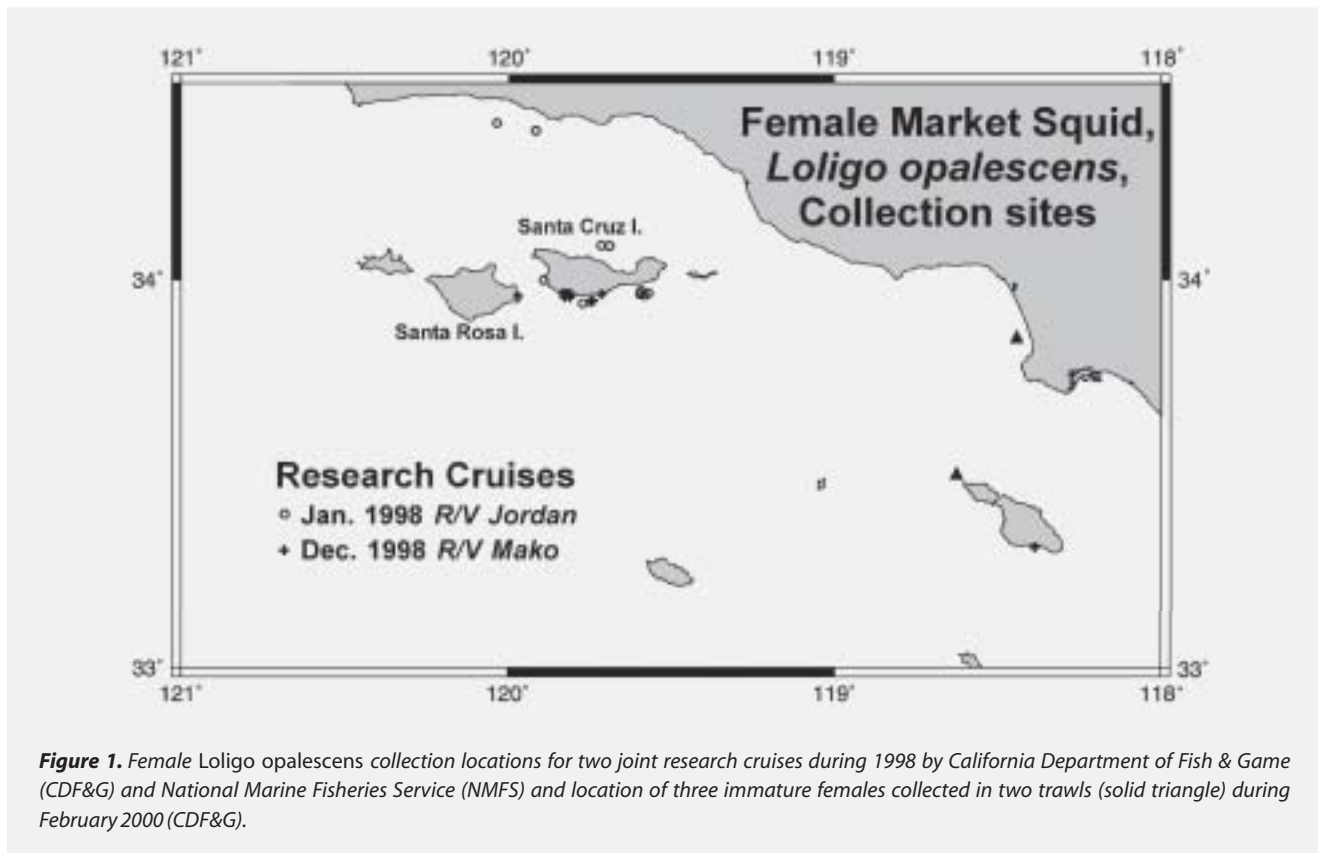
*Loligo opalescens*, market squid, live less than a year and die after a short spawning period, before all oocytes are expended. The maximum lifetime fecundity of a female can be estimated by counting all oocytes in the ovary just before initial ovulation (potential fecundity,  $E_p$ ) and subtracting from  $E_p$  the number of oocytes remaining in the ovary (residual fecundity,  $E_r$ ) at the end of the spawning period. During the spawning period no oögonia are produced, hence the standing stock of oocytes declines as they are ovulated. This decline in the number of oocytes is correlated with a decline in mantle condition and an increase in the size of the smallest oocyte. For a *L. opalescens* female of 129 mm dorsal mantle length  $E_p$  was estimated to be 3844 oocytes and  $E_r$  was estimated as 834 oocytes; thus our estimate of maximum lifetime fecundity ( $E_p - E_r$ ) is 3010 oocytes. The dry weight of 3844 eggs was equivalent to 65.6% of the dry body weight of a 129 mm female at the beginning of the spawning period. The close agreement between the decline in body weight and the standing stock of oocytes during the spawning period indicates that the production of eggs is largely, if not entirely, financed by the conversion of tissue to eggs.

As the fishery for *L. opalescens* occurs only on their spawning grounds, and market squid die after completing all spawning (all bouts of egg depositions), it is possible to calculate the fraction of the potential fecundity that escapes the fishery. To do this we used an indirect method to compute the mean fecundity in the catch and then subtracted this average total fecundity from the estimated, average potential fecundity. These calculations indicate that, during the period December 1998 through December 1999, 32.6% of the reproductive potential of the catch escaped capture. This indicates that it may be practical to use escapement fecundity as a way to monitor the California fishery for *L. opalescens*.

## INTRODUCTION

Market squid, *Loligo opalescens*, is the most valuable fishery resource in California waters and is monitored under the Coastal Pelagics Species Fishery Management Plan of the Pacific Fishery Management Council. *L. opalescens* off the California coast is short lived (Butler et al. 1999) and may die after spawning (McGowan 1954; Fields 1965). This population is entirely dependent upon the reproductive output of the preceding generation. Thus, the potential and maximum lifetime fecundity of *L. opalescens* are critical life history traits and fecundity must be known to estimate the biomass using either egg deposition or larval production methods (Hunter and Lo 1997).

Traditional squid fecundity methods assume that counts of the standing stock of oocytes in the ovary and/or ova in the oviduct of animals taken on the spawning grounds is equivalent to the lifetime fecundity of the animals. As Laptikhovskiy (2000) postulated, such estimates will be biased if the females selected for fecundity estimates spawned some eggs prior to capture, if some of the oocytes remain in the ovary after the female dies, or if some of the standing stock of oocytes is lost due to atresia. The first analyses of *L. opalescens* fecundity taking such biases into account and an evaluation of the use of fecundity as a tool to examine egg deposition, mortality, and to monitor egg escapement from the fishery, were recently completed (Macewicz et al. MS). Our objectives here are to present information on maturity, spawning and age, and to review fecundity of *L. opalescens*. We will present the following aspects of *L. opalescens* fecundity: potential, the standing stock of oocytes of all development stages in the ovary just prior to ovulation of the first batch



**Figure 1.** Female *Loligo opalescens* collection locations for two joint research cruises during 1998 by California Department of Fish & Game (CDF&G) and National Marine Fisheries Service (NMFS) and location of three immature females collected in two trawls (solid triangle) during February 2000 (CDF&G).

**Table 1.** Classification system for the gross anatomical characteristics of the reproductive system of female and male market squid, *Loligo opalescens*.

Female Organs	Character	Grade
Nidamental gland	length	millimeters
Accessory nidamental gland	color	0=clear, 1=whitish, 2= pink, 3=peach, 4=reddish-orange
Oviduct	number of large clear ova	1=none, 2=1-20, 3=21-200, 4= >200
Ovary	number of large clear oocytes	1=none, 2=1-20, 3=21-200, 4= >200
Ovary	number of opaque or white oocytes	1=none, 2=1-20, 3=21-200, 4= >200
Male Organs	Character	Grade
Testis	length and width	millimeters
Spermatophoric (Needham's) sac	number of spermatophores	1=none, 2=1-20, 3=21-100, 4= >100
Terminal spermatophoric duct	number of spermatophores	1=none, 2=a few, 3=some, 4=many

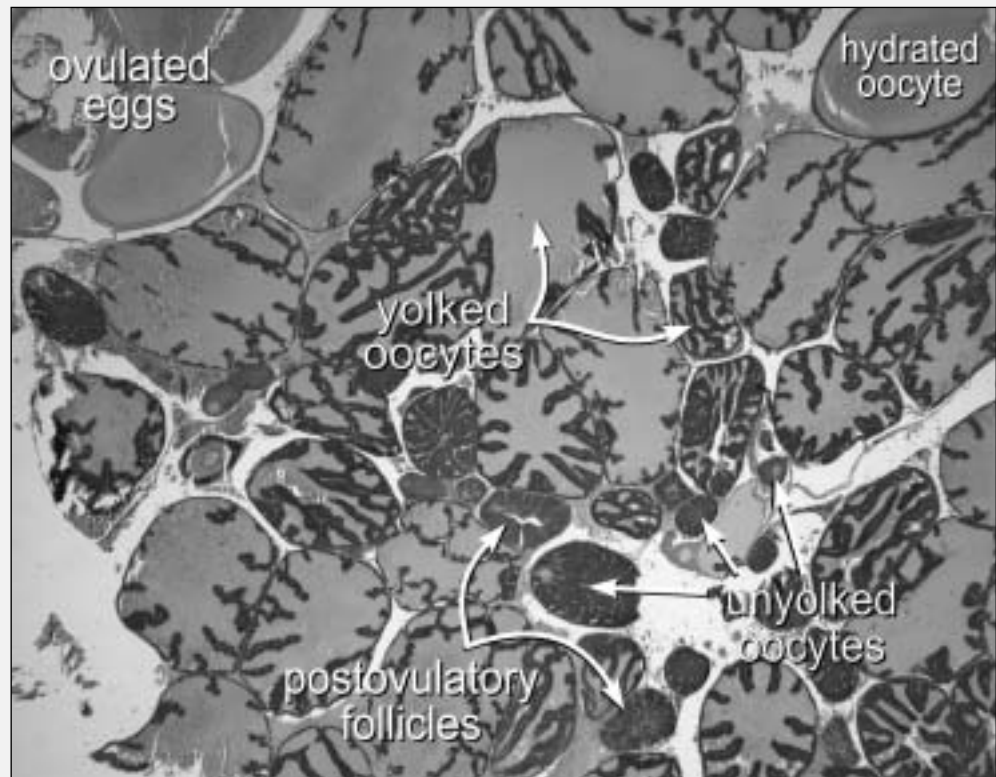
of mature oocytes into the oviduct; **batch**, the number of mature oocytes (stage VI of Knipe and Beeman 1978) in the ovary that are ready to ovulate; **minimum residual**, the minimum standing stock of all oocytes that might be expected to remain in the ovary at death; **maximum lifetime**, the maximum number of eggs a female might be expected to deposit in a lifetime equivalent to the potential less the minimum residual; **oviduct fecundity**, the number of ova (ovulated mature oocytes) present in

the oviduct; and **total fecundity**, the sum of the standing stock of oocytes in the ovary and the standing stock of ova in the oviduct present in a female regardless of when she is sampled.

#### METHODS

*L. opalescens*, were collected jointly by California Department of Fish & Game (CDF&G) and National Marine Fisheries Service (NMFS) in 1998 (Figure 1).





**Figure 2.** Histological H&E stained slide of the ovary of a mature spawning female *L. opalescens*.

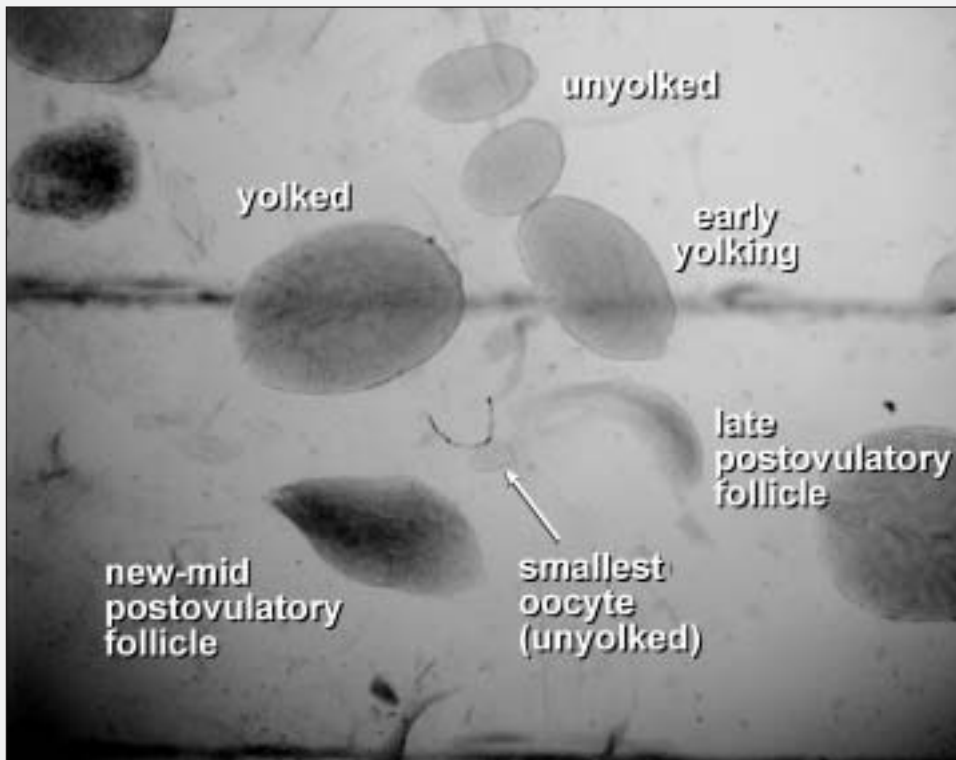
**Table 2.** Definition of three groups of female maturity and spawning based on the histology of their ovaries (numerical stages from Knipe and Beeman, 1978).

REPRODUCTIVE STATE	HISTOLOGICAL CHARACTERISTICS
IMMATURE	Ovary contains only unfoliated oocytes; oocyte development ranged from stages I (oogonia) to IV (follicular invagination oocyte)
MATURE PREEVULATORY	No postovulatory follicles are present. Ovaries must contain oocytes with yolk (stages V-VI, yolking begins about 1.1 mm in size); ovary usually also contains unfoliated oocytes.
SPAWNING MATURE	Ovary contains postovulatory follicles of any age indicating that the female has ovulated; degree of degeneration of postovulatory follicles varies from none to extensive; the female is considered to be "spawning". Oocyte development stages III-VI are often present but stages Ic-II are rare (late stage Ic oocytes were present in 2% of the ovaries) and the earliest stages Ia and Ib are absent.

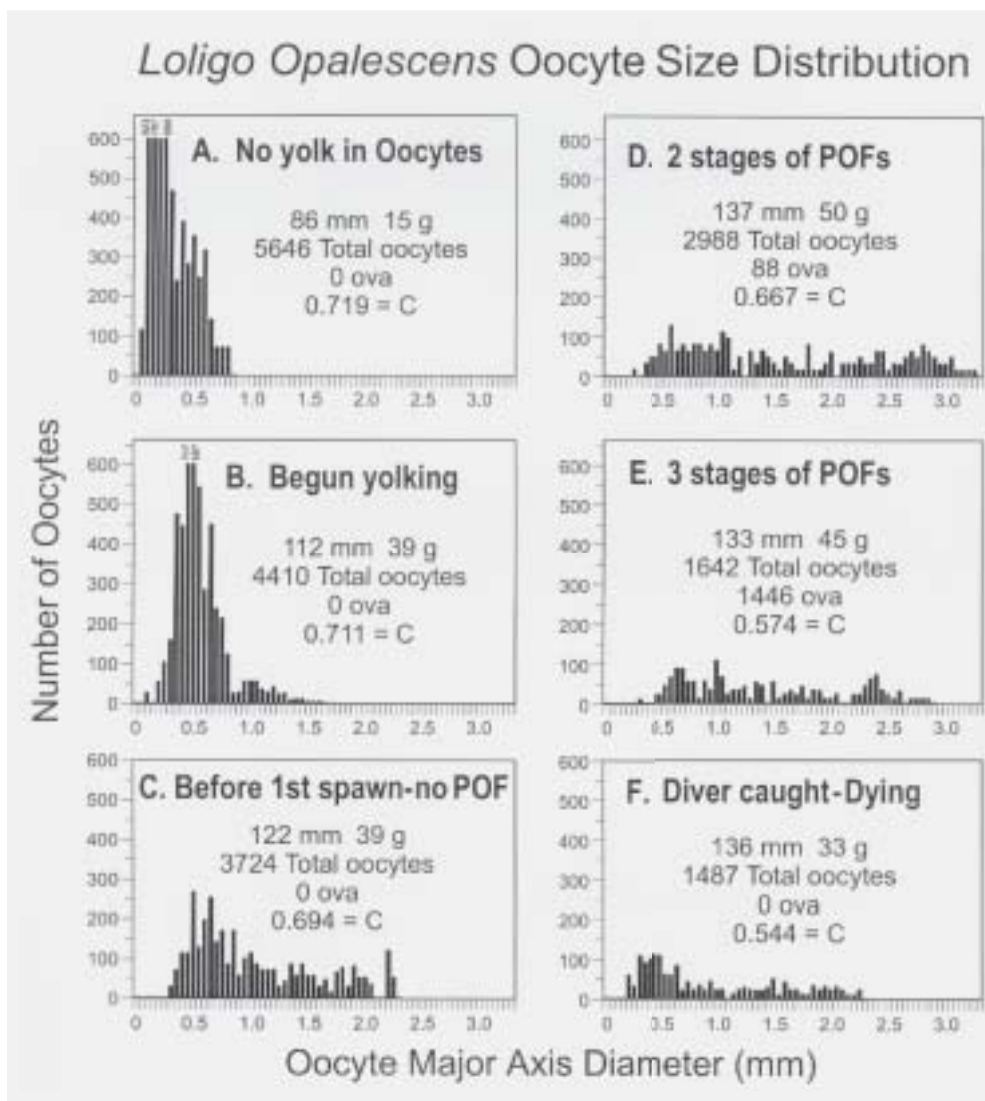
Immediately after capture, sex, dorsal mantle length (mm), whole body weight (g), and characteristics of the reproductive systems (Table 1) of each individual were recorded. A standard size disc of tissue was cut out of the mantle and frozen, and later dried. Statoliths were dried and aged following Butler et al. (1999). The reproductive organs that were preserved in the field in 10% formalin were weighed. The ovaries from 135 female *L. opalescens* collected in January and 117 females from December were

analyzed histologically (Figure 2) to determine maturity and spawning condition (Table 2).

All oocytes in weighed ovarian tissue samples were macroscopically identified (Figure 3) and counted for 98 females (37 from January and 57 from December 1998, 4 from 2000). Mean number of oocytes in the ovary was estimated using the gravimetric method (Hunter et al. 1985, 1992). The diameter of the major axis (D, in mm) of the smallest



**Figure 3.** Whole *L. opalescens* oocytes as viewed under a dissection microscope used for counting and classifying oocytes.



**Figure 4.** Oocyte size distribution of six female market squid, *Loligo opalescens*. Dorsal mantle length (mm), body weight (g), the total number of oocytes in the ovary, the number of ova, and the mantle condition index (C, mg/mg<sup>2</sup>) are indicated for each specimen. Female A is immature. Females B and C are considered to be mature-preovulatory because neither has postovulatory follicles (POFs) in her ovary nor ova in her oviduct. While female B has begun yolkling its oocytes, female C has well-yolked oocytes and is close to her first ovulation. Females D and F are mature-spawning females and their ovaries contained postovulatory follicles. Female F was caught by a scuba diver and appeared to be dying.

oocyte was measured. The number of ova in the oviduct was also counted either directly (usually when N was less than 300) or the mean estimated using the gravimetric method. Finally, all the oocytes in one tissue sample were measured for six females and whole-ovary, oocyte frequency distributions were estimated (Figure 4); for this work we used a video coordinate digitizer linked by a NTSC video camera to a dissection microscope with a resolution in vertical lines of 5µm and of 7.69µm in horizontal lines.

In addition, samples were routinely taken from the landed squid catch by CDF&G port samplers. Length, weight, and reproductive characteristics were recorded and mantle sample discs were collected and dried for 1275 mature female squid caught by the fishery December 1998 to December 1999 in the southern Californian Bight. We selected 60 of these females and used the gravimetric method to directly estimate fecundity (number of oocytes) of their ovaries while the number of ova in the oviducts was calculated indirectly from their oviduct weight using the linear equation developed from the research survey females ( $Y = 245W_{\text{oviduct}}$ , pseudo  $r^2 = 0.98$ ).

## RESULTS

### Gross anatomical and aging

We found that the gross anatomical characterization could distinguish immature from mature females by the absence of large clear oocytes in the ovary and the absence of large clear ova in the oviduct, but mature females could only be separated into preovulatory or spawning categories with analysis of ovarian histology slides. Therefore numerous maturity stages are not necessary. Although nidamental gland length (NGL) showed a change from immature to mature, the mature females could not be further separated

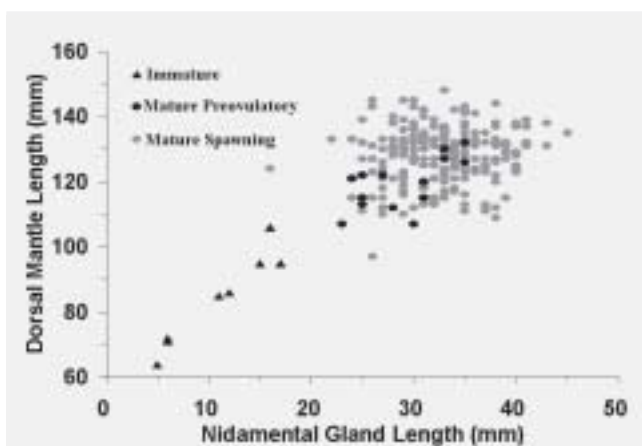
using this character (Figure 5) and we do not recommend measuring NGL since presence of large clear oocytes or ova is quicker in identifying mature females. Immature female market squid are generally smaller and younger than mature females. Age of mature females is highly variable per length and mature-preovulatory females are scattered among the size classes of mature-spawning females, indicating that fecundity may not be age specific (Figure 6).

### Batch fecundity

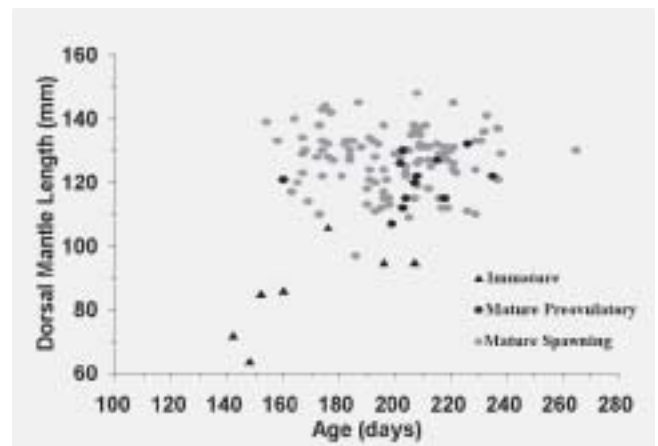
Although spawning females had postovulatory follicles of distinctly different stages, indicating that ovulation is not a continuous process but are events separated by enough time to produce the distinct stages of degeneration, batches of mature oocytes are not spawned as soon as they are ovulated. No evidence exists for the production of ova in large batches: 1) flat oocyte distribution in spawning squid (Figure 4D, E, F); 2) ovaries had small batches of mature oocytes (range 5-246 oocytes per batch, mean of 50); and 3) maximum batch in the ovary was never close to the maximum (1726) ova in the oviduct. Ova may build up in the oviduct as the result of a series of waves of ovulations since spawning females with 900 or more ova in their oviduct had, in every case, three or more different stages of post-ovulatory follicles in their ovaries (Table 3). Hence, batch fecundity is not useful in estimating daily egg deposition in *L. opalescens*.

### Potential fecundity

The immature ovary has a narrow size range of unyolked oocytes but as oocyte maturation proceeds, D (smallest oocyte size) increases and the total numbers of oocytes of all sizes decline markedly after the onset of spawning (Figures 4 and 7). This supports the conclusions of Knipe and



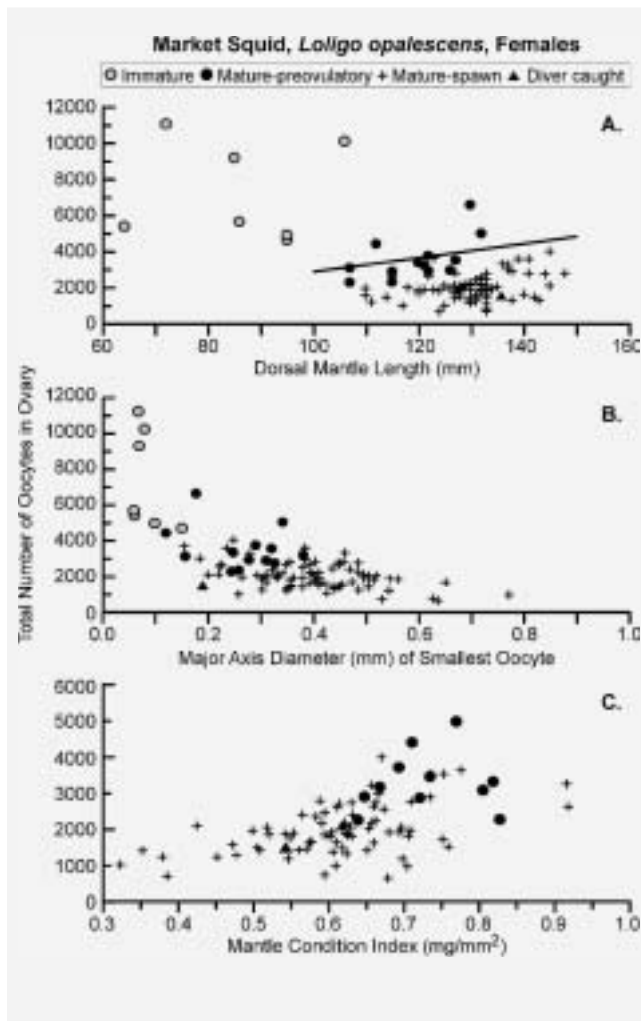
**Figure 5.** Nidamental gland length as a function of dorsal mantle length for each female *Loligo opalescens* (N=255) sampled during research surveys. Reproductive state of females identified by symbols.



**Figure 6.** Age from daily rings on statoliths as a function of dorsal mantle length for each female *Loligo opalescens* (N=129) sampled during research surveys. Reproductive state of females identified by symbols.

**Table 3.** Percentage of spawning female market squid classed by the number of eggs in their oviducts and by the number of ages of postovulatory follicles (POF) in their ovaries.

Number of Eggs in the Oviduct	Number of Females	Percentage of Females	
		1- 2 ages POF	≥3 ages POF
0	1	100	0
1 - 300	36	22	78
301 - 600	20	35	65
601 - 900	10	20	80
901 - 1200	7	0	100
1201 - 1500	2	0	100
1501 - 1800	2	0	100



**Figure 7.** The number of oocytes in ovaries of 98 *Loligo opalescens* as a function of dorsal mantle length (A), the diameter of the major axis of the smallest oocyte (B), and mantle condition index (the dry weight per surface area of a mantle tissue disc, C). Line in A expresses potential fecundity as a function of dorsal mantle length ( $E_p = 29.8L$ ) for the 13 mature-pre-ovulatory females.

Beeman (1978) and our own histological analysis that new oocytes (primary oögonia, stage I) are not recruited during the spawning period, and therefore supports the notion of a fixed potential fecundity in *L. opalescens*. Ovaries of immature females contain an excess of oocytes, some of which undergo atresia. Potential fecundity ( $E_p$ ) can be estimated only by using mature females prior to first ovulation. Classification of mature-preovulatory females is certain only with histology to ensure absence of postovulatory follicles. Result of simple regression of dorsal mantle length ( $L$  in mm) on the number of oocytes from 13 mature-preovulatory females (Figure 7) was  $E_p = 29.8L$  with a pseudo  $r^2$  of 0.92. A 129 mm mature female has a potential fecundity of 3844 (SE 317) oocytes.

### Residual fecundity

Few if any *L. opalescens* live to realize their full potential fecundity. Knipe and Beeman (1978) indicated “spawned out”, dying, or dead had some oocytes remaining in their ovaries when examined histologically. During the present study divers collected a dying female with 1487 oocytes remaining in her ovary (Figures 4F, 7). Mantle disc dry weight in milligrams per square millimeter of disc surface area was used as a crude index of mantle condition ( $C$ ) of mature females. Early in the spawning period, when  $C$  is decreasing from 0.8 to 0.6 mg/mm<sup>2</sup> (Figure 7C), the number of oocytes in the ovary declines rapidly but later on the number of oocytes declines more gradually. To provide quantitative estimates of the rate of decline of fecundity through the spawning period we fitted a nonlinear model to the fecundity data of 75 mature-spawning females from our research cruises (Figure 8). We assumed that a female squid meeting extreme conditions, with the most advanced ovary ( $D=0.771$ ) and the thinnest mantle ( $C=0.323$ ) observed, would not spawn again and a female of 129 mm  $L$  may have a residual fecundity ( $E_r$ ) of 834 oocytes (c.v. = 0.12).

### Maximum lifetime fecundity

A 129 mm female *L. opalescens* might be expected to release a maximum of about 3010 eggs ( $E_p - E_r = 3844 - 834$ ) in her lifetime: about 78% of her  $E_p$ . However, very few females would be expected to realize 78% of their potential since this maximum is based on extreme values for both mantle condition and ovarian maturation. In fact in the overall spawning population (Table 4), only 1.5% of the females had  $C \leq 0.349$  mg/mm<sup>2</sup>.

### Direct estimation of fecundity of 60 females from landed catch

Ovaries from females landed by the squid fishery are not useable for fine-scale histological analysis to determine preovulatory females. Since the 13 mature-preovulatory females had a mean  $C$  of 0.73 mg/mm<sup>2</sup> (2SE was  $\pm 0.02$ ),

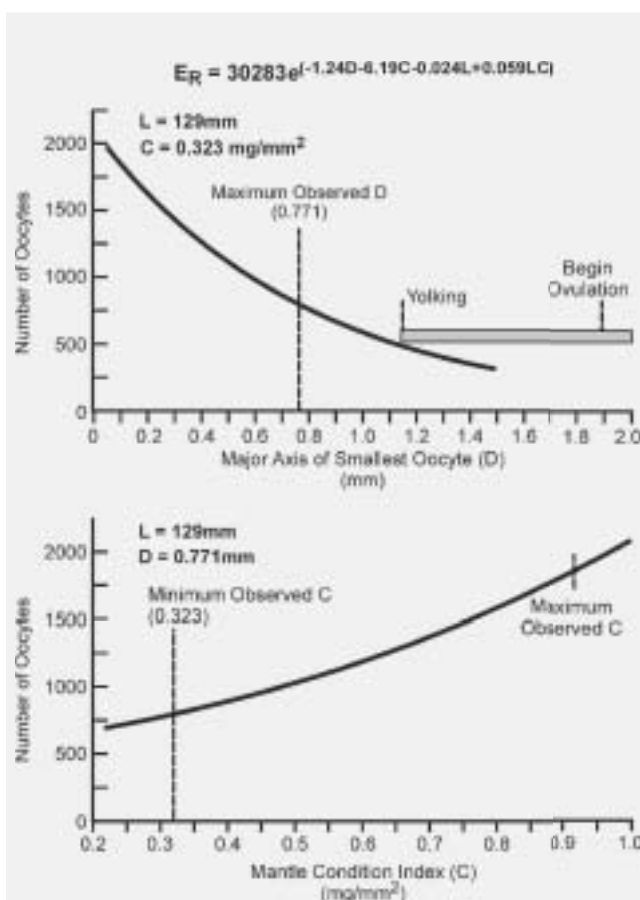
**Table 4.** Distribution of mantle condition index for 1275 mature female *L. opalescens* sampled from the landed catch during December 1998 to December 1999.

Mantle Condition Index (mg/mm <sup>2</sup> )	Mature Females Number Percentage	
	0.263 - 0.299	4
0.300 - 0.349	15	1.2
0.350 - 0.399	29	2.3
0.400 - 0.449	54	4.2
0.450 - 0.499	91	7.1
0.500 - 0.549	128	10.0
0.550 - 0.599	207	16.2
0.600 - 0.649	210	16.5
0.650 - 0.699	216	16.9
0.700 - 0.749	137	10.7
0.750 - 0.799	94	7.4
0.800 - 0.849	53	4.2
0.850 - 0.899	18	1.4
0.900 - 0.949	10	0.8
0.950 - 0.999	6	0.5
1.000 - 1.043	3	0.2

we used values of  $C \geq 0.7$  mg/mm<sup>2</sup> to identify 22 of the 60 females as being close to the preovulatory state. Indeed the mean total fecundity (3890 oocytes and ova) for the 22 females was within 5% of the computed potential fecundity (4083) based on their mean length (137mm). This mean is a minimum value because some of the ova in oviducts may have already been deposited in egg capsules on the spawning grounds. Two additional classes were arbitrarily set using values of  $C \leq 0.49$  mg/mm<sup>2</sup> and  $C = 0.5$ - $0.69$  mg/mm<sup>2</sup>. We used fecundity data for these 60 females with the research survey data for subsequent analyses.

#### Endogenous support of oocyte maturation

The oocyte maturation and egg deposition of the potential fecundity (3844) for a 129 mm female would require 65.6% ( $6.8/10.37 \times 100$ ) of her preovulatory dry-body weight; calculations are  $6.8g = 3844 \times 0.00177$  (the dry weight of a squid egg including its portion of the capsule) and  $10.37g = 0.24$  (conversion factor from wet to dry mantle disc weight)  $\times 43.2g$  (preovulatory wet-body weight estimated from the relation of mantle length and body weight for immature and mature-preovulatory females of  $W_w = 0.000051L^{2.8086}$ ). If we convert the decline in body weight loss from release of eggs to proportional changes in  $C$  (starting at  $C = 0.798$ ,

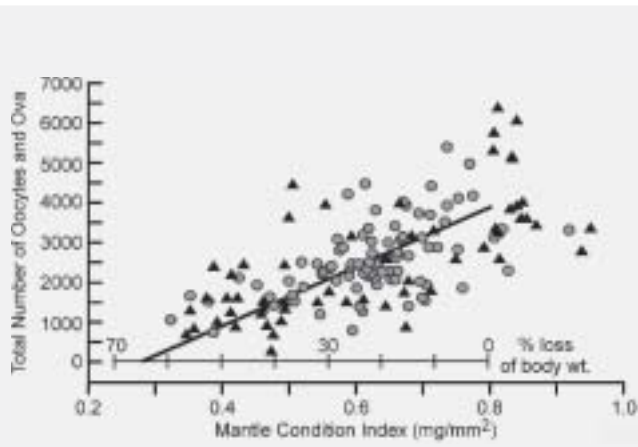


**Figure 8.** Changes in the standing stock of oocytes predicted by the equation when the major axis of smallest oocyte ( $D$ ) is varied and mantle condition index ( $C$ ) held constant (upper panel), and when  $C$  is varied and  $D$  held constant (lower panel). Major axis size of oocyte when yolking begins and when ovulation begins is also indicated, as is the maximum observed  $D$  and minimum observed  $C$ , respectively. Substitution of the latter two values into the equation yields the standing stock of oocytes of females close to the end of their reproductive activity, and is considered to be a minimum estimate of residual fecundity.

the mean for values of  $C \geq 0.7$ ), we find that the hypothetical line follows the general trend in the decline of total fecundity with  $C$  (Figure 9) indicating that the production of eggs could be largely, if not entirely, financed by conversion of mantle tissue to eggs, although some feeding has been observed on the spawning ground (Butler, pers. comm.).

#### Average total fecundity and the fraction of potential fecundity released before capture

The total fecundity ( $E_{YD}$ , total number of oocytes and ova present inside the female) of those sampled from landed fishery catches can give us fecundity remaining in the catch. The estimated average total fecundity for the stock caught and sampled during December 1998-1999 is about 2599 oocytes and ova which is about 0.674 of the potential fecundity (3859) of the average size female taken in the fishery (129.5 mm dorsal mantle length). This estimate



**Figure 9.** Standing stock of oocytes and ova as a function of mantle condition index for 60 mature females taken in the fishery (triangles) and 87 mature females taken in research surveys (circles). The hypothetical line indicates the expected relation if losses in body weight were equivalent to the weight of the spawn released. Line is computed for a 129 mm female where the starting point is her potential fecundity of 3844 oocytes and a mantle condition index (C) of 0.798, and the ending point is 0 eggs and C of 0.275 mg/mm<sup>2</sup>.

was calculated by weighting our combined data (Table 5) of mean total fecundity by the frequency of C observed in the 1275 females sampled from the catch (Table 4) during 1998-1999. Therefore the average number of eggs released was 1260 ( $E_p - E_{YD} = 3859 - 2599$ ), equivalent to 0.326 of the average potential fecundity escaping capture.

**Proxies for measuring fecundity of the catch**

We investigated three methods for indirectly estimating  $E_{YD}$  of mature females caught and landed by the fishery, because directly estimating oocytes and ova is not practical as *L. opalescens* spawn year round. The best method (pseudo  $r^2 = 0.6$ ) used C and G (gonad weight: ovary and oviduct weighed together), as predictors:  $E_{YD} = 378e^{(2.33C + 0.2447G - 0.24CG)}$ . Another method used C and L to predict standing stock of oocytes ( $E_Y = 220.453e^{(1.99C + 0.0079L)}$ ) but it explained only 33% of the variability and an estimate of the number of ova remaining in the oviduct would be needed to obtain the total sum of oocytes and ova remaining in the female. A third relationship used only C to predict the total stock of oocytes and ova:  $E_{YD} = 728.82e^{(1.95C)}$  but was less precise ( $r^2 = 0.42$ ) than using both G and C.

**Table 5.** The mean fecundity for various classes of mantle condition for spawning *Loligo opalescens* and the grand mean weighted by the frequency of mantle condition classes in fishery samples 1998-1999.

Mantle Condition Index (mg/mm <sup>2</sup> )	Number of Spawning Females	Mean Fecundity (SE)			Dorsal Mantle Length (mm)	Mean Potential Fecundity <sup>a</sup> ( $E_p$ ) (SE)	Number of Eggs Deposited ( $E_{SP} = E_p - E_{YD}$ )	Weighting Factors (Table 4)
		Oocytes in ovary ( $E_Y$ )	Ova in oviduct ( $E_D$ )	Total ( $E_{YD}$ )				
≤0.499	31	1212 (93)	207 (40)	1419 (100)	133 (2.08)	3954 (384)	2535	0.151
0.500-0.699	70	2008 (84)	437 (49)	2496 (100)	128 (1.20)	3813 (340)	1317	0.597
≥0.700	34	2571 (202)	1073 (109)	3657 (210)	135 (2.37)	4020 (385)	363	0.252
0.323-0.951	135	1967 (83)	544 (47)	2541 (102)	131 (1.01)	3897 (336)	1356	
				2599 <sup>b</sup>	129.5	3859 (320)	1260 <sup>c</sup>	

a Potential fecundity estimated by:  $E_p = 29.8L$ , where L = dorsal mantle length in millimeters

b Product of population  $E_p$  and weighted average of the fraction of potential fecundity remaining in spawning females):  $3859 \times [(1419/3954 \times 0.151) + (2496/3813 \times 0.597) + (3657/4020 \times 0.252)] = 2599$ ; or the population  $E_p$  less  $E_{SP(weighted)}$  which is  $3859 - 1260$

c Weighted average of the number of eggs deposited  $[(2535 \times 0.151) + (1317 \times 0.597) + (363 \times 0.252)]$

## DISCUSSION

Estimation of the egg deposition rate and mortality of adult market squid on the spawning grounds are important life table parameters that must be considered to calculate the proportion of potential fecundity escaping the fishery. Neither mortality nor egg deposition rates have been measured directly. We are investigating indirect sources of information to help delimit the general domain of these variables. Additionally, the proportion of immature and mature- preovulatory females in the catch should be monitored because if either increases dramatically then release of eggs may drop to a level of concern. We think estimation of fecundity of the catch is important in monitoring the fishery and egg escapement.

## ACKNOWLEDGMENTS

This was a cooperative project between the California Department of Fish & Game (CDF&G) and the National Marine Fisheries Service (NMFS) from start to finish. We worked closely with CDF&G personnel throughout the study with port-sampling data and cruise time, and CDF&G provided partial financial support.

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# Some aspects of estimating proportions mature and potential implications for stock predictions

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## ABSTRACT

Some aspects of estimating proportions mature are shown using North East Arctic haddock (*Melanogrammus aeglefinus* L.) as an example. Data from the Norwegian Barents Sea bottom trawl surveys in February from 1989 through 2001 are used together with data from an acoustic survey off Lofoten in the end of March. Proportions mature at age differ when comparing proportions of numbers with proportions of biomass. Observed proportions of female spawners in the total spawning stock are also varying. The overall picture is further confused by different yearclasses having possible differences in their geographical distribution and migratory patterns.

## INTRODUCTION

Haddock is the second most important of the commercially exploited groundfish species in the Barents Sea. In the years covered by the analyses presented in this paper, landings have ranged from 26 000 tons in 1990 to a high of 173 000 tons in 1996. Previous catch statistics show an all time high of 322 000 tons landed in 1973.

Previous studies have shown large fluctuations in maturity at length and/or maturity at age somehow connected to the large variations in year class strength (Templeman et al., 1978; Beacham, 1983; Tormosova, 1983; Kovtsova, 1993; Korsbrekke, 1999). Large fluctuations in recruitment have also led to large fluctuations in spawning stock size. The main spawning areas are on the continental slopes in the western part of the Barents Sea and as far south as Røstbanken and spawning takes place from the end of March, but mostly towards the end of April (Solemdal et al., 1989; Solemdal et al., 1997).

Estimating the spawning biomass (or the spawning potential) of any commercially exploited stock is considered important for assessment purposes. Traditional estimation uses observed fractions, but these can contain so much noise that a modelled maturity ogive (smoothed ogive) will be preferred.

## MATERIAL AND METHODS

### The Barents Sea survey

Data from the Norwegian Barents Sea bottom trawl survey collected from 1989 to 2001 are used in this study. The survey is a combined acoustic and bottom trawl survey for demersal fish and is conducted annually from the end of January to the beginning of March. The main aim of the survey is to map the spatial distribution and obtain indices of abundance for the most important commercially exploited species in the Barents Sea. The target species are cod (*Gadus morhua*), haddock, golden redfish (*Sebastes marinus*), beaked redfish (*Sebastes mentella*) and Greenland halibut (*Reinhardtius hippoglossoides*). The survey area consists of 23 strata and these are grouped into 7 subareas (A, B, C, D, D', E and S). The strata system together with the bottom trawl stations taken in 1996 are shown in Figure 1.

### The Lofoten survey

Data from the acoustic survey off Lofoten collected from 1989 to 2001 are used in this study. This acoustic survey is mainly targeting the spawning stock of North East Arctic cod. The survey area is the continental shelf from around 70°N south to and including Røstbanken and the Vestfjord area from the Lofoten islands and down to approximately 200 meters depth. This survey consists of equidistant paral-

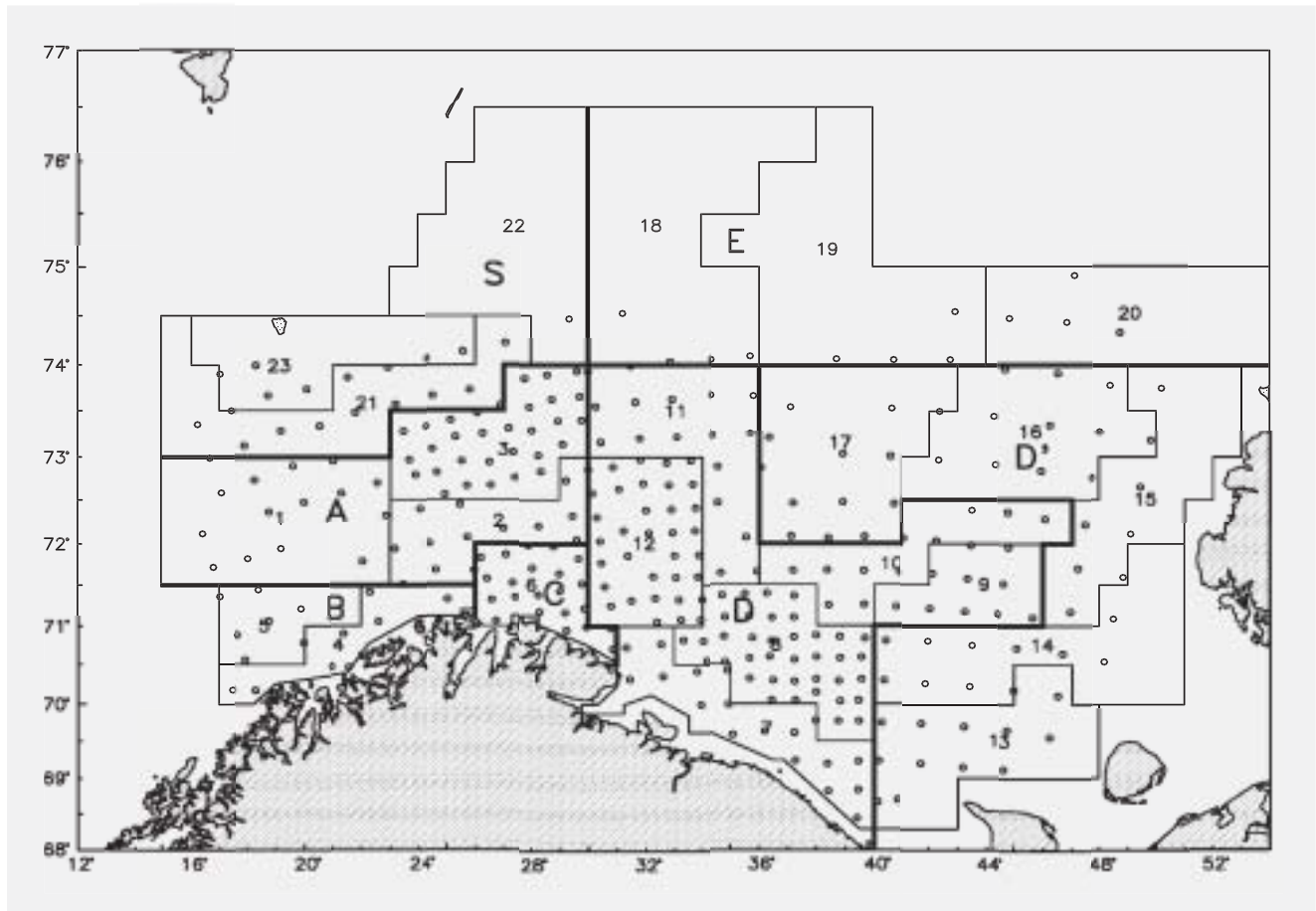


Figure 1. The Barents Sea survey with strata system and trawl stations (1996 survey).

lel acoustic transects with bottom trawl stations (and some pelagic trawl station) to estimate population composition and parameters. Observations indicate that most of the haddock in the survey area are in the echosounder dead-zone. Trawl stations are therefore used to produce traditional swept area estimates of abundance. Each station is treated as a random sample (this will not hold for cod, but could be a fair assumption for haddock). The survey tracks (1999) are shown in Figure 2. A further description of the survey can be found in Korsbrekke (1997).

**Determination of maturity stages and number of spawnings**

The macroscopic determination of maturity stages used in both surveys is given in the following table:

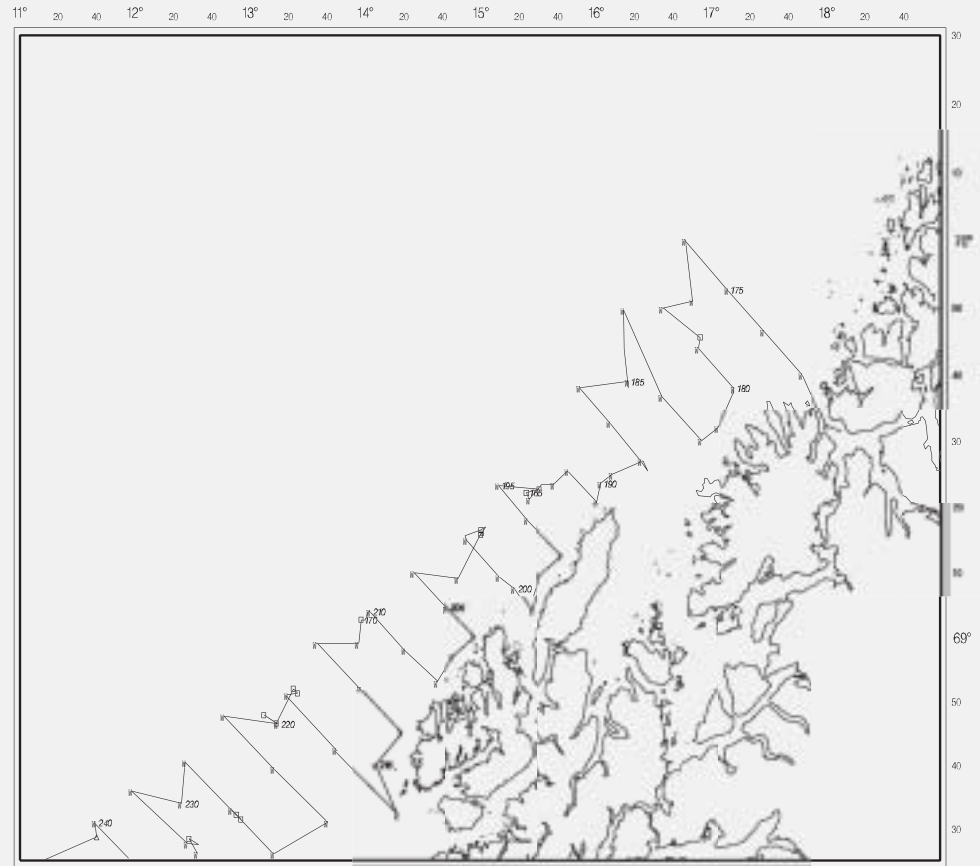
Samples with stages 2, 3 and 4 were treated as sexually mature fish while samples classified as uncertain (stage 5) were deleted from further analysis. All samples classified as maturing (stage 2) were clearly going to spawn that year. Only a limited number of spent (stage 4) were found and mainly in the Lofoten survey.

During age reading of otoliths the occurrence of narrow zones was assumed to be signs of previous spawning. No further investigations were performed to validate this.

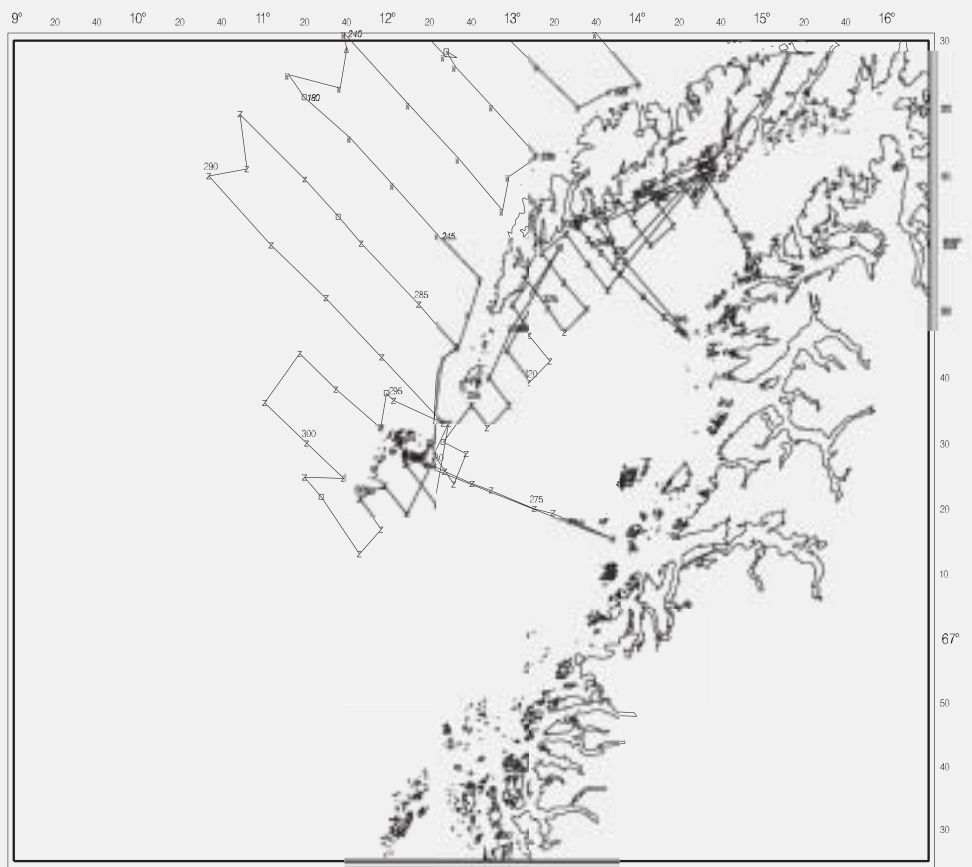
**Swept area estimation and age-length keys**

The bottom trawl stations taken during the Lofoten survey are assumed to represent fairly random stations relatively to the haddock occurring in the survey area. Swept area

Stage	Description
1	<b>Immature</b> , gonads are small, no visible eggs or milt
2	<b>Maturing</b> , gonads are larger in volume, eggs or milt are visible but not running
3	<b>Spawning</b> , running gonads, light pressure on the abdomen will release eggs or milt
4	<b>Spent</b> , gonads small, loose and/or bloody, regeneration starting, gonads somewhat larger and fuller than stage 1, no visible eggs or milt
5	<b>Uncertain</b> , used only when difficult to distinguish between stages 1 and 4



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Figure 2. Survey tracks from the Lofoten survey (1999).

estimates of abundance are calculated in the same way as for the Barents Sea survey. Length-based abundance indices are estimated in 5-cm length groups as

$$L_{p,l} = \frac{A_p}{N_p} \cdot \sum_{i=1}^{N_p} \rho_{i,l} \tag{1}$$

where

- $L_{p,l}$  is the abundance index, stratum  $p$ , and for length group  $l$
- $A_p$  is the area of stratum  $p$
- $N_p$  is the number of stations in stratum  $p$  and
- $\rho_{i,l}$  is the single station point observation of fish density.

Details on the calculation of point observations of density including the use of a length-dependent fishing width of the trawl can be found in Jakobsen *et al.* (1997). Length-stratified biological samples are given a weighting factor:

$$w_{p,l} = \frac{L_{p,l}}{n_{p,l}} \tag{2}$$

where

- $n_{p,l}$  is the number of biological (age) samples in stratum  $p$  and length group  $l$ .

Traditional age-length keys are extended to subarea-based age-sex-maturity-length keys using the following proportion:

$$P_{a,m,s}^{(l)} = \frac{\sum_p n_{p,a,m,s,l} \cdot w_{p,l}}{\sum_{p,a,m,s} n_{p,a,m,s,l} \cdot w_{p,l}} \tag{3}$$

where

- $P_{a,m,s}^{(l)}$  is the weighted proportion of age  $a$ , maturity  $m$  and sex  $s$  within length group  $l$  (for the subarea in question).
- $n_{p,a,m,s,l}$  is the number of biological samples in stratum  $p$  of age  $a$ , maturity  $m$  in length group  $l$ .

Indices of abundance are calculated by multiplying the proper proportion with  $L_{p,l}$  and then sum up over all length groups. Population parameters are calculated as weighted means. This can be done at different levels and not only at age. It is quite straightforward to calculate for example mean length at age and sex and also mean weight at age, sex and maturity. Such increased resolution in population

parameters can be used in future studies to further improve the goodness of maturity models. Such studies are also likely to gain more knowledge of the underlying processes leading up to variations in maturation.

**Estimation of maturity ogives**

Proportions sexually mature are modelled as a response probability using the logit link function (Nelder and Wedderburn, 1972):

$$\log\left(\frac{P}{1-P}\right) = \alpha + \beta^T x \tag{4}$$

Since the different age groups have a different maturation pattern relative to length (Korsbrekke, 1999) a model with different age-dependent intercepts was used:

$$\log\left(\frac{P}{1-P}\right) = \alpha_i + \beta^T x \tag{5}$$

$3 \leq i \leq 8$  being the different age groups analysed in this study. There was no indication in the material of any specimen being sexually mature at the age of 2 and no immature fish were found at age 9 or older. The only continuous explanatory variable used was weighted mean length at age using the combined data from both surveys. Thus, the model can be rewritten as:

$$P_i = \frac{\exp(\alpha_i + \beta L_i)}{1 + \exp(\alpha_i + \beta L_i)} \tag{6}$$

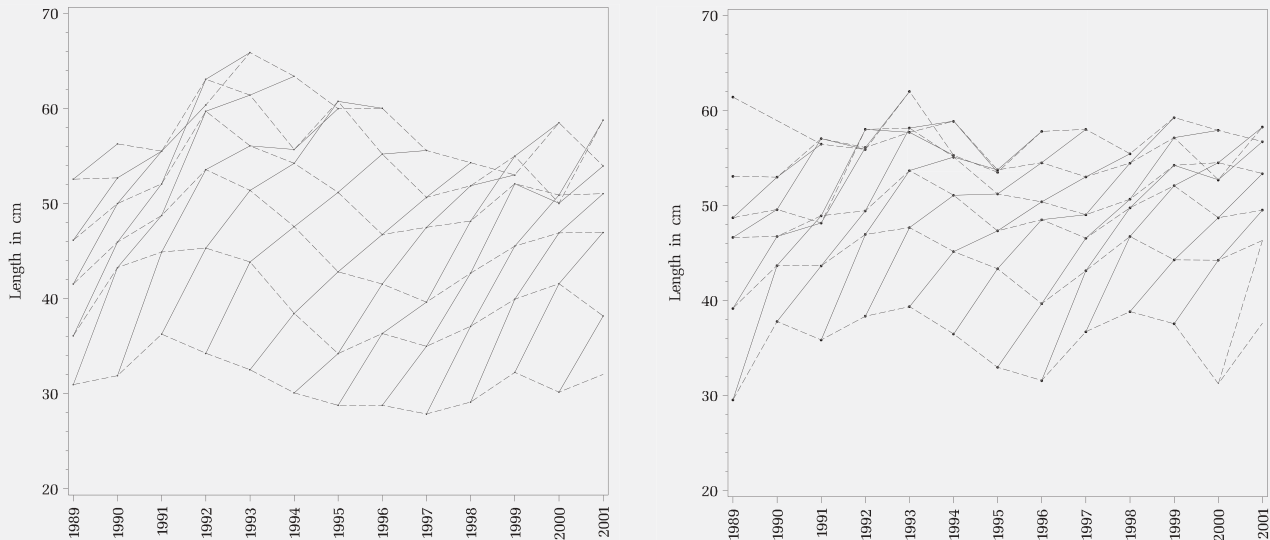
The regression parameters were estimated using weighted maximum likelihood. Each observation was given a weight as given in Equation (2) or the same weights multiplied with biomass. Thus, the weighting factors are equal to the swept area estimates (abundance indices) or biomass indices.

A number of four different models were fitted to the data. Model 1 and 2 are using the proportion of spawners as the dependent variable while model 3 and 4 are using the proportion of female spawners. Model 1 and 3 are using swept area estimates of numbers as weighting factors while model 2 and 4 are using swept area estimates of biomass as weighting factors.

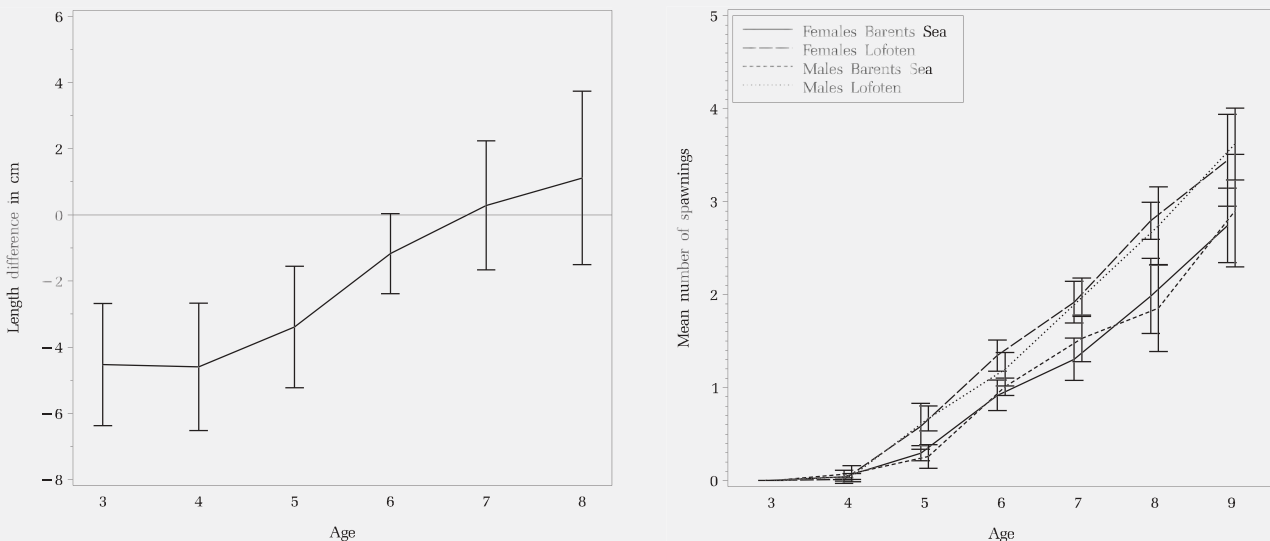
**RESULTS**

**Mean length at age and differences in abundance**

Mean length at age (and cohort) from both surveys is shown in Figure 3. Most age groups seem to peak at a maximum length at around 1992 or 1993. The younger age groups



**Figure 3.** Mean length at age (3-8) observed in the Barents Sea survey (left) and the Lofoten survey (right).



**Figure 4.** Difference in mean lengths observed in the Barents Sea survey and in the Lofoten survey (left). Mean number of spawnings observed in both surveys (by sex) are shown in the right panel. Vertical lines connect  $\pm 2$  SD.

have a higher length in the Lofoten survey, but the growth rates appear to be higher in the Barents Sea.

The higher growth rate observed in the Barents Sea can also be seen in Figure 4 (left panel) where the arithmetic mean of yearly differences in mean lengths is shown. There is an overall tendency that the mean length at age 3 is more than 4 cm higher in Lofoten than in the Barents Sea. This difference is reduced with age and while age groups 7 are of about the same length the mean length in age group 8 is higher in the Barents Sea than in Lofoten.

Repeat spawners shows reduced growth as compared to the immature part of the population. Figure 4 (right panel)

illustrates the difference in mean number of spawnings at age (spawning checks) for the two areas. Differences in observed length at age is result of different growth rates and possible migrations between the areas. The arithmetic mean of the difference between the (log) abundance indices from the two areas is shown in Figure 5. The Barents Sea indices are at times many hundred times higher than the Lofoten indices (especially at age 3 or 4), but sometimes are the indices higher in Lofoten (7 and 8 year olds).

#### Modelling proportions sexually mature

The above outlined 4 different models were applied to the combined datasets. The results are summarized in the following table:

Model no.	$\hat{\beta}$	$\hat{\alpha}_3$	$\hat{\alpha}_4$	$\hat{\alpha}_5$	$\hat{\alpha}_6$	$\hat{\alpha}_7$	$\hat{\alpha}_8$	Weighing factor	$r^2$
1	0.2050	-11.21	-10.55	-10.26	-9.83	-8.16	-5.95	Numbers	0.7713
2	0.1859	-10.13	-9.48	-9.10	-8.70	-6.98	-5.27	Biomass	0.5948
3	0.1766	-11.79	-10.70	-10.12	-9.68	-9.47	-10.11	Numbers	0.8686
4	0.1542	-10.45	-9.42	-8.73	-8.35	-8.07	-8.72	Biomass	0.7049

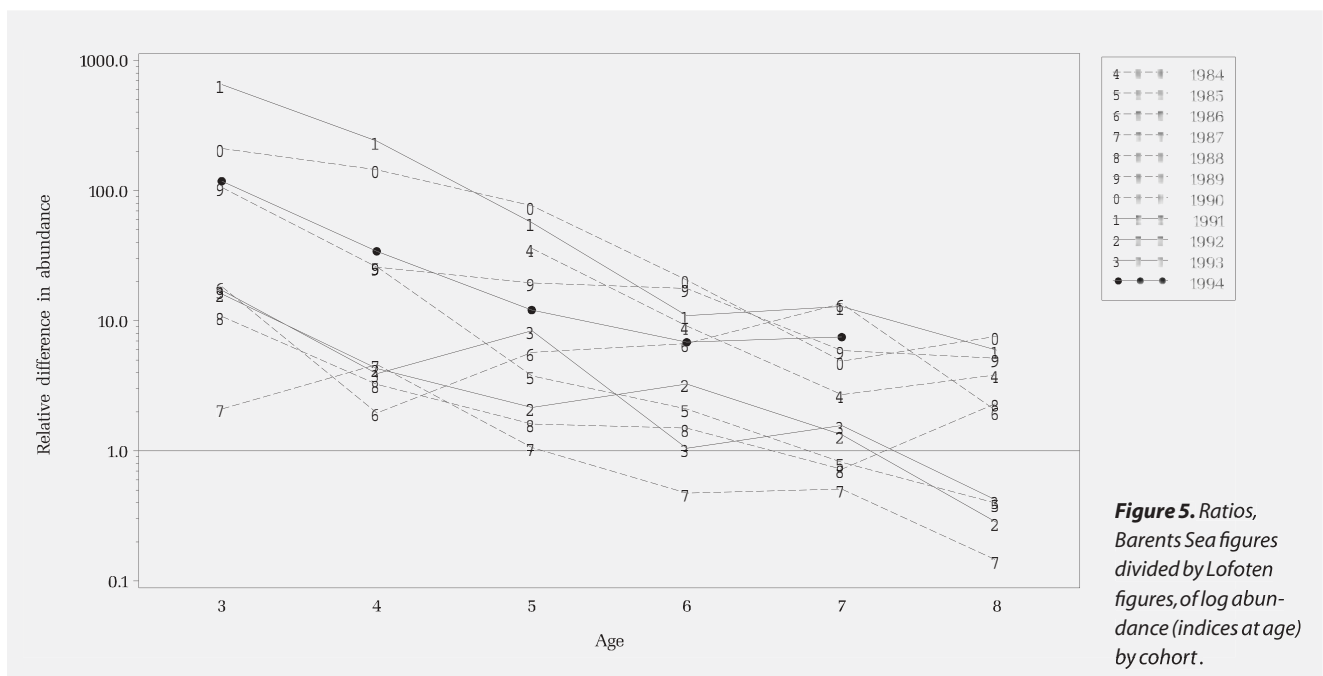
The best fit was achieved when modelling proportions of numbers, while the modelling of female spawners gave a better fit than modelling all spawners. Model 4 is visualized in Figure 6A where the lines connect minimum and maximum observed length at age. Age groups 3-5 shows a nice fit to the observed proportions of female spawning biomass, while age groups 6-8 have a reduced fit. Predicted and observed proportions of female spawning biomass at ages 6-8 are compared in Figure 6B-D. The predicted proportions by age and year are shown on the left hand side of Figure 7 and compared with the proportions used by the Arctic Fisheries Working Group (AFWG) on the right hand side. Applying these proportions on stock numbers at age yields the results shown in Figure 8.

**DISCUSSION**

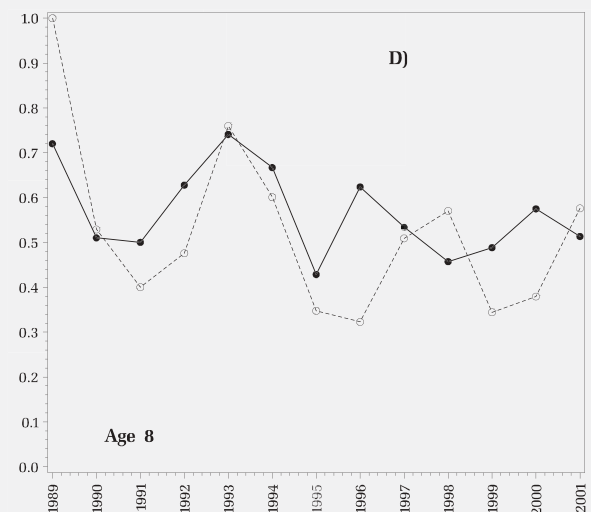
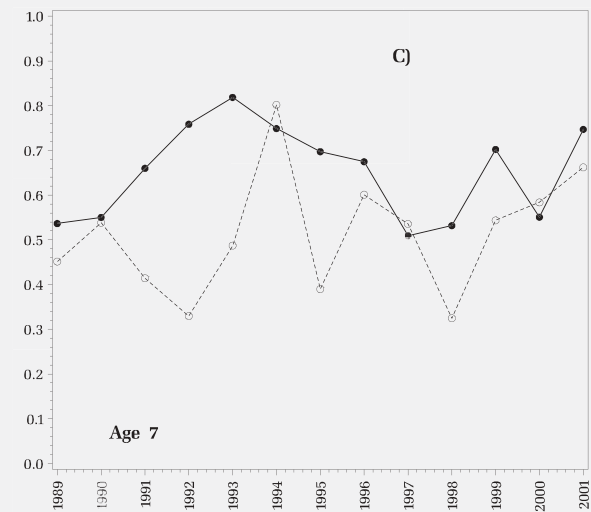
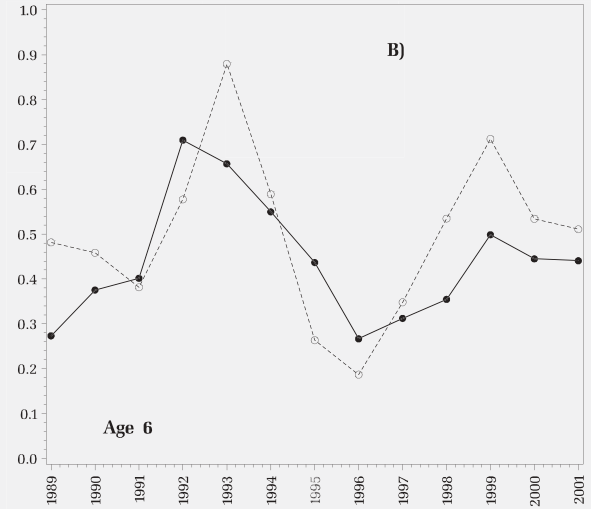
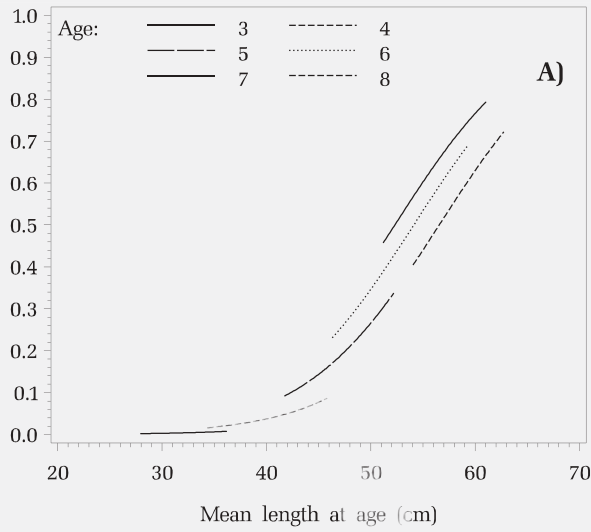
Length at age shows clear and similar trends in both areas (Figure 3). The variation is larger in the Barents Sea, and both surveys demonstrate clear cohort effects in addition to year effects. The cohorts 1991 and 1992 show reduced growth relative to most other cohorts and this should be viewed in relation to possible density dependent effects since 1989, 1990 and 1991 are the three largest yearclasses observed in this material. Figure 4 (left panel) shows that

there is an overall tendency for younger fish to be larger in the Lofoten area than in the Barents Sea; 3 and 4 year olds are on average more than 4 cm longer in the Lofoten area, while 7 year olds and older are of similar length and possibly longer in the Barents Sea. The observed differences in length at age is a consequence of at least two factors:

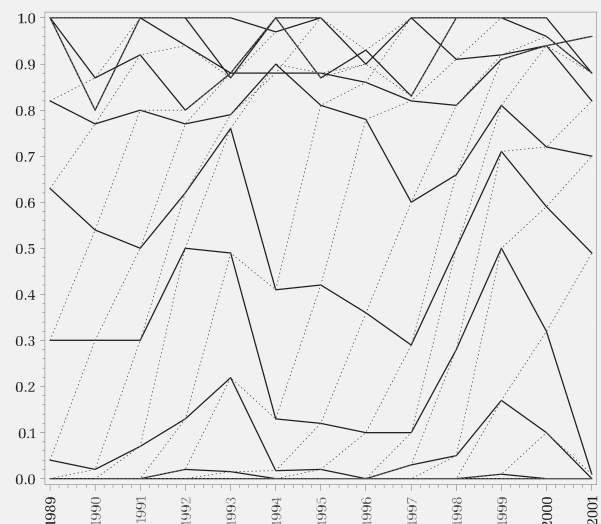
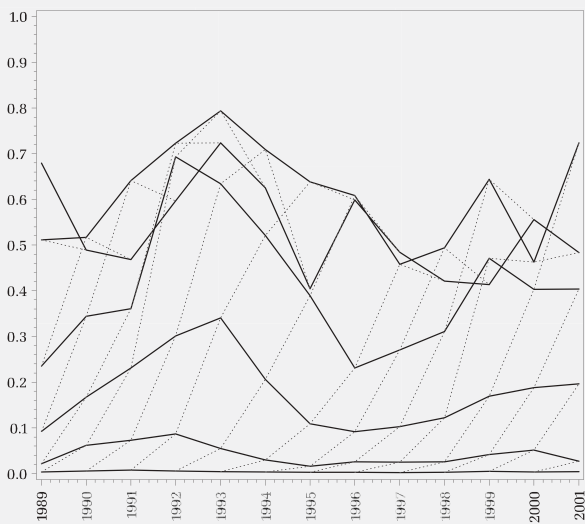
1. The true underlying growth rates are likely to be different in the two areas due to different climatic conditions and prey availability. This would imply earlier maturation in the Lofoten area (higher growth of immature fish) and reduced growth after maturity due to the diversion of energy to reproduction.
2. Figure 5 clearly demonstrates a migration with age towards the Lofoten area and if this (possibly spawning) migration is size dependent the largest individual in the youngest migrating age groups will show up in that area and thus affect the observed length at age. The migratory trend is very clear for all cohorts except for the weakest 1986 yearclass. The strongest yearclasses in this material are the 1989-1991 yearclasses and they also show a larger difference between the two areas than weaker yearclasses. This points to a reduced or delayed migration for these yearclasses and, as point-



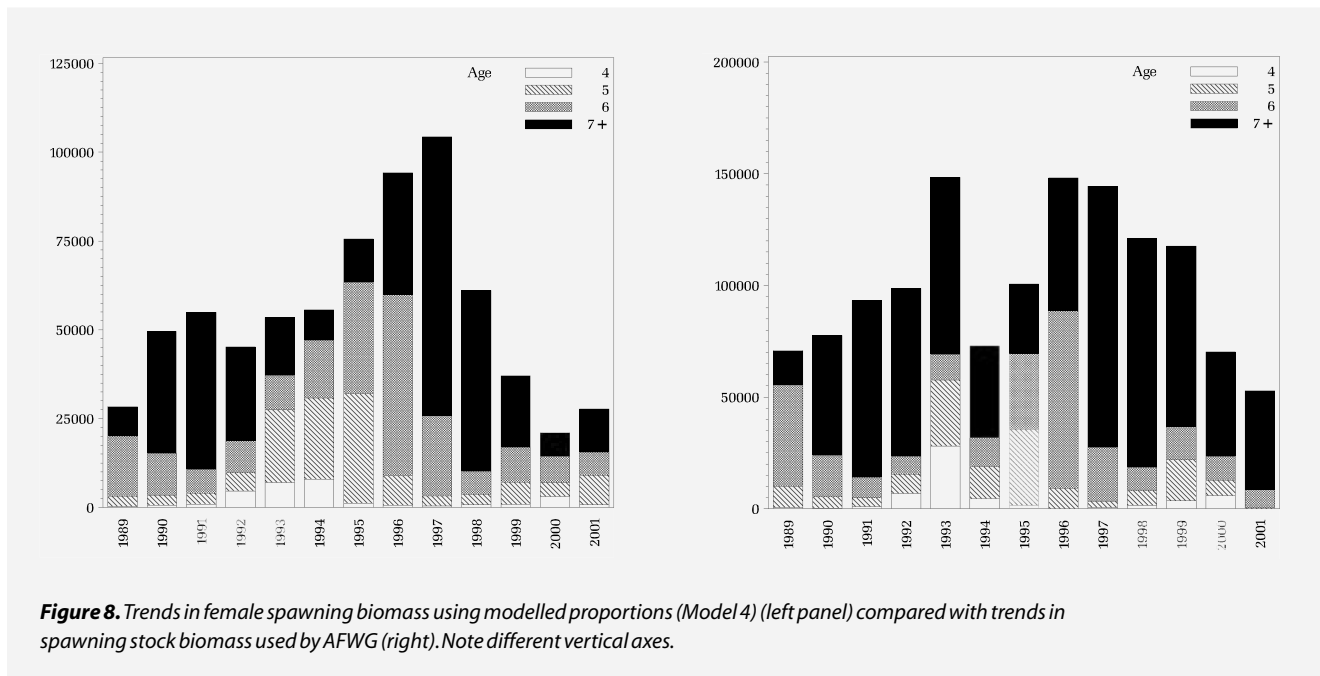
**Figure 5.** Ratios, Barents Sea figures divided by Lofoten figures, of log abundance (indices at age) by cohort.



**Figure 6.** A) Predicting the proportion female spawning biomass; visualization of model 4, lines connect minimum and maximum observed length at age. B), C) and D) Predicted proportion female biomass (solid line) compared with observed proportions (dotted line) for age groups 6, 7 and 8, respectively.



**Figure 7.** Predicted proportions female spawning biomass by age (solid line) and cohort (dotted line) (left panel) compared with proportion spawners used by AFWG (right panel).



**Figure 8.** Trends in female spawning biomass using modelled proportions (Model 4) (left panel) compared with trends in spawning stock biomass used by AFWG (right). Note different vertical axes.

ed out earlier, maybe due to lower growth. Figure 5 quantifies the change in relative abundance over time and it is clear that the migration of haddock from the Barents Sea to the west and south will be a dominating factor.

The spawners observed in the Lofoten area have a higher number of previous spawnings than spawners observed in the Barents Sea (Figure 4, right panel). Haddock is known to spawn along the continental edge from Lofoten to Bear Island, but it is not known how much the preferred spawning ground may change between years or if some year classes are spawning on different spawning grounds than others.

The mean weight of spawners (at age) is different from mean weight at age in the population. This difference is largest for the youngest fish in the spawning stock (ages 3-5) and could possibly lead to a large bias in the spawning stock biomass calculation for a large year class. The four different models presented tabled above illustrate clearly the difference between modelling proportion spawners in numbers and proportion spawners as proportion of total biomass. The biomass weighted models show a poorer fit than the others. This is to be expected due to the inclusion of one additional (stochastic) variable, namely the average weight at age. This would increase the level of “noise” and must be a part of the explanation for the reduced goodness of fit.

Previous work (Korsbrekke, 1999) has shown that males sexually mature approximately one year earlier than females. This will lead to trends in female spawning stock biomass that are somewhat delayed relative to the total

spawning stock biomass. This means that the use of female spawning stock biomass as an indicator of spawning potential yields a different perception than the use of traditional spawning stock biomass. This is partly demonstrated in Figure 8 where the female spawning stock biomass has a larger dynamic range and is also suggesting differences in trends. This could be of importance when the stock is assessed. Replacing the estimated spawning stock biomass with an other indicator of spawning potential will lead to revisions of limit reference points and could also give a different perception of the current stock status.

Observed and predicted proportion female spawning biomass is at times very high for agegroups 6 to 8. This problem can be linked to strong intra haul correlation, but could also point at a substantial and possibly varying spawning mortality for recruit male spawners. Future work should look into this problem in further details.

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# Fecundity regulation of wild Atlantic herring through resorption of atretic oocytes throughout maturation cycle

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## INTRODUCTION

Some recent reports suggest that reproductive traits of spawning fish likely affect recruitment dynamics (Kjesbu *et al.*, 1996; Marteinsdottir and Thorarinsson, 1998; Slotte and Fiksen, 2000). In addition, spawning stock biomass (SSB) is not necessarily proportional to population egg production because relative fecundity (fecundity/body weight) may be dependent on the age composition and/or nutritional condition of spawning fish (Hunter *et al.*, 1985; Trippel *et al.*, 1997; Kjesbu *et al.*, 1998,). Marshall *et al.* (1998) also showed that total egg production is a better index of recruitment potential than SSB for Atlantic cod, *Gadus morhua*, indicating the importance of accurate estimation of egg production to better understand the spawning - recruitment relationship.

Current life history and migration patterns of Norwegian spring spawning (NSS) herring, *Clupea harengus*, are as follows (Fig. 1). NSS herring spawn once a year from late February to early April on stony or rocky bottom below 250 m depth along the Norwegian coast (58 – 70 °N). They spawn for the first time in their life (recruit spawners) at an age of 3 – 5 years and 27 – 31.5 cm in total length (TL), after which (≥32 cm TL) they spawn every year (repeat spawners) (Slotte, 1999). After spawning, the fish feed in the Norwegian Sea, but migrate in September to a restricted coastal overwintering area within the Vestfjorden system (67 – 68 °N). They stay in this area until mid-January without taking food, followed by spawning migration. During overwintering and spawning migration, body reserves accumulated during the previous summer feeding season are the only source of energy for reproduction, migration, and routine metabolism (Slotte, 1999).

The objectives of this contribution are to establish relationships between oocyte growth (maturation cycle) and realized fecundity (i.e., subtracting for atresia) and to indicate when and which condition indices should be measured to reflect reproductive investment.

## RESULTS AND DISCUSSION

### Oocyte growth

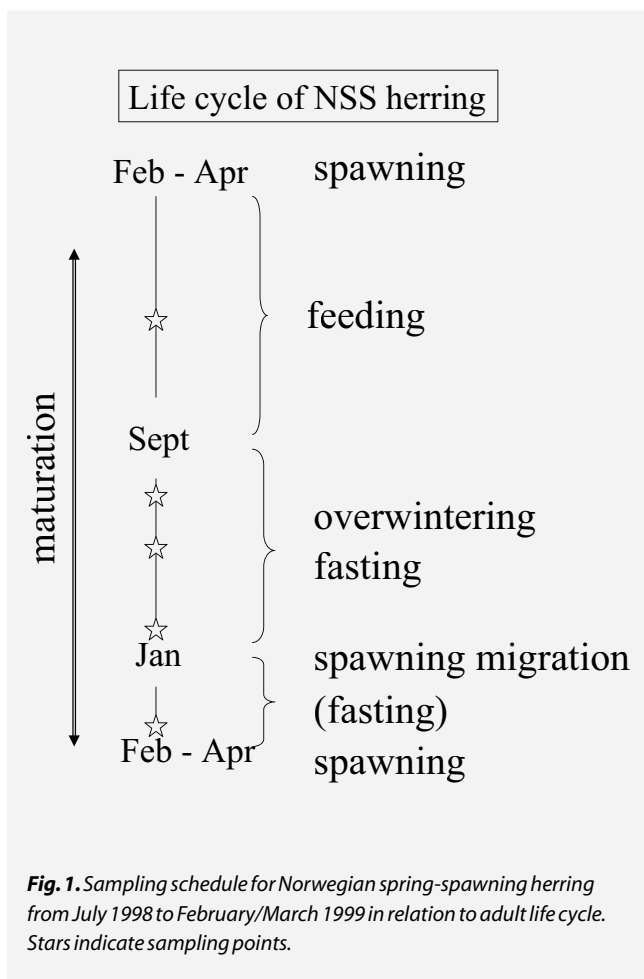
Our sampling schedule (Fig. 1) almost covered the maturation cycle especially for vitellogenesis because one third (15/45) of fish still had oocytes in cortical alveolus stage in July and 18% (7/40) had hydrated oocytes in February/March. Monthly mean oocyte diameter (OD, 314-1390 μm) except for hydrated oocyte changed according to the relationship:

$$OD = 3.75 \times ED + 402 \quad (r^2=1.00, n=5),$$

where ED is elapsed days from 1 July (Kurita *et al.*, 2003). As expected, oocyte volume increased to the power of three against diameter. Single oocyte weight also grew to the power of three against diameter because it was proportional to oocyte volume. Since gonad weight can be approximately expressed as a product of oocyte weight and fecundity, it can be expressed as a function of oocyte diameter and fecundity. Therefore GSI can be expressed with oocyte diameter, fecundity, and somatic weight (Fig. 2). Hydrated oocytes or ovaries should be dealt with more carefully in the estimations because oocyte weight increases rapidly with the extent of hydration.

### Fecundity regulation by atresia throughout maturation cycle

Fecundity of repeat spawners, which were larger than 32 cm TL, increased positively with body size (Kurita *et al.*,



**Fig. 1.** Sampling schedule for Norwegian spring-spawning herring from July 1998 to February/March 1999 in relation to adult life cycle. Stars indicate sampling points.

2000). When comparing fecundity of 32–34 cm fish, which was found to be independent of TL ( $p > 0.05$ ), fecundity decreased significantly from  $104,600 \pm 19,700$  (mean  $\pm$  SD,  $n=24$ ) in July to  $44,700 \pm 9,400$  ( $n=23$ ) in February/March, i.e., to about 43 % of that in July.

The noticed decrease in fecundity was concurrent and associated with seasonal changes in oocyte resorption. We counted only  $\alpha$ -stage atresia as an atretic oocyte (see Witthames, this volume). Alpha-stage atresia of vitellogenic oocytes was easily recognizable (Kurita *et al.*, 2003).

In an earlier phase of  $\alpha$ -stage atresia, chorion was distorted and fragmented, but in position (Fig. 3). Follicle cells became enlarged and yolk granules were disintegrated. Chorion gradually moved into deeper layers and follicle cells phagocytized yolk granules, and the oocyte *proper* became smaller. When all yolk was resorbed, the term  $\beta$ -atresia was, in agreement with traditional protocols, used. Both prevalence ( $P$ : (number of fish having atresia)/(total number of fish examined)) and average relative intensity of atresia among fish having atresia ( $I_a$ : geometric mean of relative intensity of atresia among fish having atresia) were zero in July when fish had just started vitellogenesis (Table 1). In contrast active resorption of developing oocytes occurred in October and November, i.e., almost all fish had atresia, and average relative intensity of atresia among all examined fish ( $= I_a \times P$ ) reached around 4 %. Resorption declined in January with prevalence decreasing to 35 % and average relative intensity of atresia to only 0.8 %, and very low in February/March. Average relative intensity of atresia for each oocyte diameter at 50  $\mu\text{m}$  intervals was high in October and in November (Fig. 4). Particularly, all fish with oocytes between 800 and 1000  $\mu\text{m}$  had atresia and average relative intensity exceeded 3 %.

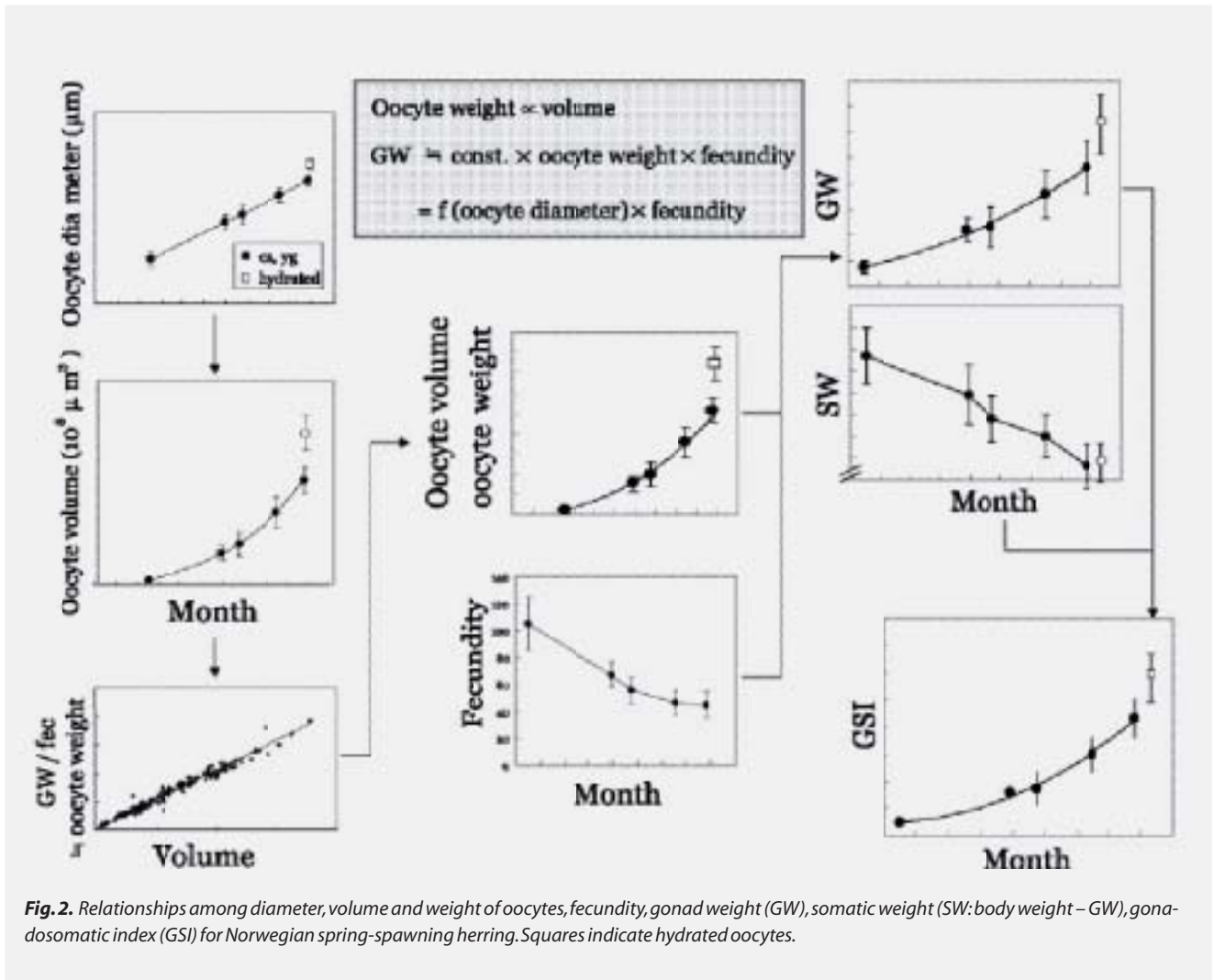
The duration of early  $\alpha$ -stage atresia was estimated at 4.3, 5.6, 7.3, and 6.6 days between July and October, October and November, November and January, and January and February/March, respectively (Table 2). For further information, see Kurita *et al.* (2003). These are comparable to 8 days for northern anchovy, *Engraulis mordax*, at  $15.5 - 16.5^\circ\text{C}$  (Hunter and Macewicz, 1985).

#### Condition control of reproductive investment

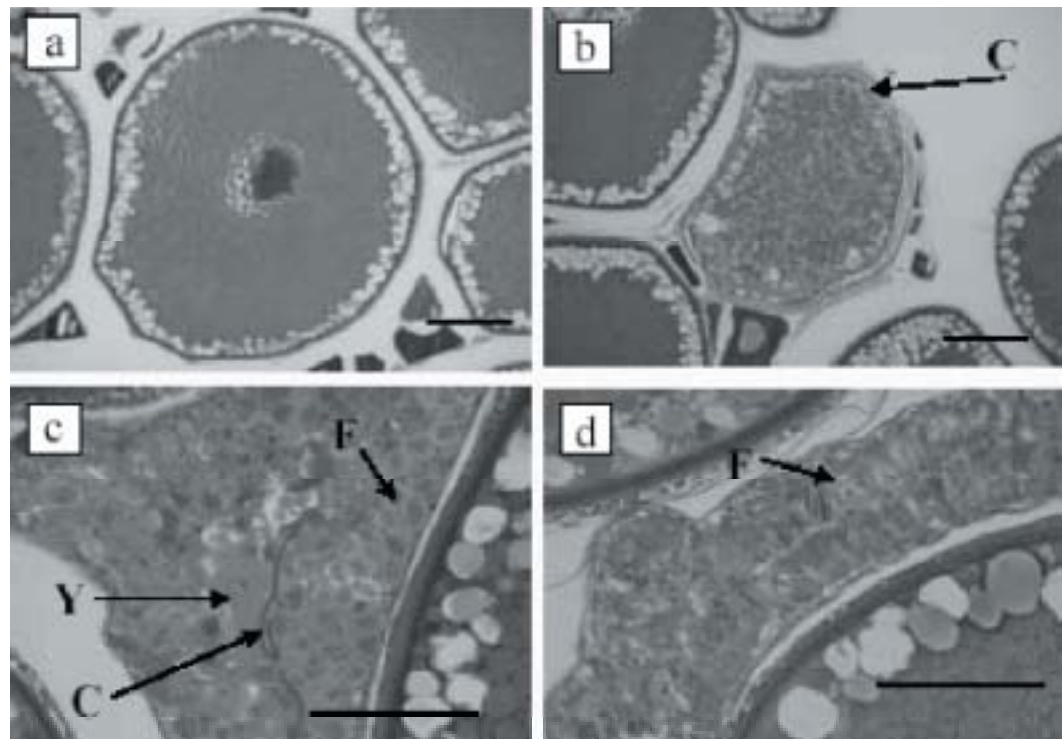
Fecundity for 32–34 cm fish in February/March varied from 26,200 to 63,400, and a strong condition effect was observed, i.e., 51% of fecundity variance was explained by condition factor (CF;  $100 \times (\text{body weight})/\text{TL}^3$ ;  $r^2 = 0.51$ ,  $p < 0.01$ ,  $n=22$ ; Fig. 5). Moreover, a condition effect on relative fecundity was observed for CF ( $r^2 = 0.18$ ,  $p = 0.05$ ,

**Table 1.** Characteristics of atresia from July 1998 to Feb/Mar 1999 for Norwegian spring spawning herring of 32–34 cm TL (based on Kurita *et al.*, 2003). Refer to the text for definition of each characteristic

Month	Number of females	Prevalence (%)	Average relative intensity (%)	
			among fish with atresia	among all fish
July	51	0	0	0
October	40	98	3.9	3.8
November	47	89	4.8	4.3
January	40	35	2.2	0.8
Feb/Mar	40	10	1.7	0.2



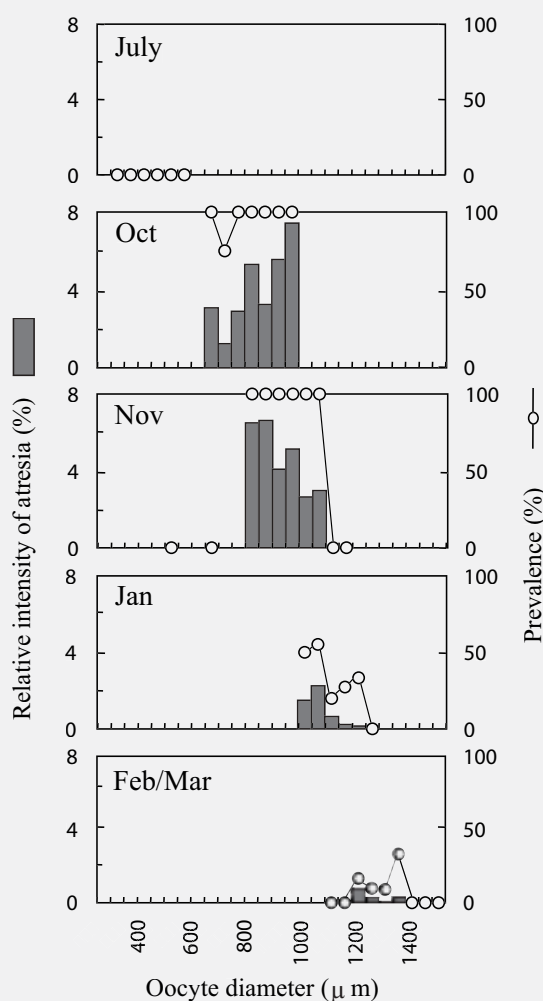
**Fig. 3.** Light micrographs of (a) a normal vitellogenic oocyte; (b) an early  $\alpha$ -stage; (c) a late  $\alpha$ -stage, and (d) a  $\beta$ -stage atretic oocyte of Norwegian spring-spawning herring. C, chorion; Y, yolk; F, follicle cell. Bars for (a) and (b) = 200  $\mu\text{m}$ , and for (c) and (d) = 100  $\mu\text{m}$ .



**Table 2.** Estimated duration of  $\alpha$ -atresia for Norwegian spring-spawning herring of 32–34 cm TL (based on Kurita et al., 2003).

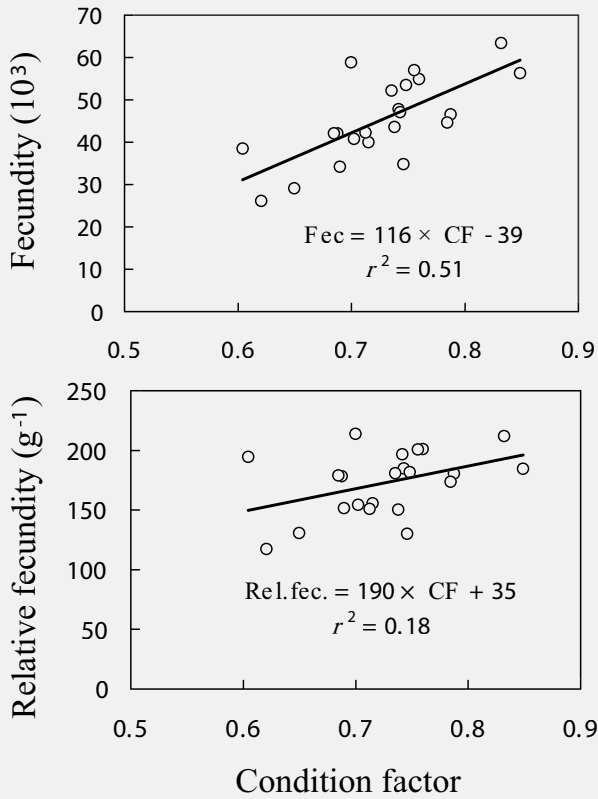
Date	Interval (day)	Temperature at 20 m deep	Number of females	Estimated fecundity	A (day)	Relative intensity of atresia (%)		Duration (day)
						for each month	average between successive months	
20 Jul		4.2 - 11.3	24	104600		0		
	100				0.0045		1.92	4.3
28 Oct		(9 - 10) <sup>a</sup>	27	66900		3.83		
	26				0.0073		4.04	5.6
24 Nov		6.8 - 7.2	40	55300		4.25		
	52				0.0035		2.52	7.3
15 Jan		5.8 - 6.6	29	46100		0.78		
	42				0.0007		0.48	6.6
26 Feb		5.8 - 6.7	28	44700		0.17		

A = coefficient of decrease.

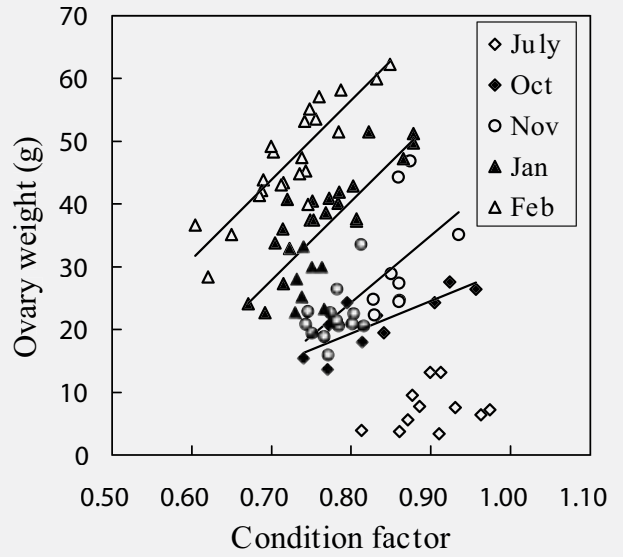
<sup>a</sup> Sea surface temperature informed by fishermen.**Fig. 4.** Changes in average relative intensity of atresia among fish having atresia and prevalence of atresia against each 50- $\mu$ m interval of oocyte diameter through maturation cycle (July 1998–February/March 1999) for Norwegian spring-spawning herring of 32–34 cm TL (Kurita et al., 2003).

$n=22$ ). Relative fecundity was found to vary by a factor of 1.3 (max/min). These variations in relative fecundity support previous results that total egg production spawned by populations even with the same SSB differ depending on fish condition (Óskarsson *et al.*, 2002). Moreover, spawning fraction in population may differ with condition (Óskarsson *et al.*, 2002). Age composition may also affect total egg production. Therefore, fecundity variation due to maternal traits should be considered in the study on population dynamics.

Single regression analyses between gonad weight (GW) and condition factor (CF) for a 33-cm standardised fish for each month from July to February/March suggested that the condition effect on GW had occurred before November because the slope of CF increased from July to November, but then stayed constant (Fig. 6). Therefore reproductive investment in the current spawning season appears to be established according to accumulated body reserves at around September and October (Fig. 7). For those fish which continue feeding during spawning season and the amount of food intake or nutritional condition in the spawning season immediately influence their reproductive output, *e.g.*, northern anchovy (Hunter and Macewicz, 1985) and Pacific anchovy, *Engraulis japonica* (Tsuruta and Hirose, 1989), somatic condition in the spawning season should be a better index for reproductive investment. On the other hand, for those fish which stop feeding in the middle of the maturation cycle, and which establish the possible amount of reproductive investment far before spawning season, like NSS herring, somatic weight or body condition as an index of reproductive investment should be examined at a period just before main regulation of reproductive invest-

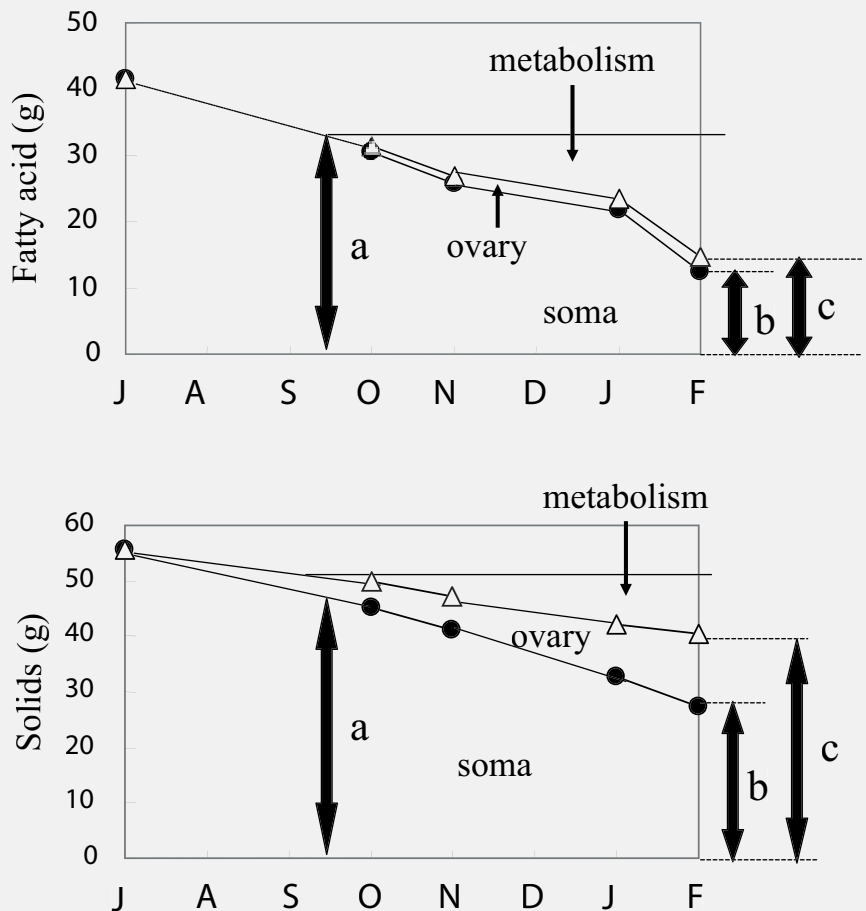


**Fig. 5.** Fecundity and relative fecundity against condition factor in February/March 1999 for Norwegian spring-spawning herring of 32-34 cm TL.



**Fig. 6.** Changes in relationships between gonad weight and condition factor through maturation cycle (July 1998 – February/March 1999) for Norwegian spring-spawning herring of 32-34 cm TL.

**Fig. 7.** Seasonal dynamics of fatty acid and solids (= water and fat free) weight in soma and ovary through maturation cycle (July 1998 – February/March 1999) for Norwegian spring-spawning herring. Arrows (a) indicate somatic weight around September and October, arrows (b) somatic weight in the spawning season, and arrows (c) body weight in the spawning season.



ment occur (arrow "a" in Fig. 7), *i.e.*, around September and October for NSS herring. However, somatic weight at spawning (arrow "b" in Fig. 7) for NSS herring shows only the remainder of body reserves, following investment in the gonad. Although it is not clear whether body weight at spawning (arrow "c" in Fig. 7) is proportional to somatic weight around September and October (arrow "a"), CF in the spawning season is considered to be a useful index of reproductive investment.

To summarize, fecundity regulation of NSS herring can be expressed as follows. During summer feeding season when the amount of materials to be invested in reproduction has not yet been established, fish produce high quanta of small vitellogenic oocytes independently of condition, while in autumn when the possible amount of investment is established and much more materials are needed for further oocyte growth, fecundity starts to be regulated depending on nutritional condition. Going through this active down-regulation period, only selected oocytes can be supported by surplus nutrition to reach final maturation and to be spawned.

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# Reproductive characteristics of the ommastrephid squid *Todarodes pacificus*

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## ABSTRACT

This paper reviews previous and present studies on the reproductive characteristics of the ommastrephid squid *Todarodes pacificus*, including captive studies of the mating and spawning behavior, maturation process, fecundity and egg development, and field surveys near the spawning grounds. These and other observations on the importance of seawater temperature near the spawning grounds on stock size are discussed. We then discuss the relationship between recent seasonal and annual changes in the inferred extent of the spawning grounds based on water temperatures around Japan and review a hypothesis explaining how climatic regime shifts might affect the reproductive process and result in stock fluctuations.

Key words: *Todarodes pacificus*, maturation, mating, spawning, egg production, stock fluctuation, climate change

## INTRODUCTION

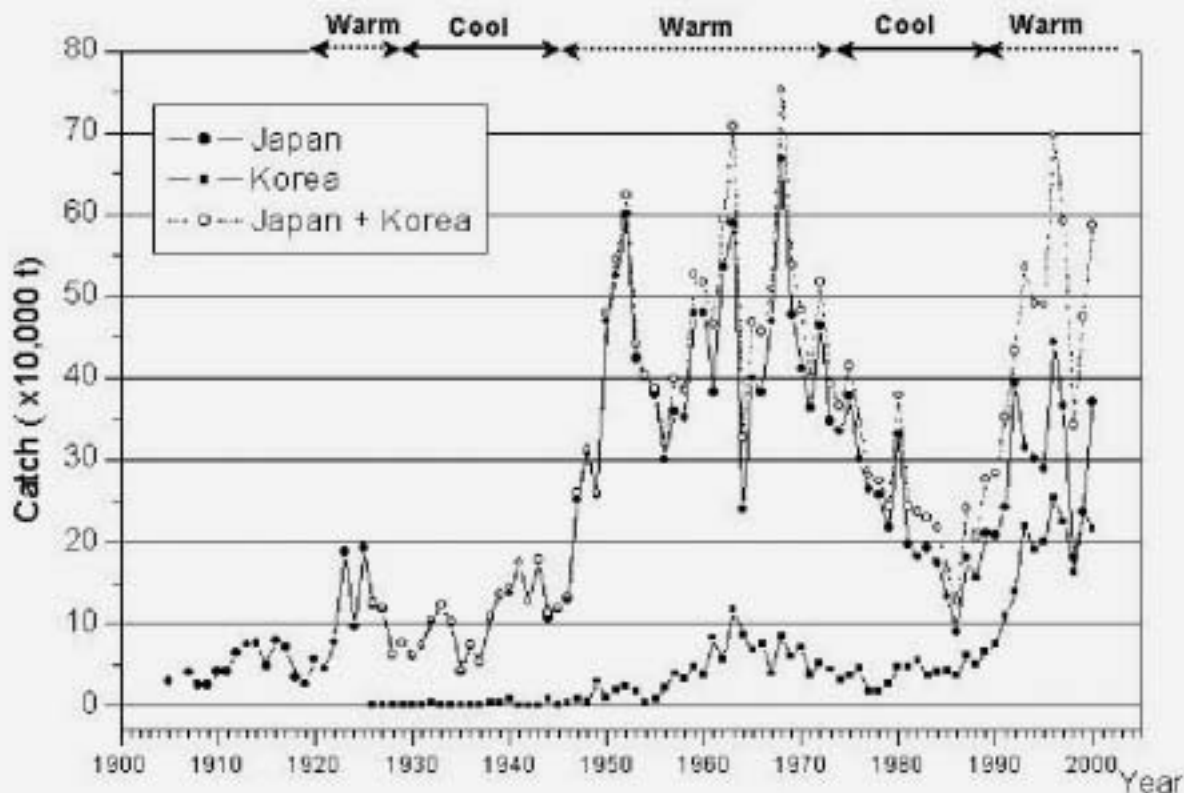
Cephalopods generally grow fast, reproduce once and then die (Mangold, 1987). This life cycle can be summed up in the phrase “live fast and die young” (O’Dor and Wells, 1987). In such short-lived species, reproductive and recruitment success is thought to depend heavily on the physical and biological environments they experience throughout the life cycle (Lipiński *et al.*, 1998). As groundfish landings have decreased and cephalopod landings have increased globally (Caddy and Rodhouse, 1998), understanding how environmental conditions affect squid populations has become increasingly important. However, a major impediment to such research includes our lack of knowledge of the basic biology of many squid species.

*Todarodes pacificus* (family Ommastrephidae) is a commercially important squid in Japan. Annual catches in Japan have fluctuated widely, with a marked increase occurring after the late 1980s; this increase appears to have been related to a climatic shift from a cool to a warm regime that occurred in 1988/89 (Sakurai *et al.*, 2000, 2002). Scientists from throughout Japan have studied the fishery biology of this species (reviewed by Murata, 1990), but most studies of its reproduction (Ikeda *et al.*, 1993a,b; Bower and Sakurai, 1996; Sakurai *et al.*, 1996; Watanabe *et al.*, 1996, Sakurai *et al.*, 2000) and gonad development (Ikeda *et al.*, 1991a,b) have been conducted at Hokkaido University.

This paper reviews previous and present studies on the reproductive characteristics of *T. pacificus* based on captive experiments and field surveys near its spawning grounds. We also review a hypothesis explaining how climatic regime shifts might affect the reproductive process and result in stock fluctuations.

## REPRODUCTIVE CHARACTERISTICS

*Todarodes pacificus* is a neritic-oceanic squid distributed in waters around Japan and Korea. Annual catches increased markedly after 1989 and have fluctuated widely since the 1990s (Fig. 1). Three populations with different peak spawning seasons (summer, autumn and winter) migrate seasonally between the Sea of Japan and the Pacific Ocean, with most spawning occurring near Kyushu Island and the Tsushima Strait (Fig. 2, Murata, 1990). The life span has been estimated to be one year based on analysis of statolith increments (Nakamura and Sakurai, 1991; 1993).



**Fig. 1.** Annual fluctuations in *Todarodes pacificus* catches of Korea and Japan during the 20<sup>th</sup> century. (Data derived from the Japan Sea National Fisheries Institute, Japan and the National Fisheries Research and Development Institute, Korea). Horizontal lines indicate interdecadal regime shifts in water temperature in the western North Pacific (Minobe, 1997). (Sakurai et al., 2002)

### GONAD DEVELOPMENT

The maturation process in *T. pacificus* has been studied by histological observations (Takahashi and Yahata, 1973; Ikeda *et al.*, 1991a,b). Takahashi and Yahata (1973) divided the process of oocyte maturation into eight stages from the oogonium production stage to the maturation and ovulation stage (Takahashi and Yahata, 1973), and Ikeda *et al.* (1991a) divided female maturation into six stages based on histological observation of ovaries. The stage composition in oocytes in ovaries of maturing females shows asynchronous development (Table 1). The female maturation process consists of two phases, and ovary and oviduct development are correlated with nidamental-gland development. In the first phase, ripe ova are produced in the ovary with rapid development of the nidamental gland, and in the next phase, ripe ova are transferred into the oviduct and stored there until spawning.

Ikeda *et al.* (1991b) divided male maturation into five stages. Spermatozoa are produced in the testis even when the testis is relatively small, and the male maturation process consists of two phases. In the first phase, spermatozoa are

produced in the testis, and in the next phase, spermatozoa are transferred into the accessory gland, where they are packed in spermatophores and stored until mating.

### MATURITY CRITERIA

A clear definition of the different maturity stages is of great importance for fisheries biologists, particularly for recognizing spawning populations. In *Illex illecebrosus* (another ommastrephid squid), Durward *et al.* (1979) showed that the relative length of the nidamental glands is well correlated with clearly defined stages in ovary development. Ikeda *et al.* (1991a) also showed that female maturity of *T. pacificus* is well correlated with both the gonad somatic index (*GSI*: ovary and oviduct weight as a percentage of body weight) and nidamental gland index (*m*: a ratio of nidamental-gland length to mantle length). Table 2 shows a comparison of female maturity conditions of *T. pacificus* between the morphological maturity scale used by the Japanese Fisheries Experimental Station and the histological maturity scale described by Ikeda *et al.* (1991a). In immature squid (i.e., those in maturity stages I-III), the *GSI* is <1.0% and *m* is <0.21. In maturing squid, the *GSI* is

**Table 1.** Summary of the stage composition in oocytes of *Todarodes pacificus* (Ikeda et al., 1991a)

Date	DML <sup>*1</sup> (mm)	Ovary weight (100mg)	GSI <sup>*2</sup> (%)	Number of eggs examined	Percentage of oocytes at each stages <sup>*3</sup>					
					EYL	LYL	EYF	MYF	LYF	M
9 Aug. '88	214	12	0.6	320	79.7	20.3				
6 Sep. '88	213	12	0.7	318	80.5	19.5				
20 Jul. '88	215	14	0.7	325	70.2	27.4	2.5			
9 Aug. '88	225	18	0.8	337	70.9	25.8	3.3			
9 Aug. '88	206	25	1.2	341	71.0	24.0	3.5	1.5		
8 Jun. '88	186	22	1.6	301	69.4	19.9	8.0	2.7		
23 Aug. '88	221	19	0.9	434	62.0	27.2	2.5	3.0	5.3	
23 Aug. '88	285	57	1.3	406	69.2	20.0	4.2	3.2	3.4	
23 Aug. '88	262	118	3.1	473	53.3	17.5	2.5	1.7	24.1	0.8
1 Sep. '88	238	57	2.1	424	52.6	21.7	1.9	1.7	21.7	0.5

<sup>\*1</sup> DML: dorsal mantle length,

<sup>\*2</sup> GSI: gonad somatic index (ovary and oviduct weight as a percentage of body weight)

<sup>\*3</sup> Oogenetic stage: EYL and LYL, early and late yolless stage; EYF, MYF and LYF, early, middle and late yolk formation stage; M: mature stage.

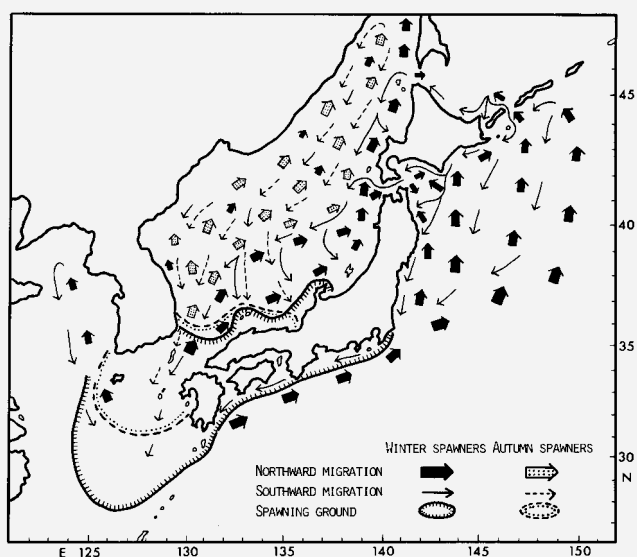
between 1.0 and 2.6% and  $m$  is between 0.21 and 0.29. In mature squid, the  $GSI$  is  $>2.6\%$  and  $m$  is  $>0.29$  (Fig. 3).

In male *T. pacificus*, Ikeda et al. (1991b) showed that maturity could be expressed numerically using the testis somatic index (TSI: testis weight as a percentage of body weight) and accessory gland somatic index (AGSI: accessory gland weight as a percentage of body weight). These numerical values are  $TSI > 0.5\%$  and  $AGSI > 0.1\%$  in the maturing stage, when there are no spermatophores and the vas deferens is

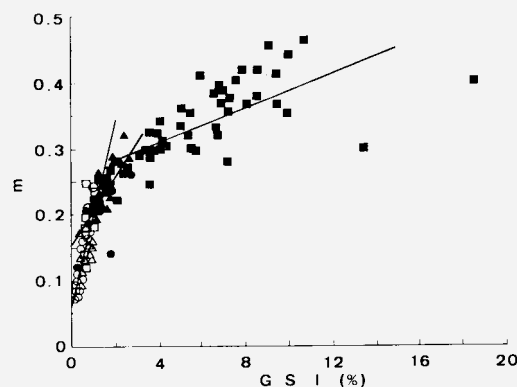
white, and  $TSI > 2.0\%$  and  $AGSI > 1.0\%$  in the mature stage, when spermatophores are present in the spermatophore sac and penis (Table 3 and Fig. 4).

#### MATURATION, MATING AND SPAWNING IN CAPTIVITY

Since 1988, Hokkaido University scientists have conducted captive experiments to clarify the reproductive characteristics of *T. pacificus* in a filtered, recirculating raceway tank (5.5 m in length, 2.5 m in width, 1.2 m in depth, and 15,000 L in capacity) at the Marine Biological Station of Hokkaido



**Fig. 2.** *Todarodes pacificus* spawning grounds and typical migration (winter and autumn spawning groups). (Murata, 1990)



**Fig. 3.** Relationship between the gonad weight and nidamental gland length during maturation in *Todarodes pacificus*.  $GSI$ , gonad somatic index (ovary and oviduct weight as a percentage of body weight).  $m$ , maturation index (the ratio of nidamental gland length to mantle length). Symbols indicate histological maturity; (○) early immature stage, (△) late immature stage, (□) early maturing stage, (●) mid maturing stage, (▲) late maturing stage, (■) mature stage. Three solid lines show the regression lines. (Ikeda et al., 1991a)

**Table 2.** Comparison of female maturity conditions of *Todarodes pacificus* between morphological maturity scale that is commonly used in the Japanese Fisheries Experimental Station (maturity A) and histological maturity scale (maturity B) by Ikeda et al. (1991a)

Maturity scale A	Maturity scale B
Immature (without ripe eggs)	I. Early immature stage II. Late immature stage III. Early maturing stage (GSI>1.0, m>0.21) IV. Mid maturing stage V. Late maturing stage
Mature (ripe eggs are present in the oviduct)	VI. Mature stage (GSI>2.6, m> 0.290)

GSI: see Table 1, m: maturation index (the ratio of nidamental gland length to dorsal mantle length)

University (Sakurai *et al.*, 1993). Immature squid have been collected from inshore waters of southern Hokkaido, Japan, and maintained in the tank, where they mature, mate and spawn. Figure 5 shows an example of a captive experiment conducted during 1988 (Ikeda *et al.*, 1993a).

Figures 6 and 7 show the progress of male and female sexual maturation in captive squid. At the start of the experiment, males were immature and maturing, and females were immature. Males matured earlier than females. Males began mating with immature females about two weeks after the beginning of the captive experiment and continued to do so through the end of the experiment (Fig. 6). Females matured two or three weeks after mating was first observed, stored ripe ova in their oviducts, spawned, and then died (Fig. 7).

During mating, a *T. pacificus* male deposits spermatophores on the buccal membrane of a female, and spermatozoa are stored in the female's seminal receptacles for several weeks until spawning (Fig. 8, Ikeda *et al.*, 1993a,b). During mating, the male quickly approaches the female from below and grasps her around the head and mantle. The hectocotylus (the male's fourth right arm) then picks up spermatophores and drives them into the buccal membrane of the female within a few seconds (Fig. 9).

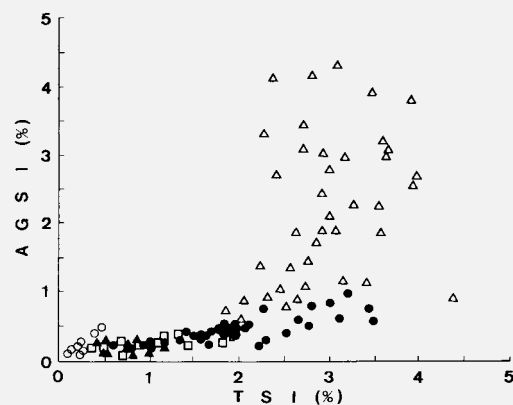
Generally about two days before spawning, females stop feeding and often rest on the tank bottom (Bower and

**Table 3.** Comparison of male maturity conditions of the Japanese common squid, *Todarodes pacificus* between morphological maturity scale that is commonly used in the Japanese Fisheries Experimental Station (maturity A) and histological maturity scale (maturity B) by Ikeda et al. (1991b)

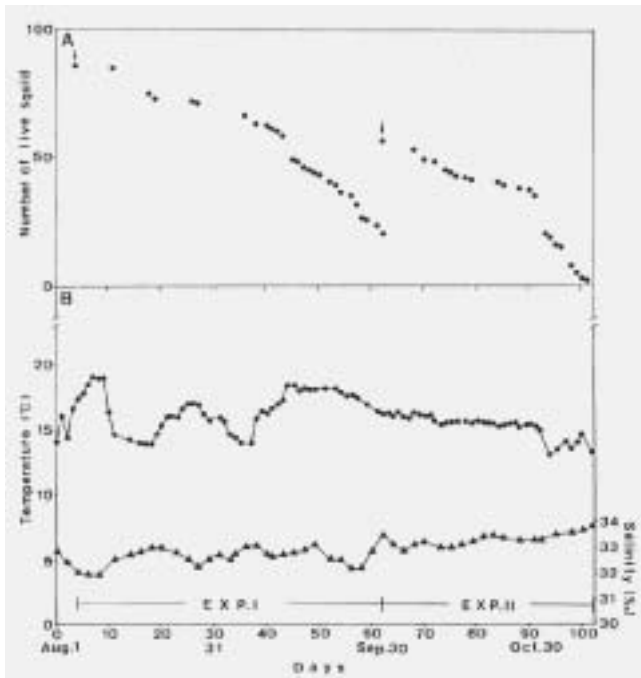
Maturity scale A	Maturity scale B
Immature (with- out spermatophores)	I. Spermatogonial proliferation stage II. Early maturing stage (TSI> 0.5, AGSI> 0.1) III. Mid maturing stage
Maturing (without spermatophores; the vas deferens white)	VI. Late maturing stage
Mature (spermatophores are present in the spermatophoric sac)	V. Mature stage (TSI> 2.0, AGSI> 1.0)

TSI: testis somatic index (testis weight as a percentage of total body weight)

AGSI: accessory gland somatic index (accessory gland weight as a percentage of total body weight)

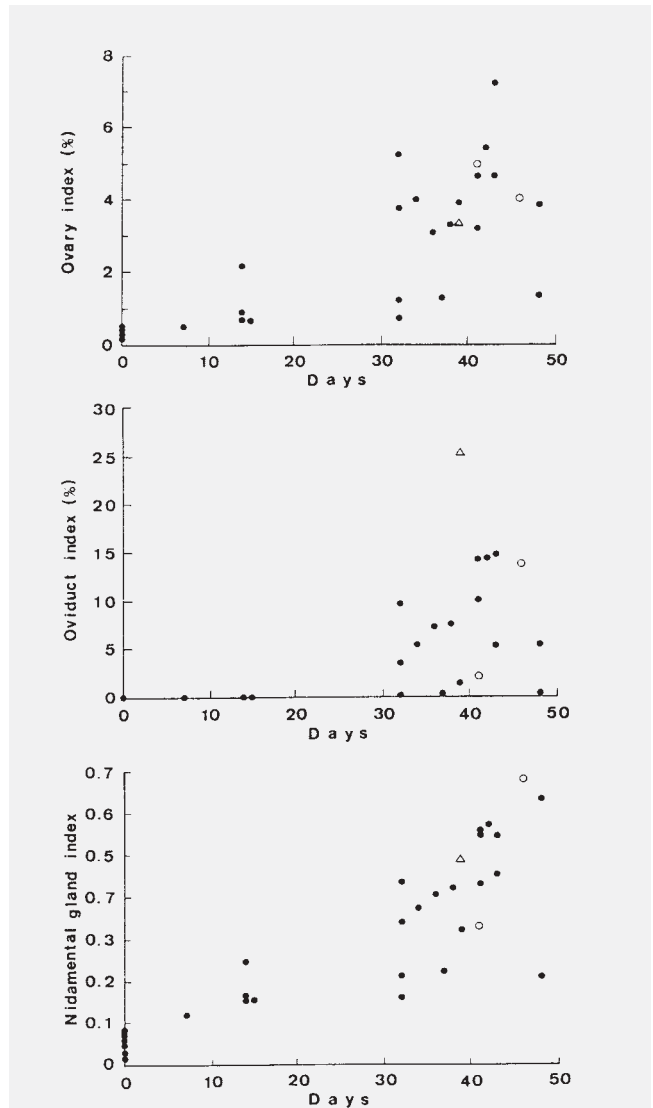


**Fig. 4.** Relationship between the testis weight and the accessory gland weight during maturation in *Todarodes pacificus*. TSI, testis somatic index (testis weight as a percentage of body weight). AGSI, accessory gland somatic index (accessory gland weight as a percentage of body weight). Symbols indicate histological maturity; (○) spermatogonial proliferation stage, (▲) early maturation stage, (□) mid maturation stage, (●) late maturation stage and (△) mature stage. (Ikeda et al., 1991b)



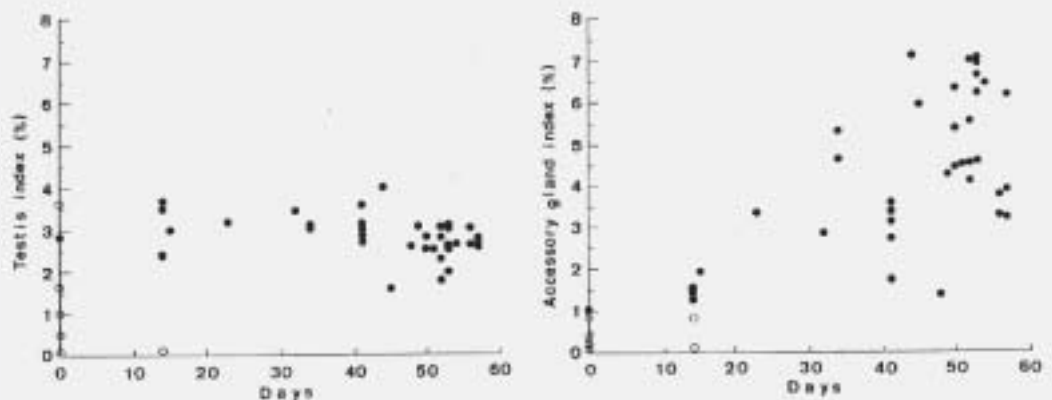
**Fig. 5.** Physical conditions and survival of *Todarodes pacificus* during the 1988 experiments. (A) Number of live squid. Arrows indicate the onset of Experiment I and Experiment II. (B) Water temperature (●—●) and salinity (▲—▲) in the breeding tank. (Ikeda et al., 1993a)

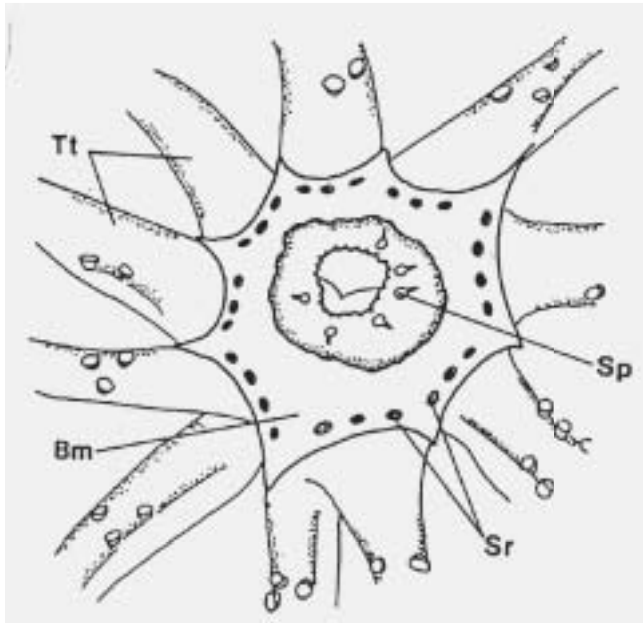
Sakurai, 1996). While resting, their chromatophores flash rapidly over the entire body surface; this characteristic is now known to be a sign that spawning is imminent. Spawning has been observed only once (Fig. 10). The female's arms just prior to spawning were slightly flattened and lowered. After one minute in this posture, the arms opened gradually, allowing the small egg mass to be formed and held within the arms (Fig. 10-A). The egg mass expanded and was not clearly visible during the spawning (Fig. 10-B,C,D). Egg-mass formation resembled the swelling of a balloon and lasted about seven minutes (Fig. 10-E). The spawning behavior was similar to that of *Illex illecebrosus* (O'Dor et al., 1982).



**Fig. 7.** The progress of female sexual maturation in *Todarodes pacificus* held in captivity in 1988. Ovary index (ovary weight as a percentage of body weight); Oviduct index (oviduct weight as a percentage of body weight); Nidamental gland index (see m, maturation index in Fig. 3). ●: Pre-spawning female (ripe ova are present in the oviduct); ○: Post-spawning female (spawned female); △: Exhausted female just prior to spawning (the total percentage of gonadal weight is almost 50% of total body weight). (Ikeda et al., 1993a)

**Fig. 6.** The progress of male sexual maturation in *Todarodes pacificus* held in captivity in 1988. Testis somatic index and accessory gland somatic index (see TSI and AGSI in Fig. 4). ○: Immature to maturing male; ●: Mature male with spermatophores in the accessory gland. (Ikeda et al., 1993a)

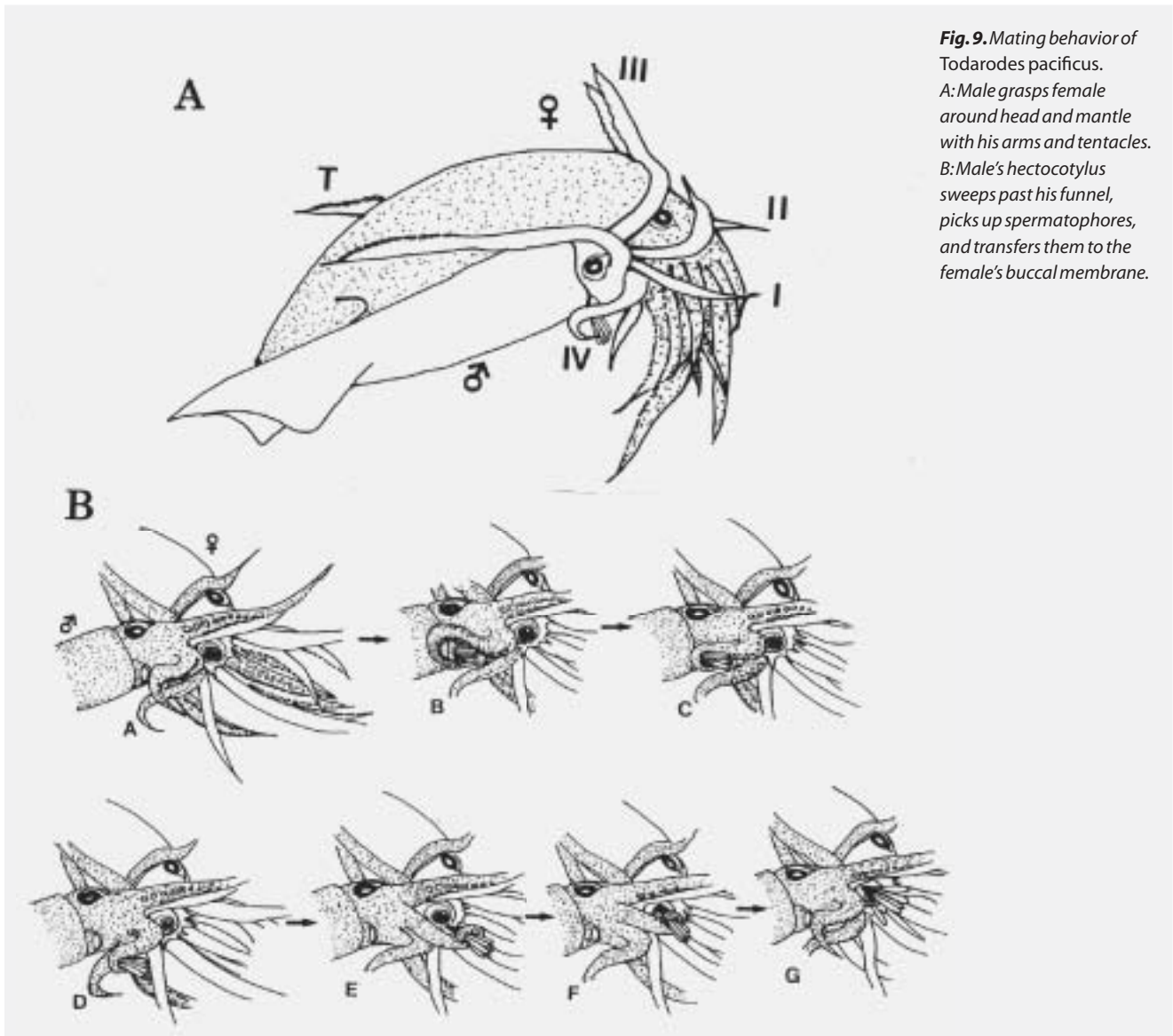




**Fig. 8.** Diagram of copulated *Todarodes pacificus* female buccal area. Bm, buccal membrane; Sp, embedded spermatophore by copulation; Sr, seminal receptacle; Tt, tentacle. (Ikeda et al., 1993b)

**EGG MASS, FECUNDITY AND HATCHING**

Bower and Sakurai (1996) described the characteristics of two *T. pacificus* egg masses spawned in captivity. The spherical egg masses were nearly neutrally buoyant and found floating near the surface of the tank. Externally, the masses were covered with a jelly-like secretion, presumably from the nidamental glands, and the interior of the masses, which contained eggs measuring 0.9 mm in diameter, consisted of a jelly presumably secreted by the oviducal glands. The larger mass measured 80 cm in diameter and contained approximately 200,000 eggs. More than 90% of the eggs within this mass were fertilized. The egg-mass surface layer effectively prevented crustaceans, protozoans, and bacteria from infesting the masses. Egg were positioned 0.4-2.0 cm apart throughout the inner mass. The chorion surrounding each egg expanded to diameters of 1.9-2.3 mm. Paralarvae hatched after 4-6 days at 18-19°C and actively swam at once, with many individuals swimming the surface. The egg masses disintegrated soon after hatching occurred.



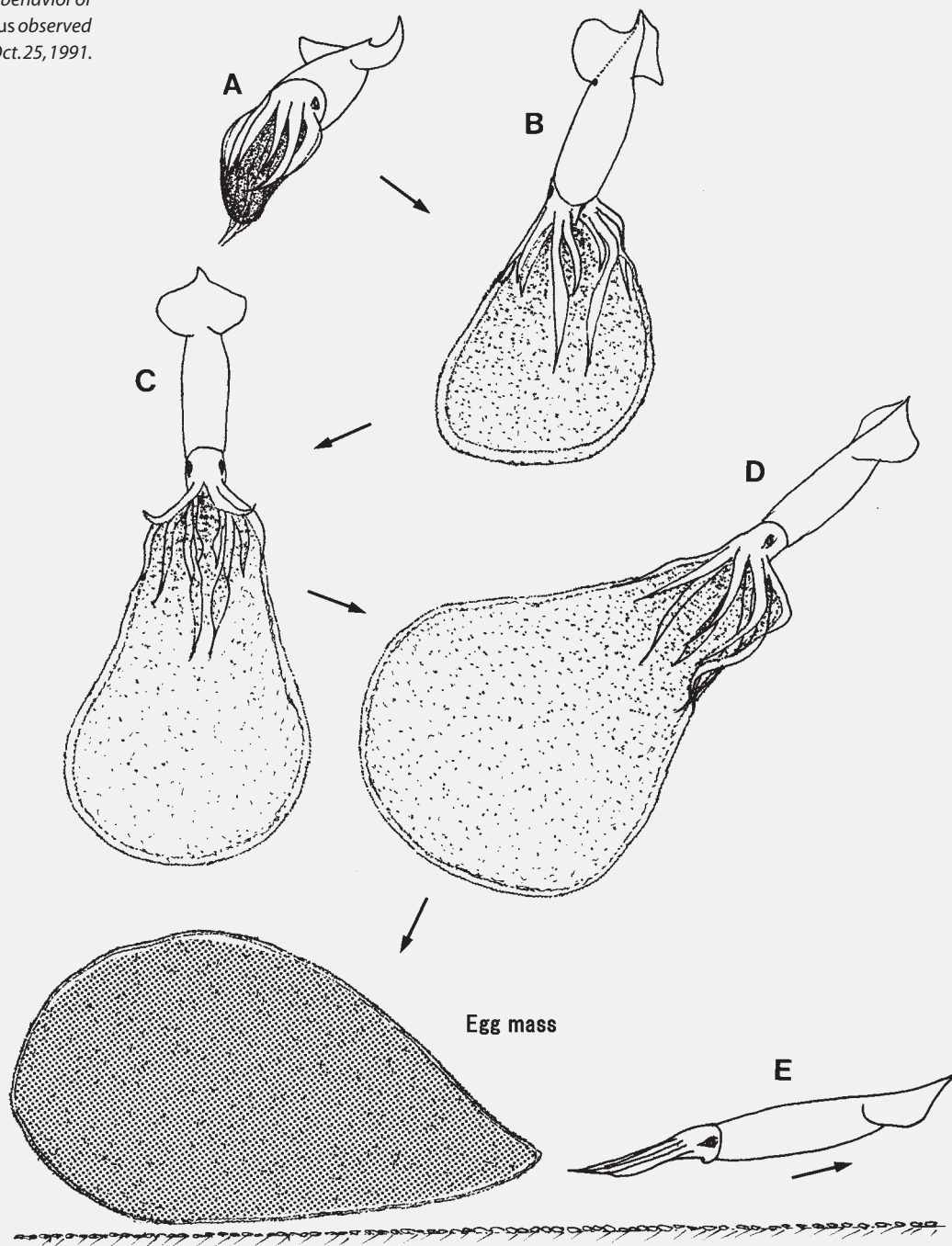
**Fig. 9.** Mating behavior of *Todarodes pacificus*. A: Male grasps female around head and mantle with his arms and tentacles. B: Male's hectocotylus sweeps past his funnel, picks up spermatophores, and transfers them to the female's buccal membrane.

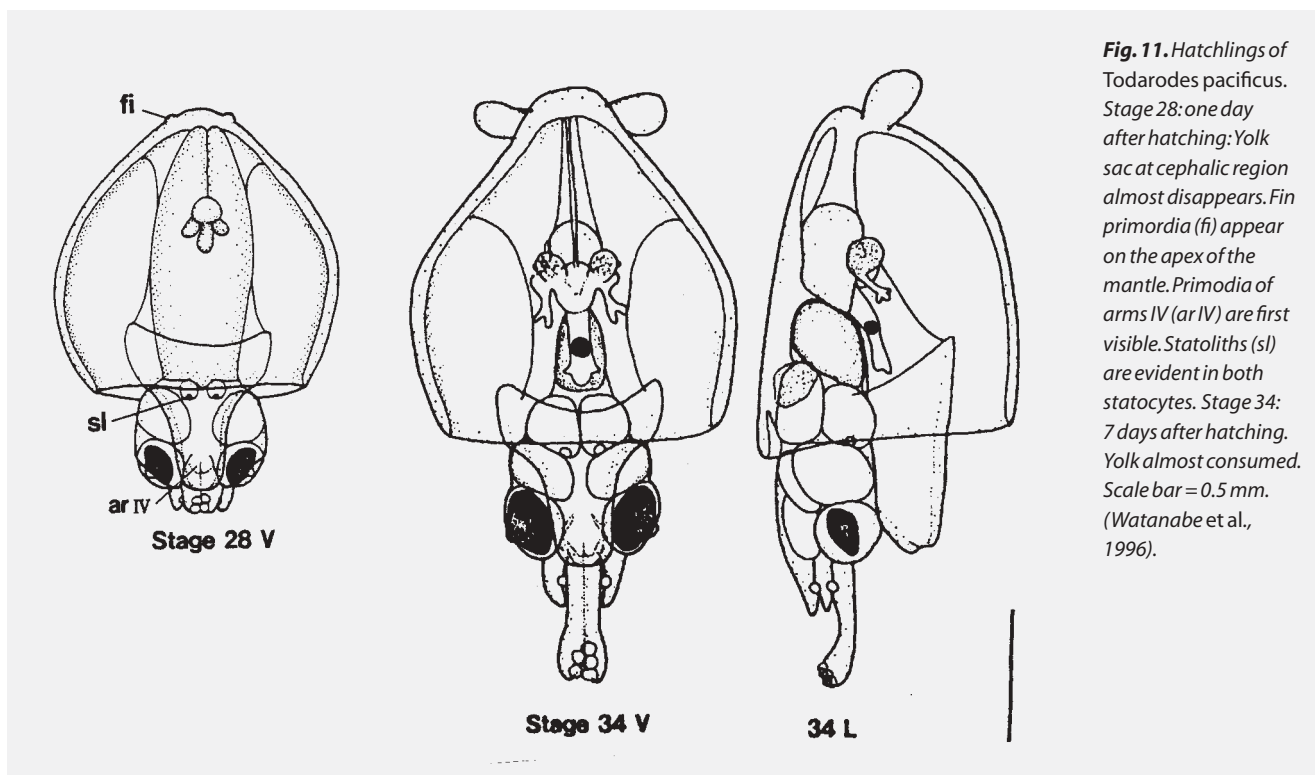
The results of our captive experiments suggest that mature females usually spawn once and then die, but some females spawned twice in a week when their spawning was disturbed (Ikeda *et al.*, 1993a). The fecundity of *T. pacificus* based on counts of ripe ova in oviducts of pre-spawning females is estimated to be between 320,000 and 470,000 (Soeda, 1956). However, in the ovaries of spawning females, oocytes occurred in all stages of development, from yolkless to mature stages and in exhausted females with thin mantles, the ovary and oviducts composed 28 % of the total body

weight (see Fig. 7, Ikeda *et al.*, 1993a). The oviducts of dead post-spawning females still contained many ova (Bower and Sakurai, 1996) suggesting that females do not necessarily spawn all ova before dying.

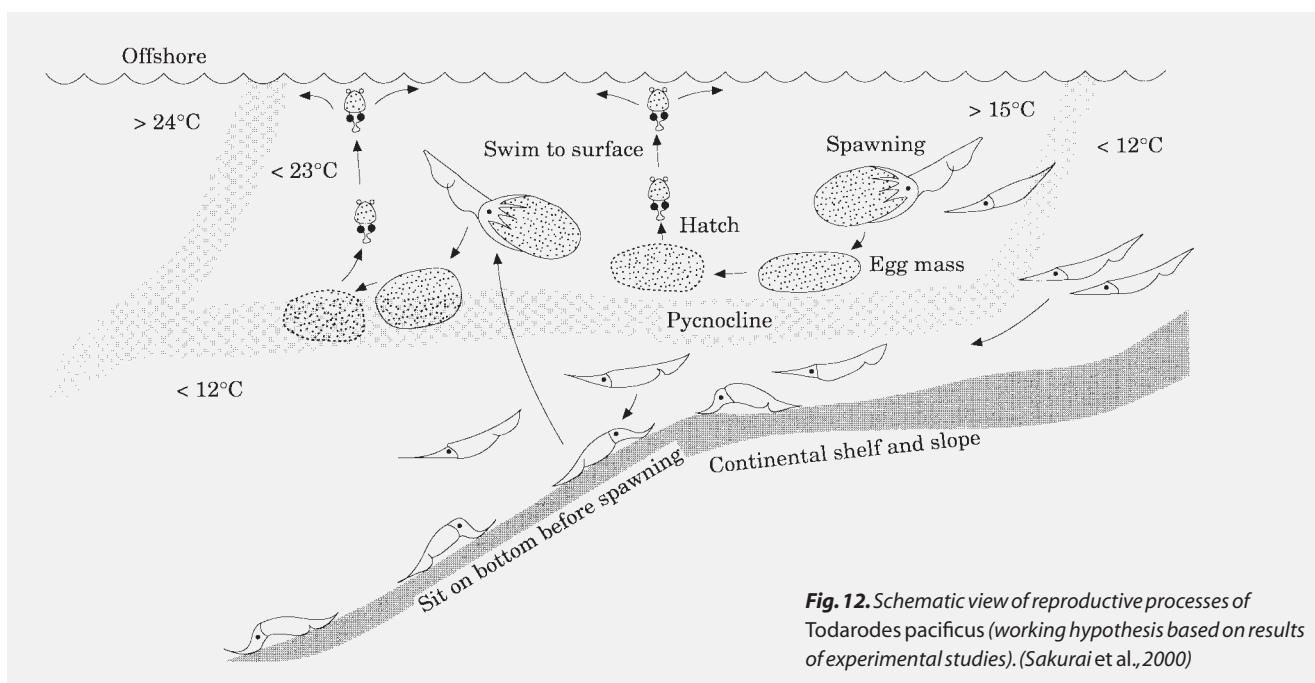
The development of a technique of artificial fertilization for ommastrephid squids (Sakurai *et al.*, 1995) has made it possible to examine the development and early life stages of *T. pacificus* (Watanabe *et al.*, 1996, Sakurai *et al.*, 1996). After hatching, paralarvae were maintained for up to seven

**Fig. 10.** Spawning behavior of *Todarodes pacificus* observed in captivity, Oct. 25, 1991.





**Fig. 11.** Hatchlings of *Todarodes pacificus*. Stage 28: one day after hatching: Yolk sac at cephalic region almost disappears. Fin primordia (fi) appear on the apex of the mantle. Primordia of arms IV (ar IV) are first visible. Statoliths (sl) are evident in both statocytes. Stage 34: 7 days after hatching. Yolk almost consumed. Scale bar = 0.5 mm. (Watanabe et al., 1996).



**Fig. 12.** Schematic view of reproductive processes of *Todarodes pacificus* (working hypothesis based on results of experimental studies). (Sakurai et al., 2000)

days without being fed while the internal yolk was completely absorbed (Watanabe *et al.*, 1996). Paralarval mantle lengths measured 0.95 mm at hatching and 1.25 mm after seven days (Fig. 11). To date, there have been no successful long-term rearing experiments, as all attempts to feed the hatchling paralarvae have failed.

#### WORKING HYPOTHESIS OF REPRODUCTION PROCESSES

Sakurai *et al.* (2000) proposed a working hypothesis of the reproduction process of *T. pacificus* based on results of experimental studies (Fig. 12). *T. pacificus* produces

gelatinous, nearly buoyant egg masses (Bower and Sakurai, 1996). The temperature range for normal embryonic development is 15–23°C (Sakurai *et al.*, 1996), while most hatchlings collected off southern Japan occur at sea surface temperatures of 17–23°C (Bower *et al.*, 1999). Embryonic development in an egg mass over a temperature range of 15–23°C is estimated to last 4.0–9.5 days (Sakurai *et al.*, 1996). Temperatures at 50-m depth were used to estimate the range of spawning grounds, since most paralarvae occur at 25–50 m depth (Watanabe, 1965) and gelatinous structures resembling egg masses have been observed within the pycnocline (70–100 m depth) using an ROV (first author, pers.



obs.). Spawning is assumed to occur above the continental shelf and slope around Japan, because captive females regularly sit on the tank bottom just before spawning (Bower and Sakurai, 1996). Also bottom trawls often collect exhausted spent females on the shelf and slope at 100-500 m depth (Hamabe and Shimizu, 1966).

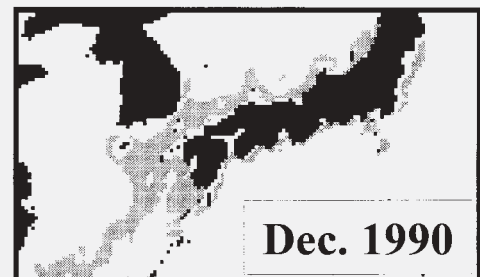
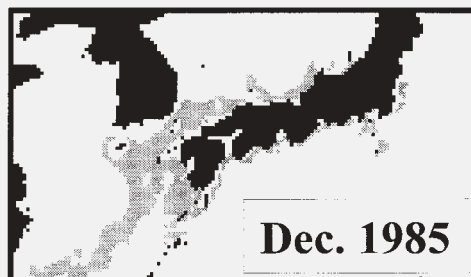
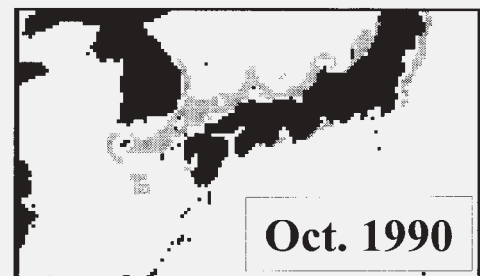
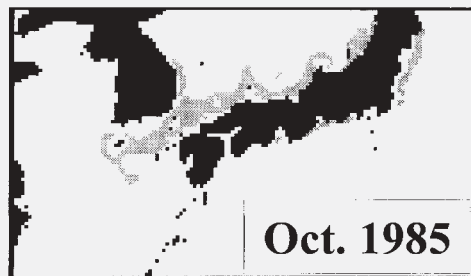
#### STOCK FLUCTUATION THROUGHOUT THE REPRODUCTION PROCESS RELATED TO CLIMATIC REGIME SHIFTS

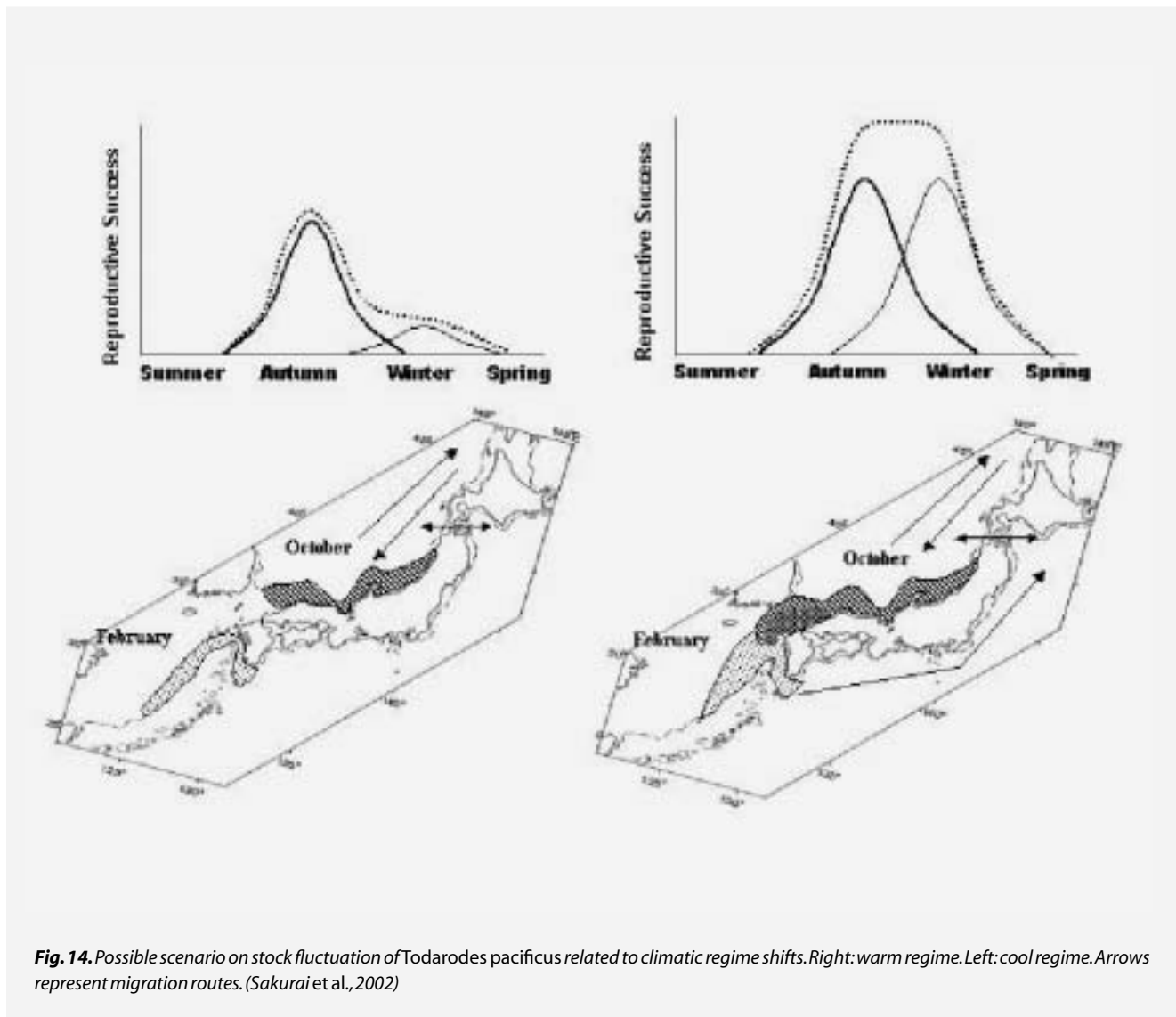
Using GIS data, Sakurai *et al.* (2000, 2002) examined the monthly and annual changes of inferred spawning areas (identified as the surface area where both temperatures at 50-m depth ranged 15-23°C and bottom depths ranged 100-500 m) during 1984 and 1999 (Fig. 13). These results

suggest that winter spawning areas in the East China Sea decreased in size when adult stocks decreased during the cool regime before 1988. During this phase, the autumn spawning group was presumably dominant, and the migration routes occurred mainly in the Sea of Japan. In contrast, the autumn and winter spawning areas are thought to have expanded and overlapped in the Sea of Japan and the East China Sea during the warm regime that occurred after 1989 (Fig. 14).

As a result, both populations of autumn and winter-spawning groups increased, and their migration routes would have expanded into Pacific waters, especially those of the winter-spawning group (*e.g.*, in 1989, 1990 and 1991). Warm winters, such as those in 1989, 1990 and 1991, may

**Fig. 13.** Comparison of seasonal shifts in inferred spawning areas (dark) between October to April in 1985/86 (cold year) and 1990/91 (warm year) based on GIS data. Black and white areas represent land and unsuitable depths/temperatures, respectively. (Sakurai *et al.*, 2000)





**Fig. 14.** Possible scenario on stock fluctuation of *Todarodes pacificus* related to climatic regime shifts. Right: warm regime. Left: cool regime. Arrows represent migration routes. (Sakurai et al., 2002)

promote an increase in stock size. However, the inferred spawning sites varied annually (*e.g.* in 1992 and 1993, there were a reduction in size of inferred spawning areas in winter), which might be a cause of the annual catch fluctuations (Fig. 1).

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# Estimation of the maturity and fecundity of tunas

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## ABSTRACT

Tuna spawning patterns are diverse and complex. All species of the tribe Thunnini spawn only at sea-surface temperatures in excess of about 24°C. Because they are repetitive broadcast spawners, tunas must have very high lifetime fecundities to be successful. This is achieved through various degrees of protracted spawning, along with a combination of frequent spawning and relatively high batch fecundities.

Maturity and fecundity estimates, and descriptions of methodologies utilized, are presented for the following species: *Thunnus albacares*, *T. maccoyii*, *T. orientalis*, *T. obesus*, *Katsuwonus pelamis*, and *Euthynnus lineatus*.

The first requirement in the process of the estimation of proportions sexually mature is to define precise criteria for the classification of maturity. The statistical procedure for deriving a maturity schedule involves fitting an appropriate weighted non-linear predictive regression model directly to the maturity data. The model can then be used to predict proportions sexually mature at specific lengths and/or ages. Also, statistical evaluations of spatial and temporal variation in maturity functions can be conducted on the data. Maturation schedules are normally estimated only for females, but procedures have also been developed for estimation of maturity for males.

Estimation of the annual fecundity in tunas requires spawning frequency estimates by length classes, and corresponding estimates of batch fecundities over the length range of mature females. Knowledge of the appearance and longevity of postovulatory follicles in ovaries of tunas after spawning is necessary for estimation of spawning frequency. The frequency at which ovaries of mature females contain postovulatory follicles has been

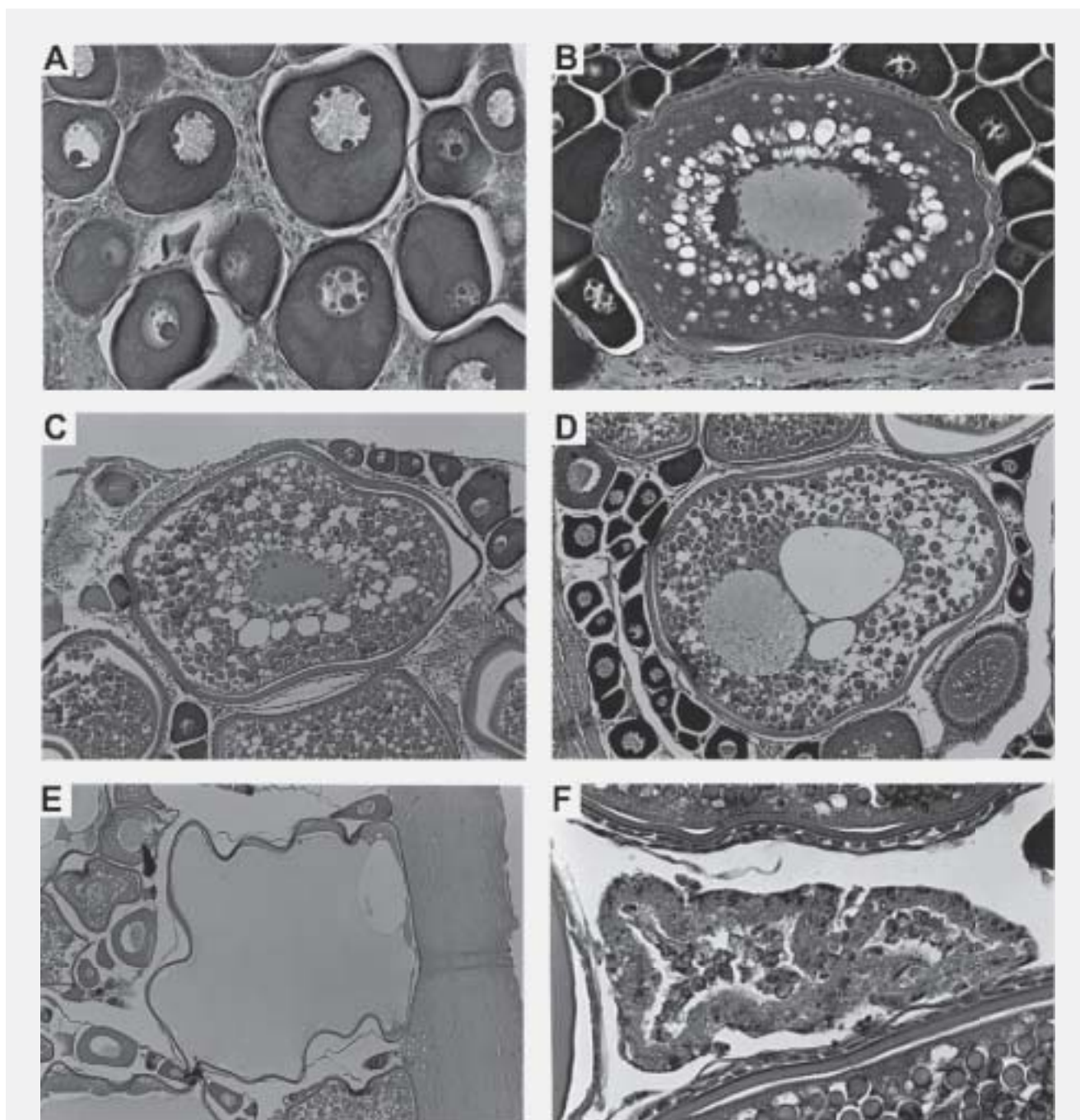
used to estimate spawning frequency for a few tunas. Only at the final stages of oocyte maturation, beginning with the migratory-nucleus phase and followed by hydration, is there a distinct hiatus in the distribution of oocytes from which the batch fecundity estimates can be derived. Spatial and temporal variation in fecundity estimates in tunas are also presented.

## INTRODUCTION

The reproductive characteristics of a stock, along with those of growth and mortality, are among the most important factors in determining the regenerative ability of a population (Quinn and Deriso, 1999). Understanding the reproductive biology of tunas and quantifying size-specific parameters provides the means, when incorporated into length- and/or age-structured models, for predicting the effects of fishing on the reproductive potential of a stock. Although there has been some progress in the past 20 years on elucidating some important life history processes in tunas, for the majority of the species our knowledge regarding reproductive patterns and parameters is meager. Accurate interpretation and classification, in recent years, of reproductive condition and estimates of spawning potential for tunas has largely been the result of utilizing histological techniques and appropriate classification criteria.

## OVERVIEW

Tunas are oviparous, have asynchronous oocyte development, and are considered to be multiple or batch spawners, shedding their gametes directly into the sea, where fertilization occurs. Spawning patterns within the tribe are diverse and complex. There are three types of spawning patterns exhibited by the tunas: 1) confluent throughout tropical and subtropical regions (*Katsuwonus pelamis*, *Thunnus*

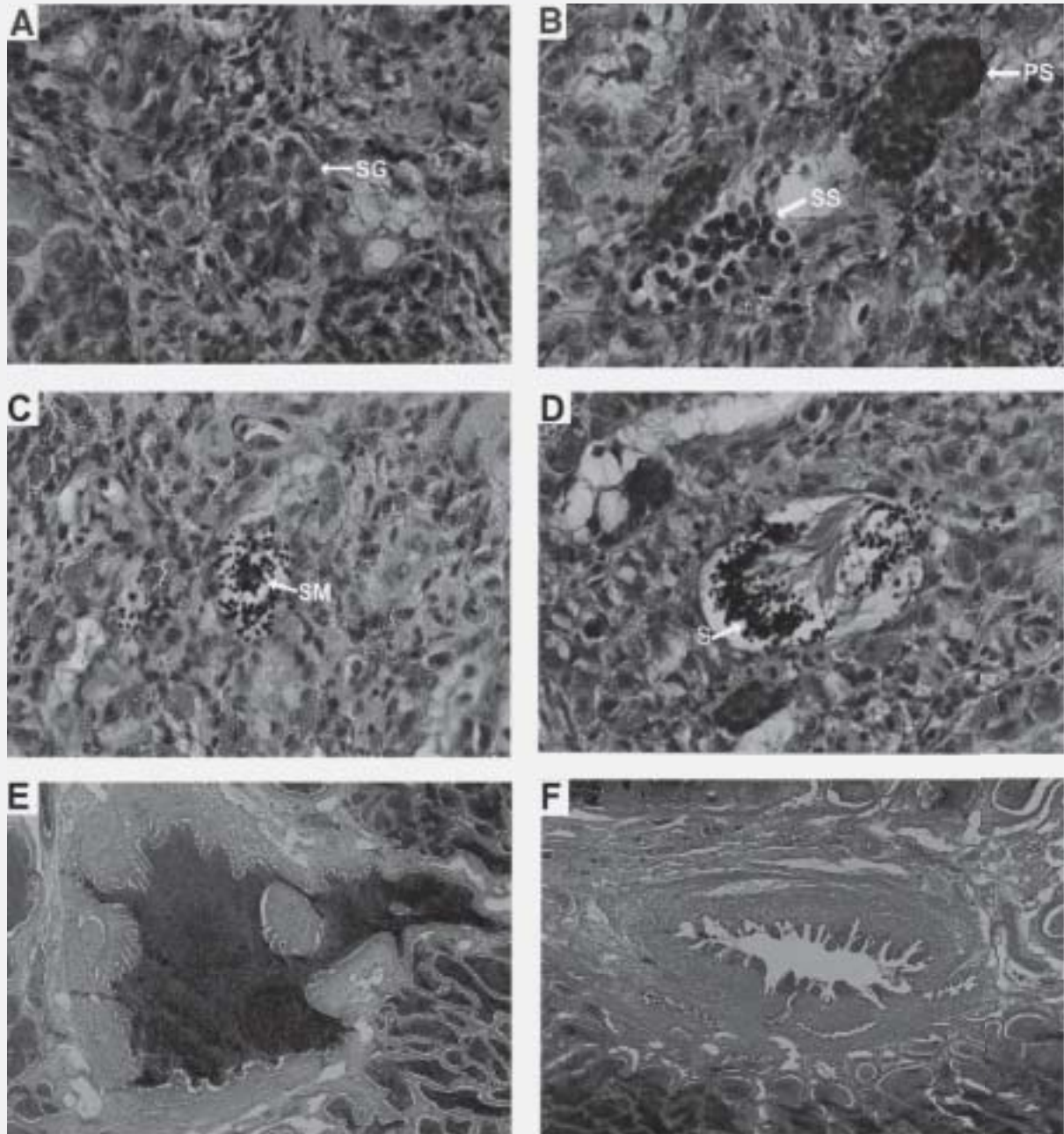


**Fig. 1.** Developmental stages and oogenic cells observed in *Thunnus albacares* ovaries. (A) unyolked oocyte (x160); (B) early yolked oocyte (x100); (C) advanced yolked oocyte (x40); (D) migratory-nucleus stage oocyte (x40); (E) hydrated oocyte (x25); (F) postovulatory follicle less than 12 h after ovulation (x160). Reprinted from Schaefer (2001b), with permission from Elsevier Science.

*albacares*, and *T. obesus*), 2) regionally-confined and protracted (*Auxis* spp., *Euthynnus* spp., *Thunnus atlanticus*, and *T. tonggol*) and 3) migratory and spatiotemporally-confined (*Thunnus alalunga*, *T. maccoyii*, *T. orientalis*, and *T. thynnus*). Common to all these species within the tribe is the relationship between spawning activity and sea-surface temperatures in excess of about 24°C.

Most studies of gonadal development in tunas, intended to describe maturation and spawning distributions, have been based on ovaries and have utilized various formulations of

the gonosomatic index for classification of condition. If a gonosomatic index is calibrated, for instance through use of histology, it can potentially be used for determination of spatiotemporal spawning distributions, but it is not accurate for classification of maturity or reproductive activity (de Vlaming *et al.*, 1982). Other methodologies that are appropriate for interpretation and classification of the gonadal development and reproductive activity of individual fish include the use of oocyte diameters from the most advanced batch of oocytes present in an ovary (Buñag, 1956; Yoshida, 1966; Schaefer, 1987; Ramon and



**Fig. 2.** Developmental stages and spermatogenic cells observed in *Thunnus albacares* testes. (A) primary spermatogonia (sg) (x240); (B) primary spermatocyte (ps) cells and secondary spermatocyte (ss) cells (x240); (C) spermatids (sm) (x240); (D) spermatozoa (s) (x240); (E) prespawning stage in mature testis with vas deferens filled with spermatozoa (x10); (F) postspawning stage (< 12 h) in mature testis with the vas deferens almost completely devoid of spermatozoa (x10). Reprinted from Schaefer (2001b), with permission from Elsevier Science.

Bailey, 1996; Schaefer, 2001a) and histology of ovarian and testicular tissues (Cayré and Farrugio, 1986; Goldberg and Au, 1986; Hunter *et al.*, 1986; McPherson, 1991; Nikaido *et al.*, 1991; Batalyants, 1992; Timohina and Romanov, 1996; Farley and Davis, 1998; Schaefer, 1998). Histological examination of microscopic slides of ovarian and testicular tissues provides the greatest precision for classification of reproductive state of tunas (Schaefer, 1998).

The histological classification of tuna ovaries for assessments of reproductive biology should be based on the sys-

tem applied successfully by Hunter and Macewicz (1985), and following some adjustments by Schaefer (1996, 1998). Oogenesis begins with the proliferation of oogonia by mitotic divisions within the oogonial nest, which become primary oocytes (Figure 1A). The oocytes considered within this developmental category are unyolked. In early yolked oocytes (Figure 1B) numerous euvitelline nucleoli appear homogeneously around the nuclear membrane, with no true nucleolus present. There is an increase in the lipoid vesicles and yolk granules, and the zona radiata can be distinguished in the follicular epithelium as develop-

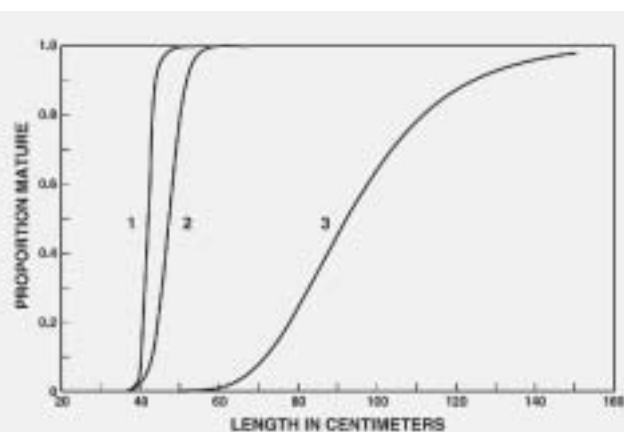
ment proceeds (Figure 1C). The first observed histological change associated with the final maturation of the oocyte is the migration of the nucleus (germinal vesicle) toward the animal pole, where the micropyle is located. During this process coalescence of the lipid vesicles takes place within the cytoplasm (Figure 1D). The final maturation of the oocyte occurs during the later stage of hydration, when the yolk plates completely fuse and form a homogeneous yolk mass (Figure 1E), during which time the oocyte significantly increases in size due to hydration or the uptake of fluid by the oocyte (Wallace and Selman, 1981). This fusion of yolk granules and hydration gives the oocyte a translucent (hyaline) appearance in fresh or preserved whole oocytes (Schaefer, 1998). Upon completion of maturation the hydrated oocytes are expelled through a rupture in their surrounding follicles into the ovarian lumen. The postovulatory follicle remains as a distinct involuted structure within the ovigerous lamellae. The postovulatory follicle is transitory, and in *Katsuwonus pelamis* and *Thunnus albacares* by 24 h ( $@>24^{\circ}\text{C}$ ) after ovulation postovulatory follicles cannot be accurately identified (Hunter *et al.*, 1986; Schaefer, 1996). The degenerating postovulatory follicle has few involutions and a follicular cavity (Figure 1F).

Tuna testes are considered as unrestricted spermatogonial testis types, because the distribution of spermatogonia may occur along the entire length of the tubule (Grier, 1981). The histological classification of tuna testes development should be based on the degree of spermatogenesis, as described by Grier (1981), and the classification system of Schaefer (1996, 1998) for assessments of reproductive biology. The four cellular stages that can be differentiated in sperm maturation are spermatogonia, spermatocytes, spermatids, and spermatozoa (Figures 2A, B, C, D). Primary spermatogonia, distributed along the lobule lengths, undergo a series of mitosis, which produce cysts containing several spermatogonial cells, called secondary spermatogonia. The primary spermatogonia are spherical and acidophilic, and each possesses a single prominent nucleolus. These are the largest germ cells in the testis (Figure 2A). A second series of mitotic divisions results in the formation of cysts full of primary spermatocytes (Figure 2B). The next developmental stage in the maturation process is secondary spermatocytes (Figure 2B), which are formed after the first meiotic division and are retained within the cysts until they eventually burst through the cyst's capsule into the lobular lumen, where they develop after a second meiosis into spermatids. Each spermatid (Figure 2C) develops into a spermatozoan, a process termed spermiogenesis. Mature spermatozoa possess a distinct rounded basophilic head and a long acidophilic flagellum (Figure 2D). The lobular lumens are continuous with the vas deferens, which are straight tubes with thick, muscular walls, and which merge caudally and

exit through a pore in the anal region. The lumen of the vas deferens is lined along its length with cuboidal to columnar epithelium, and varies in general appearance from smooth to convoluted (Harder, 1975; Schaefer, 1996). Histological evidence of recent spawning activity in male *T. albacares* is apparently detectable for only about 12 hours after the spawning event, based on appearance of the epithelial lining and the amount of sperm present in the vas deferens (Figure 2E, F) (Schaefer, 1996).

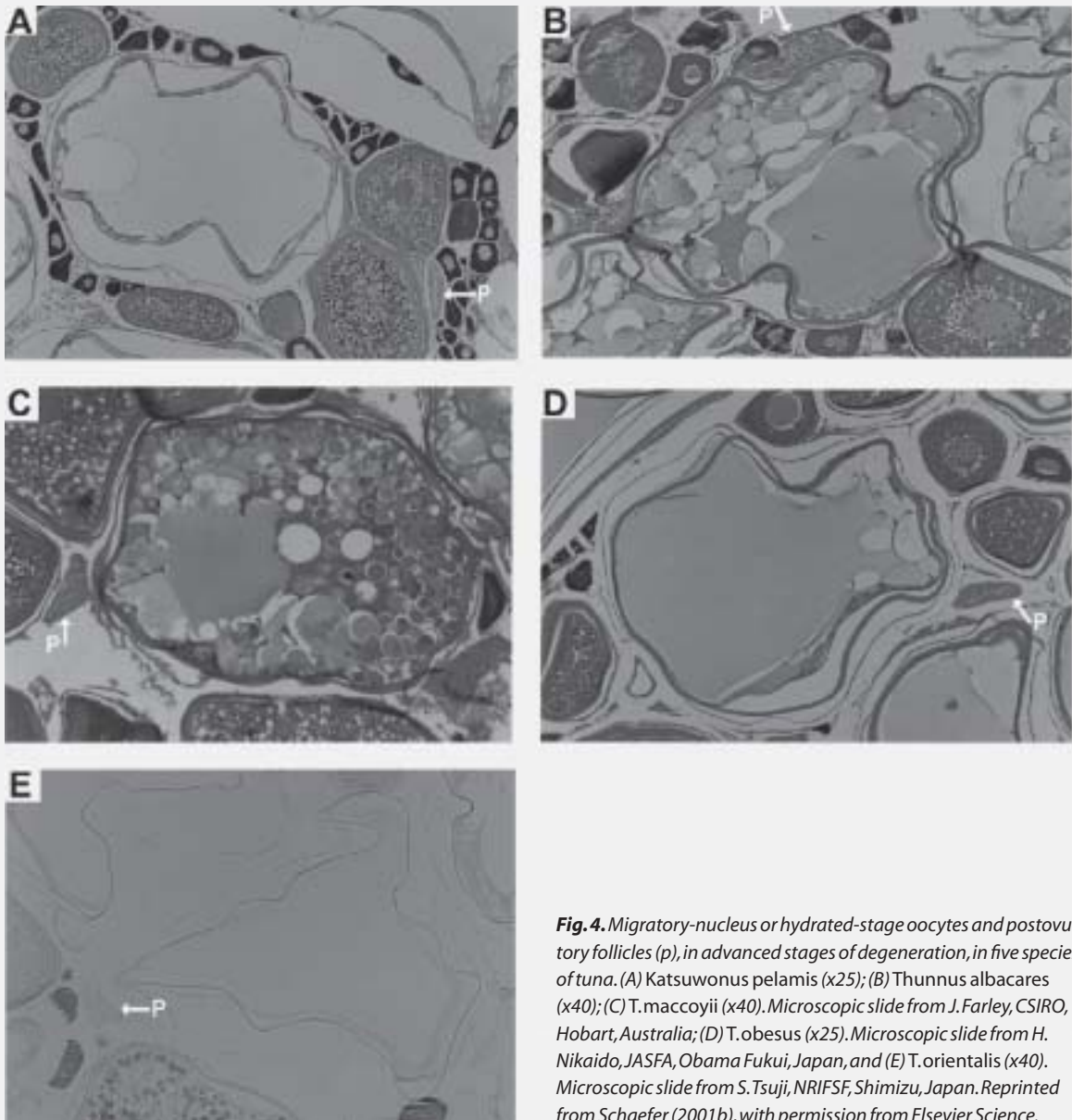
There has been a considerable amount of non-quantitative estimation of sizes and/or ages at maturity for tunas, based mostly on invalid gonosomatic indices. Many such studies have reported the apparent size at first maturity. Reporting only the size at first maturity is useless, and even misleading. Functional statistical relationships between proportion mature and size and/or age must be derived to estimate proportions sexually mature from a population.

The first requirement in the process of the estimation of proportions sexually mature is to define precise criteria for the classification of maturity. Histological examinations and criteria are necessary to attempt to correctly classify female and male tuna as to sexual maturity. Particularly for females, histological information is required because of the inadequacy of gonad indices or oocyte diameters for separating developing ovaries, in a stage of early vitellogenesis, from post-spawning ovaries, in atretic stages of resorption. The histological classification scheme used by Schaefer (1998) for female and male *T. albacares* provides the basis to distinguish between mature and immature individuals. The second consideration is the implementation of an appropriate experimental design based on previous knowledge of the spawning locations and times for the species under consideration. The final consideration should be the



**Fig. 3.** Relationship between proportion of female tunas mature and length. (1) *Katsuwonus pelamis* (Stéguert and Ramcharrun, 1996); (2) *Euthynnus lineatus* (Schaefer, 1987), and (3) *Thunnus albacares* (Schaefer, 1998). Reprinted from Schaefer (2001b), with permission from Elsevier Science.





**Fig. 4.** Migratory-nucleus or hydrated-stage oocytes and postovulatory follicles (p), in advanced stages of degeneration, in five species of tuna. (A) *Katsuwonus pelamis* (x25); (B) *Thunnus albacares* (x40); (C) *T. maccoyii* (x40). Microscopic slide from J. Farley, CSIRO, Hobart, Australia; (D) *T. obesus* (x25). Microscopic slide from H. Nikaido, JASFA, Obama Fukui, Japan, and (E) *T. orientalis* (x40). Microscopic slide from S. Tsuji, NRIFS, Shimizu, Japan. Reprinted from Schaefer (2001b), with permission from Elsevier Science.

use of an appropriate statistical procedure for evaluation and interpretation of the data. A weighted non-linear predictive regression model fit directly to these sigmoidal data is probably the most appropriate (Schaefer, 1987; 1998). The model can then be used to predict proportions sexually mature at specific lengths and/or ages for the stock being investigated.

The available estimates of lengths at 50% maturity of female tunas are given in Table 1. These estimates, for just 3 of the 15 species of Thunnini, appear to be the only reliable estimates available. The relationship between proportion of female *E. lineatus* mature and length from the eastern Pacific (Schaefer, 1987) is shown in Figure 3, and the estimated length at 50% maturity in Table 1. The estimated lengths at 50% maturity given in Table 1 for female *T. albacares* from studies conducted in two different oceanic

regions, western and eastern Pacific, are quite variable. These differences may be more a function of the experimental designs and classification procedures employed than geographic variation. The relationship between proportion of female *T. albacares* mature and length from the eastern Pacific is shown in Figure 3. In the study by Schaefer (1998) the estimated length at 50% maturity for females of 92 cm corresponds to an age of about 2 years (Wild, 1986). As an example of the size at first maturity, and the potential misuse of such information, the minimum length at sexual maturity of females was 59 cm. Males were found to mature at lesser lengths than females, with the estimated length at 50% maturity for males being 69 cm. The relationship between proportion of mature female *K. pelamis* and length, from the Indian Ocean, is given in Figure 3. The shape of the maturation curve is noticeably different than that for *T. albacares*, but similar to that for

**Table 1.** Estimates of lengths at 50% maturity for female tunas. (From Schaefer, 2001b)

Species	Length (cm)	Area	Reference
<i>Euthynnus lineatus</i>	47	Eastern Pacific	Schaefer (1987)
<i>Katsuwonus pelamis</i>	42	Atlantic	Cayré and Farrugio (1986)
<i>Katsuwonus pelamis</i>	42	Western Indian	Stéquert and Ramcharrum (1996)
<i>Katsuwonus pelamis</i>	43	Western Indian	Timohina and Romanov (1996)
<i>Thunnus albacares</i>	108	Western Pacific	McPherson (1991)
<i>Thunnus albacares</i>	92	Eastern Pacific	Schaefer (1998)

**Table 2.** Estimates of spawning frequency (mean spawning interval) for female tunas. (From Schaefer, 2001b)

Species	Days	Area	Reference
<i>Katsuwonus pelamis</i>	1.18	Western Pacific	Hunter <i>et al.</i> (1986)
<i>Thunnus albacares</i>	1.53	Western Pacific	McPherson (1991)
<i>Thunnus albacares</i>	1.52	Eastern Pacific	Schaefer (1998)
<i>Thunnus maccoyii</i>	1.62	Eastern Indian	Farley and Davis (1998)
<i>Thunnus obesus</i>	1.09	Western Pacific	Nikaido <i>et al.</i> (1991)

**Table 3.** Estimates of the relative batch fecundity (oocytes per gram of body weight) of tunas. (From Schaefer, 2001b)

Species	Fecundity	Area	Reference
<i>Euthynnus lineatus</i>	99-136	Eastern Pacific	Schaefer (1987)
<i>Katsuwonus pelamis</i>	82	Western Atlantic	Goldberg and Au (1986)
<i>Thunnus albacares</i>	67	Eastern Pacific	Schaefer (1998)
<i>Thunnus orientalis</i>	69	Western Pacific	Tanaka (1999)
<i>Thunnus maccoyii</i>	57	Eastern Indian	Farley and Davis (1998)
<i>Thunnus obesus</i>	31	Western Pacific	Nikaido <i>et al.</i> (1991)

*E. lineatus*. It appears in *K. pelamis* and *E. lineatus*, once the critical size and/or age is attained, there is a strong selection pressure for individuals to initiate maturation. The estimated lengths at 50% maturity given in Table 1 for female *K. pelamis* from studies conducted in three different oceanic regions are almost identical. For *T. maccoyii*, Davis (1995), using oocyte diameter and gonad index data, estimated the mean size at first maturity to be 152 to 162 cm. Recent analyses of size and age data for *T. maccoyii* caught on the spawning ground provide estimates of the size and age at 50% maturity of 158 – 163 cm and 11 or 12 years old (T. Davis, CSIRO, personal communication).

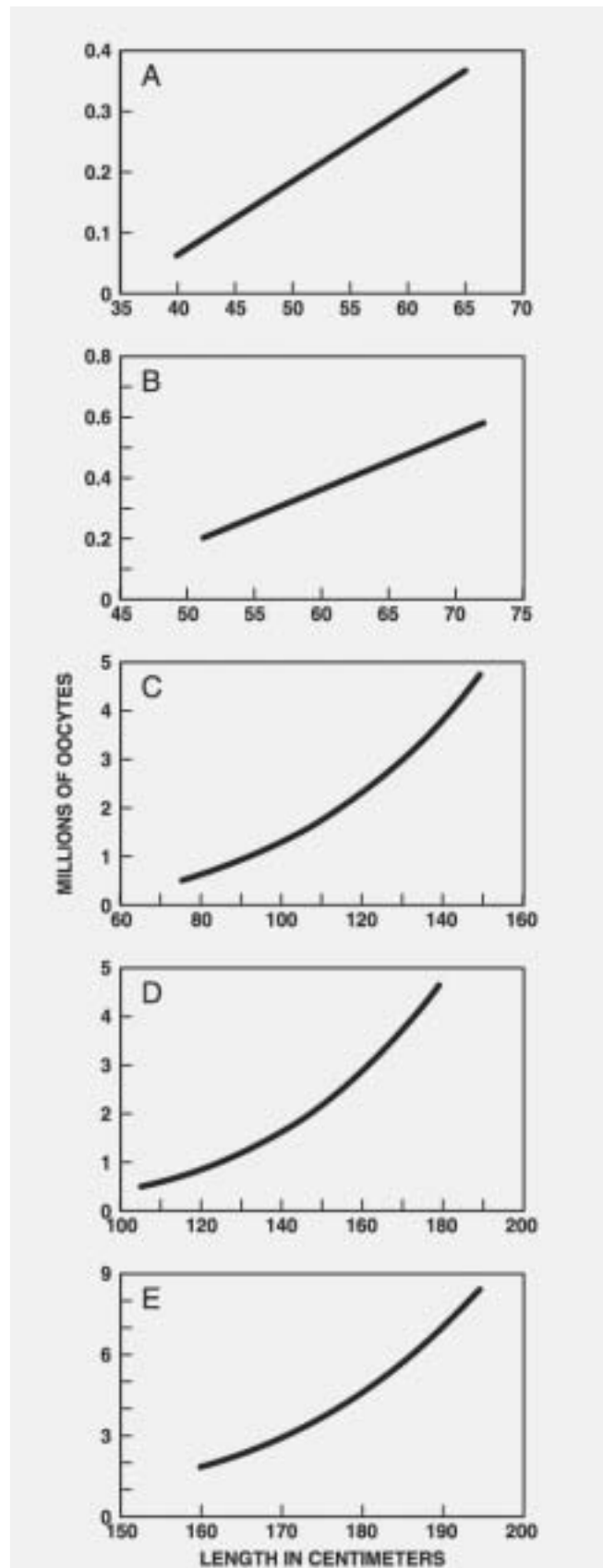
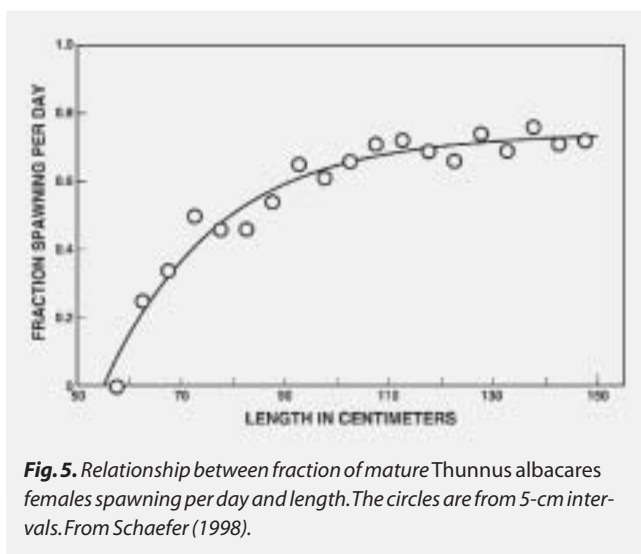
Tunas, being multiple spawners, continuously produce batches of hydrated oocytes that are released into the sea in separate spawning events. The estimation of spawning frequency, as the mean spawning interval between sequential spawning events, is essential for a comprehensive understanding of the reproductive biology of tunas. Knowledge of the appearance and longevity of postovulatory follicles in ovaries of tunas after spawning is necessary for estimation of spawning frequency (Hunter and Macewicz, 1985; Hunter *et al.*, 1986; Schaefer, 1996). The age and longevity

of postovulatory follicles have been determined only for *K. pelamis* (Hunter *et al.*, 1986) and *T. albacares* (Schaefer, 1996). However, the frequency of ovaries of mature females containing postovulatory follicles has been used to estimate spawning frequency in *K. pelamis* (Hunter *et al.*, 1986), *T. albacares* (McPherson, 1991; Schaefer, 1996, 1998), *T. obesus* (Nikaido *et al.*, 1991), and *T. maccoyii*, (Farley and Davis, 1998) (Table 2). The data in Table 2 implies that these species, when reproductively-active, spawn almost daily. The incidence of both postovulatory follicles and late-stage oocytes indicates daily spawning in tunas (Figure 4). This high frequency of spawning implies that in a reproductively-active female there is a continuous maturation of oocytes, which are recruited from the reservoir of primary oocytes. The relationship between length and the fraction of mature *T. albacares* females spawning is illustrated in Figure 5. It appears the larger females are physiologically capable of maintaining a higher spawning frequency, which is potentially related to their energy reserves.

The fecundity of tunas is not fixed at the beginning of their spawning period. Their annual fecundity is indeterminate because tunas spawn numerous times during a season

or year, and their potential annual fecundity exceeds the number of developing oocytes within the ovaries at any given time. Annual fecundity can be estimated from batch fecundity (the number of oocytes released per spawning) and spawning frequency. Only at the final stages of oocyte maturation, beginning with the migratory-nucleus phase and followed by hydration, is there a distinct hiatus in the distribution of oocytes from which the batch fecundity estimates can be derived. Migratory-nucleus and hydrated oocytes can be easily distinguished from other oocytes in ovaries of tunas by their larger size ( $\geq 0.75$  mm) and by their appearance (Schaefer, 1998). However, there is only a short period from late afternoon until about 2200 h (previous to spawning) when ovaries with migratory-nucleus or hydrated oocytes are found in *T. albacares* (Schaefer, 1996, 1998).

Estimated mean relative batch fecundities for tunas, based on counts of distinct advanced stages of oocytes, are given in Table 3. The relative fecundity estimates in Table 3 for *T. albacares* (67 oocytes/g body weight), *T. orientalis* (69 oocytes/g body weight) and *T. maccoyii* (57 oocytes/g body weight) are similar, but considerably greater than the estimate for *T. obesus* (31 oocytes/g body weight) and considerably less than the estimates for *K. pelamis* (82 oocytes/g body weight) and *E. lineatus* (99-136 oocytes/g body weight). Batch fecundity increases with body length for the species listed in Table 3 (Figure 6). Although not illustrated in Figure 6, the data for each of these species clearly indicate the high variation in batch fecundity estimates among tunas of similar size. Even with the high variability in batch fecundity estimates of fish of the same size, statistical comparisons have indicated significant spatiotemporal differences. For example, the predicted batch fecundity estimate for a 125-cm *T. albacares* was 1.45 million oocytes and the estimate for the following year was 2.50 million oocytes, within the same area of the eastern Pacific (Schaefer, 1998).



The annual fecundity is the product of the spawning frequency and the batch fecundity. The annual relative batch fecundity (number of oocytes/g body weight/year) was estimated to be 14100 and 17300 for the average 2- and 3-year-old *T. albacares*, respectively, in the eastern Pacific (Schaefer, 1998). These estimates illustrate that the annual egg production is far greater for the average 3-year-old than for the average 2-year-old. Predictive models for length at maturity and spawning frequency should be coupled with those for batch fecundity and used with abundance estimates for females to produce estimates of the potential annual egg production of tuna stocks.

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# Methods to assess maturity and realised fecundity illustrated by studies on Dover sole *Solea solea*

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## ABSTRACT

Studies on the development of potential fecundity during maturation, and its dispersal during spawning are described for *Solea solea* with reference to assessment of maturity at age and realised fecundity. Recruitment of fecundity was shown to be complete well before the start of spawning in 4 year and older fish that were probably about to spawn for the second time or more. However, the youngest group in the study (three-year-old fish) appeared to have a low spawning success as indicated by many partially developed ovaries that appeared to abort maturation though follicular atresia. In view of this partial maturity it was considered that the assessment of population maturity at age or length would be most reliable at the start of spawning and, if based on macroscopic assessment, the ovary should contain yolk oocytes visible to the unaided eye (ie oocytes >0.5mm diameter). The main obstacle to estimating realised fecundity was high levels of follicular atresia in some fish within all age groups peaking around the start and end of spawning. The selection of methods and results are discussed with reference to other teleosts and the application of fisheries independent methods based on egg production.

## INTRODUCTION

This paper describes methodology and presents results relating to the assessment of sexual maturation and fecundity (millions of eggs) in a species with a broadcast spawning strategy. Despite their high fecundity and somatic growth rates many of these species are threatened by extremely high fishing mortality on the adolescent and adult populations and stock assessments are required to provide safeguards for several reasons. These include determining at what size and age fish start producing eggs (attainment of maturity) in relation to the their life span

determined by fishing or natural mortality. More recently it has become apparent that age diversity in the spawning stock influences egg number and egg survival with older fish performing much better to maintain recruitment (Scott et al., 1999). The measurement of egg production can also provide the basis for a valuable fishery independent method of stock assessment by dividing the average individual's contribution into that of the population production over a day (Parker, 1980), period (Lo et al., 1992), or a year (Lockwood et al., 1981).

All of these assessments require a thorough understanding of the dynamics of oocyte growth (Wallace and Selman, 1983) within the follicle and the degradation of post ovulatory follicles following ovulation and the release of egg batches completing oocyte maturation. This paper therefore refers to literature describing the morphology and classification of oocytes and post ovulatory follicles that form the theoretical basis to the assessment of maturity and fecundity in female fish. Attainment of maturity is defined as the culmination of oocyte development leading to the production of ovulated eggs in batches, which may comprise all (total ovulation) or a proportion of the potential fecundity (partial ovulation). The potential fecundity equates to the number of oocytes that contain either yolk granules and or cortical alveoli and is rarely equivalent to the number of eggs spawned (realised fecundity). The difference between the two values arises because some follicles abort development (follicular atresia) after recruiting to the vitellogenic population either prior to or during spawning.

The focus of the methods and results section is on reproduction in females of a flatfish *Solea solea* because it has

**Table 1.** Details of the length ranges of fish collected at various locations off the English coast between 1984 and 1997 to complete each aspect of the study.

Date	Latitude range (degrees)	Longitude range (degrees)	Sea area	Number of fish	Length range of fish samples	Study
1984	51.5 N	15 E	Southern North Sea	2	35-38 cm	Homogeneity of the ovary
July 1993 – May 1994	50.5 N	0 to 0.5 E	English Channel	50 – 80 per month	Length stratified 25-43 cm	Development of fecundity and maturity at age
March – June 1997	53.5 N	-3 to -4 W	Irish Sea	215	Random 28-40 cm	Realised fecundity and production of atretic vitellogenic follicles

been extensively studied to estimate realised fecundity to apply the annual egg production method (ICES, 1990, Horwood, 1993, Armstrong et al., 2001). Because of the asynchronous oocyte development and long spawning season sole provide an example of several problematic aspects of maturity and fecundity assessment (Urban, 1991). In the discussion the methods and results will also be considered in relation to other species maturing over a range of ages/sizes and include round fish such as cod *Gadus morhua*. Although these species occupy European shelf edge waters the conclusions and processes should be representative of a wide range of teleosts from all over the world.

## METHODS

### Fish collections

Collections of female sole were taken mostly from the catches of commercial vessels using beam trawls working in three regions around the English coast with high sole abundance during the maturation and spawning seasons

1984, 1993-4 and 1997 (Table 1). On two occasions, one in August 1993 and the other in March 1997, the collections were taken from research vessel beam trawl catches. The total length of each fish was measured (cm) and the body cavity opened to assess the stage of sexual maturity using macroscopic criteria described in Table 2. Stage 6 fish were excluded from the ovary collections made in 1997 otherwise the ovaries were preserved in 4% (w/w) formaldehyde buffered with 0.1M sodium phosphate Ph 7.0. After removal of the ovary the remaining body was wrapped in polyethylene and transported on ice to the shore where it was weighed. Only in 1993 – 94 were the otoliths removed for age determination (Möller-Christensen, 1964). Ages given throughout this paper are based on a nominal birth date of 1 January and the birth year will be used to identify and follow each year class through the study period from August 1993 until July 1994. For example, fish spawned in 1991 (referred to as the 1991 year class) will be classed as age two in 1993 and age three in 1994.

**Table 2.** Female sole ovary maturity staging criteria

Maturity stage	Description	
1	Immature	Ovaries are small; extending more than 8 cm into the body cavity; translucent in appearance. No oocytes visible.
2	Resting	Ovaries cream coloured, extending further into the body cavity. Lumen filled with fluid. No oocytes visible.
3	Early developing	Ovaries extend to fill half the body cavity. Yellow in colour. Blood supply well developed. Some developing oocytes visible.
4	Late developing	Ovaries fill the body cavity and body is distended. Yellow in colour. Lumen and advanced yolked eggs visible.
5	Ripe	Ovary swollen and the body distended. Translucent hyaline oocytes can be seen through the ovary wall (tunica) interspersed with opaque granular oocytes and may be just a few or many but ovaries will not run even under heavy pressure.
6	Running	Ovary full becoming flaccid as spawning progresses. Lumen filled with ovulated oocytes, which can be extruded under light pressure.
7	Spent	Ovaries reduced in size and flaccid. Few eggs in state of resorption (mainly opaque eggs) and much slime in ovaries.

**Preparing the ovary for histology**

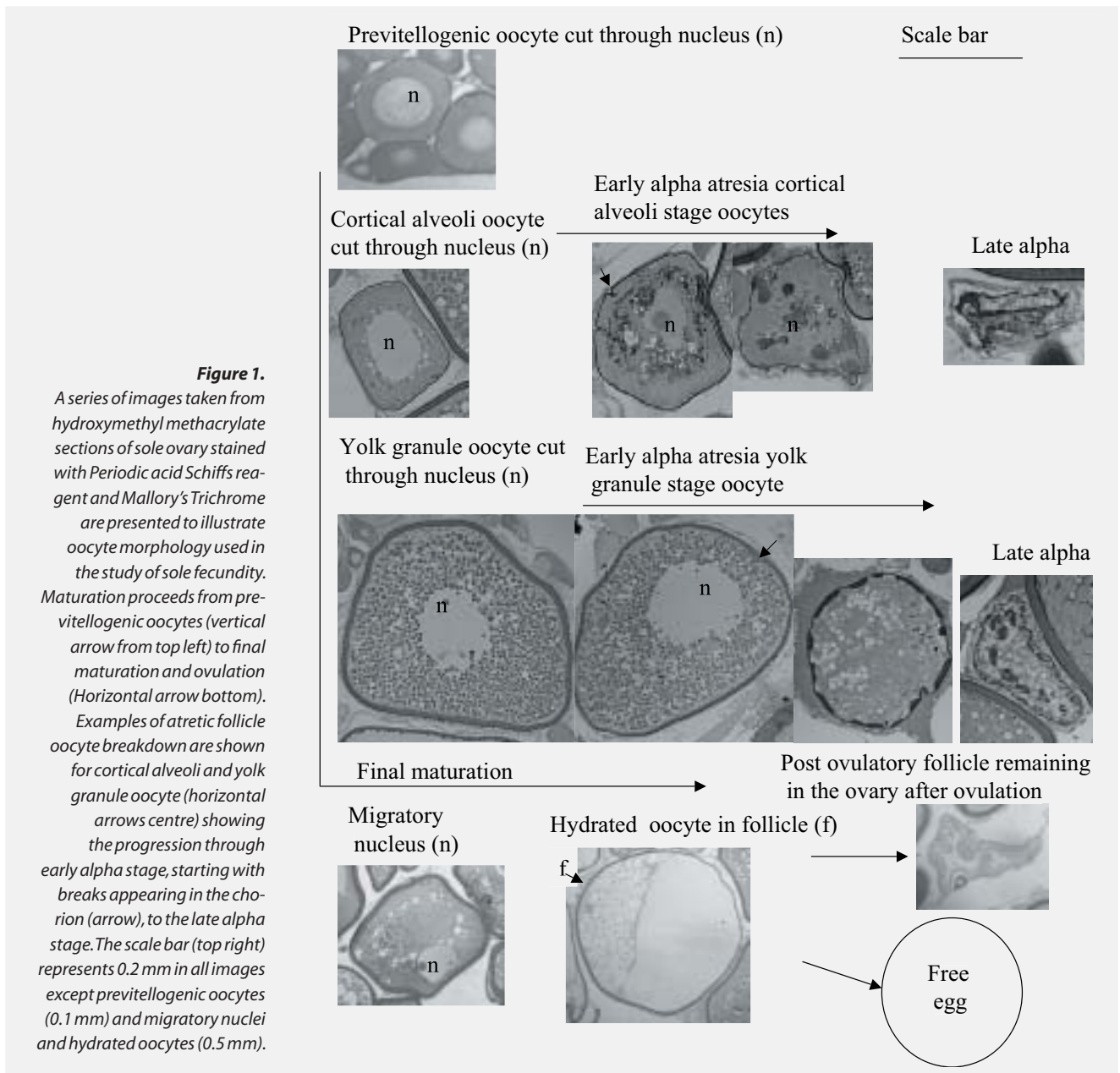
The fixed ovaries were weighed and their volume determined using a displacement method (Scherle, 1970). Transverse sections approximately 0.5 cm thick were dissected out from the midpoint of each ovary and dehydrated in ethyl alcohol prior to embedding in hydroxymethyl methacrylate resin (Technovit 7100 Taab). Each resin block was sectioned at 5 µm using a motorised microtome cooled in a refrigerated cabinet at -12°C and the sections were then stained with periodic acid Schiff's (PAS) and Mallory's trichrome to identify the oocyte development stages present in the ovary.

To study ovary morphology just prior to and during spawning freshly-caught whole fish, caught in 1984 (Table 1) were clamped flat on a metal plate and dipped into isopentane cooled to -150°C by liquid nitrogen on board ship. After 1

h the surrounding tissue was removed from the ovaries that were then mounted in a block of 2% (w/v) sodium carboxy-methyl cellulose and stored in the vapour phase of a liquid nitrogen refrigerator at about -100°C. They were transferred to a deep freeze at -20°C for transport and sectioning. Sections of 25 µm were cut from the block using a whole body cryostat (Bright LKB 2250) and supported on adhesive cellulose acetate tape whilst staining with Harris' haematoxylin and eosin. The distribution of hydrated oocytes was then described to illustrate how the batch fecundity was organised prior to and during spawning and to determine whether the hydrated oocytes were aggregated or homogenously packed in the ovary .

**Oocyte staging**

Nomenclature for staging developing oocytes (Fig. 1) in histological section within the follicle followed that



used for *Solea solea* by Ramos (1983), which was based on *Carassius auratus* (Yamamoto and Yamazaki, 1961). Oocytes that contained only cortical alveoli were included with yolk granule stage oocytes in the fecundity estimates (Khoo, 1979).

Atretic oocyte stages follow descriptions from Bretschneider and Duyvene de Wit (1947) and Lambert (1970). In the present study only oocytes in the first part of alpha atresia (Fig. 1) were included to estimate atretic oocyte abundance because their boundaries and identity were more distinct for scoring using the stereometric method described below. Normal cortical alveoli and yolk granule stage oocytes show the first indications of regression into early alpha atresia as one or more breaks appear as the chorion starts to fragment. In the cortical alveoli stage the breaks are also accompanied by a more wrinkled chorion and intense blotches of PAS staining. Atretic oocytes in which the breaks in the chorion appear to be more than 3 times its width are not included in the estimate of atresia and the oocyte is classed as in the late alpha stage. The chorion continues to fragment and like the yolk granules completely disappears leaving large vacuoles in the ooplasm and the oocyte has degraded to beta stage atresia.

### Post ovulatory follicles (POFs)

Identification of post ovulatory follicle follows morphological criteria described by Hunter and Macewicz (1985a). No attempt was made to age the postovulatory follicles (Fig. 1) based on the stages described by the above authors because POF ageing process has not been studied in *Solea solea* in experimental conditions reflecting the ambient sea water temperatures of the collection sites. Data on the persistence of POFs in cod at similar water temperatures (9°C) suggest they can be identified using PAS stain up to 6 weeks post spawning (Witthames et al., 2000).

### Oocyte development studies

Maximum oocyte size was measured in ovaries collected monthly to identify a sub set of the vitellogenic oocyte population from when they commenced vitellogenesis until final maturation. This data was used to determine oocyte growth rate from the start of fecundity recruitment (Ramsay and Witthames, 1996) and to quantify ovary development in relation to the likely onset of spawning (West, 1990).

To measure the maximum oocyte size (Method 1) a tissue sample from the centre of the preserved ovary was placed on a microscope slide and the oocytes teased apart with forceps to form a dispersed layer (whole mount preparation). The largest oocyte in the sample was measured, using a visual display system attached to the microscope, across

its longest and shortest bisecting axis and the mean taken. The procedure was repeated three times and the largest of the three values used. Hydrated oocytes were noted but not measured. Measurements of oocytes in histological section (Method 2) were only taken when the oocyte was transected through the nucleus (ie equatorially cut). The possible inaccuracy of the whole mount technique (Method 1) when applied to sole due to extreme values was examined and it was shown that the technique has a standard error of between 8.9 and 18.2 µm. Comparison (linear regression) of estimated maximum oocyte sizes with an alternative method (Method 2) based on 50 random measurements of oocytes in a histological section from the same ovary showed a high degree of consistency.

$$\text{Method 2} = 0.856 * \text{Method 1} - 0.619 \quad (r^2 = 0.95, \\ P < 0.0001).$$

The intercept was not significantly different from zero:  $P > 0.97$ .

The oocyte size frequency distribution, from a minimum diameter of 150 µm, was measured manually using Gravimetric Fecundity Analysis software written by Pilkington Image Analysis Systems (PIAS). The resolution of the video measurement system was 0.003 mm per pixel and a calibrated bar 150 µm long was used to identify and measure all oocytes larger than the bar.

### Fecundity studies

Astereological technique (Emerson et al., 1990) was carried out using Software written by Pilkington Image Analysis systems (PIAS) to estimate the standing stock of vitellogenic oocytes from when the fecundity started to develop in pre-spawning fish (from July 1993 until February 1994: Table 1). The same approach was also used to measure the decline in the standing stock of oocytes in spawning fish (1994 and 1997 samples). Using this technique it was possible to determine independently the number of vitellogenic, atretic and hydrating / hydrated follicles but not ovulated oocytes at all maturity stages excluding stage 6 running fish. The software provides a means to measure vitellogenic oocyte size frequency using 4 dots to define oocyte diameter and overlays a Weibel grid (M162) on a colour video image of the section field to perform point and profile counts. This was repeated in a number of separate fields depending on the ovary weight spread across both the dorsal and ventral ovary to determine the mean partial area and number of oocytes per field. The data was stored for analysis in a spread sheet template to calculate the numbers of oocytes in each class. Numbers of each class of oocyte were expressed independently of length or total weight by dividing the oocyte count by the length <sup>3</sup> and referred to as fecundity, atretic or hydrated oocyte condition. This approach, using length



$\hat{f}_3$  rather than relative fecundity (fecundity / total weight), to normalise for the effect of fish size on fecundity was preferred because total weight declines by 15% or more as the fish sheds its annual fecundity.

The rate of increase in the standing stock of oocytes was studied over a period after the first signs of maturation but before spawning commenced and restricting the analysis to fish of 5 years or older which were very likely to spawn. The fecundity data from selected months was modelled using a changepoint regression (Quandt, 1958) or ‘two line’ model by fitting the fecundity condition (fecundity / length  $\hat{f}_3$ ) data using the algorithm in Julious (2001). This is a linear regression where the coefficients are allowed to change at a given point. The model was:

$$\begin{aligned} \text{Fecundity condition}_i &= \alpha_1 + \beta_1 x_i & x_i \leq \delta \\ &= \alpha_2 + \beta_2 x_i & \delta \leq x_i \end{aligned}$$

Equation 1

where  $x_i$  is the time in days and  $\delta$  the unknown changepoint.

A model with  $\beta_2$  fixed at 0 was also fitted to represent the hypothesis that fecundity condition does not change with time after the changepoint. Analysis of variance was used to test the change in fit between this model and the model with  $\beta_2$  estimated.

**Spawning intensity assessment**

The start and intensity of spawning was assessed by the prevalence of fish with ovaries containing either post ovulatory follicles (POF) or hydrating oocytes (HYD). The latter group included cases where hydration was complete but not ovulated or atretic hydrated oocytes. Prevalence is defined as the number of fish with POF or HYD divided by the number of fish in the sample. To identify when 50% of the population had commenced spawning a logistic regression was used (Rijnsdorp and Verthaak, 1997) fitting the following model.

$$M_j / N_j = a + bD \tag{Equation 2}$$

where  $M_j$  = number of fish with POF at day number  $j$ ,  $N_j$  is the number of fish sampled at day  $j$ ,  $D$  = the number of days elapsed since December 31 1996.

**RESULTS**

**Histology and whole mount inter- calibration**

Samples from the 1993 – 94 collections (Table 1) were used for this study and comprised of between 50 and 80 fish each month. The leading oocyte cohort was assessed by histology to determine its stage of development and the maximum oocyte diameter recorded in whole mount preparations from the same ovary (Table 3). In all the ovaries where the maximum oocyte size was less than 231  $\mu\text{m}$ , histological examination found only previtellogenic oocytes. If the maximum oocyte size was larger than 231  $\mu\text{m}$  but smaller than 400  $\mu\text{m}$  cortical alveoli were found and these inclusions increasingly filled the oocyte cytoplasm indicating that preliminary maturation of the ovary had commenced. Oocytes larger than 400  $\mu\text{m}$  contained cortical alveoli and yolk granules indicating the start of exogenous vitellogenesis leading to the later stages of maturation. The start of final oocyte maturation, when the nucleus migrates to the oocyte periphery from a central position, occurs in smaller oocytes in 3 year old compared to 4 year and older fish (at 742 and 800  $\mu\text{m}$ , respectively). Based on this evidence it suggests that smaller fish, probably spawning for the first time, will produce smaller eggs compare to those spawning at a larger size.

**Fecundity production and oocyte growth**

This study started (day 1) on 18 July 1993 but the fish in samples taken in July (70%) and August (30%) were mostly at maturity stage 2 and their ovaries contained only previtellogenic oocytes. To focus on the rate of vitellogenic oocyte proliferation the change point regression analysis was therefore restricted to a period from September 1993 until January 1994 when most fish had started producing their annual fecundity, but before the start of spawning. One of the 10 fish in the September sample had not commenced vitellogenesis therefore only the 9 most developed fish of each monthly sample were included in the regression. It was assumed that this selection would focus on the

**Table 3.** Maximum oocyte diameter measured in whole mounts compared with the development stage of the leading cohort found in histological section based on 50 to 80 fish per month during 1993-94 (Table 1). The lower and upper 2.5% of each size range has been excluded.

Oocyte histological stage	Whole mount size range (95%) ( $\mu\text{m}$ )	Mean ( $\mu\text{m}$ )
Previtellogenic	163 - 231	215
Cortical alveoli	231 - 400	296
Yolk granule	403 - 911	703
Migratory nuclei	742 - 1030	897

**Table 4.** Details of the parameters determined by the changepoint regression analysis from Equation 1.

Model	Parameter estimates for				
	$\delta$	$\alpha_1$	$\beta_1$	$\alpha_2$	$\beta_2$
$\beta_2 = 0$	95.2	-7.60	0.153	6.93	
$\beta_2$ estimated	91.0	-7.60	0.153	5.48	0.0089

increase in fecundity in the sub set of the population that was mature in September through until January when the first spawning fish was found. The parameters (Table 4) after fitting Equation 1 suggests that fecundity condition increases quickly with time then flattens off in the period between 85 and 207 days (Fig. 2). Changes in fecundity condition with time after the changepoint are not statistically significant ( $\hat{\beta}_2 = 0.0089$ ,  $p = 0.40$ , Table 5).

The increase in maximum oocyte size with time was investigated using analysis of covariance, treating date as a covariate and year class as a factor for the months September to January (Fig. 3). To restrict this analysis to fish that were likely to spawn in 1994 and to remove non maturing fish from the data set (mostly in the 1991 year class) a fixed percentile of each months data was excluded. The percentile was based on the proportion of fish where the leading oocyte cohort in the months March and April was less than 400 $\mu\text{m}$ , which was 10% and 40% of the 1990 and 1991 year classes. A straight line was then fitted to the data and the effect of year class tested. Separate lines were fitted for three year classes: 1991, 1990 and oldest group fish pooled from 1989 or earlier classes. The analysis showed

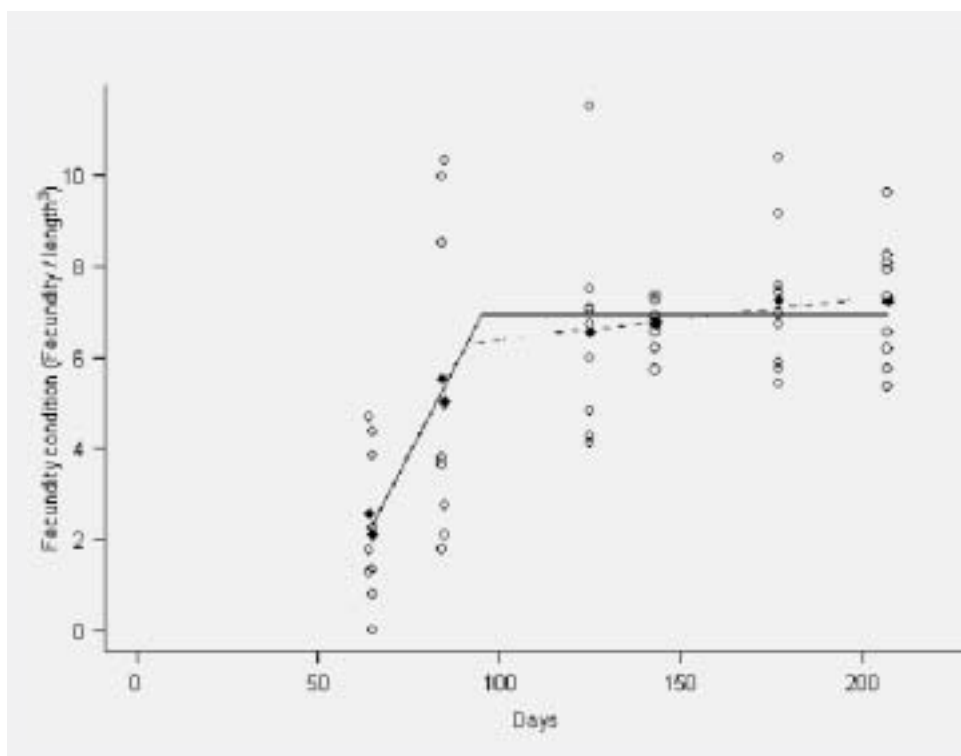
the lines were significantly different in terms of intercepts and slopes (test for different intercepts  $P < 0.01$  and different slopes  $P < 0.005$ ). The possibility of pooling all year classes older than 1991 was also tested and found them to be significantly different.

#### Oocyte size frequency distribution

Measurements of oocytes from an ovary collected in March 1997 just prior to the main spawning in the Irish Sea (Table 1) revealed a very asynchronous population of vitellogenic oocytes with the highest frequencies recorded in the largest size classes (Fig. 4). The 2 size classes on the interface between previtellogenic and vitellogenic oocytes contained very few oocytes (<0.3%) and the smallest previtellogenic group (150-175  $\mu\text{m}$  class interval) was the most abundant of any class by about 3 times.

#### Maturity assessment and abortive maturation

Hydrated oocytes were found in one age 6+ individual by January and increasingly in the 4 year and older groups during February onwards (Table 6). Three year old fish had both the lowest prevalence and contained hydrated oocytes for the shortest time (from April to June) of any age group



**Figure 2.** Observed fecundity condition  $o$ , daily means  $\blacklozenge$  and fitted changepoint model with:  $\beta_2$  estimated (dashed line) and  $\beta_2 = 0$  (solid line) using Equation 1 and parameters in Table 4. The dates shown indicate when each collection was made between July 1993 until February 1994 and include the first two samples when most fish had not commenced their annual maturation.

**Table 5.** Results of the Anova testing for changes in fecundity condition with time after the changepoint.

Model	Residual degrees of freedom	Residual sum of squares	Test degrees of freedom	$\Delta$ Sum of squares in regression	F value	Pr(F)
$\beta_2 = 0$	51	196.50				
$\beta_2$ estimated	50	193.66	1	2.84	0.73	0.40

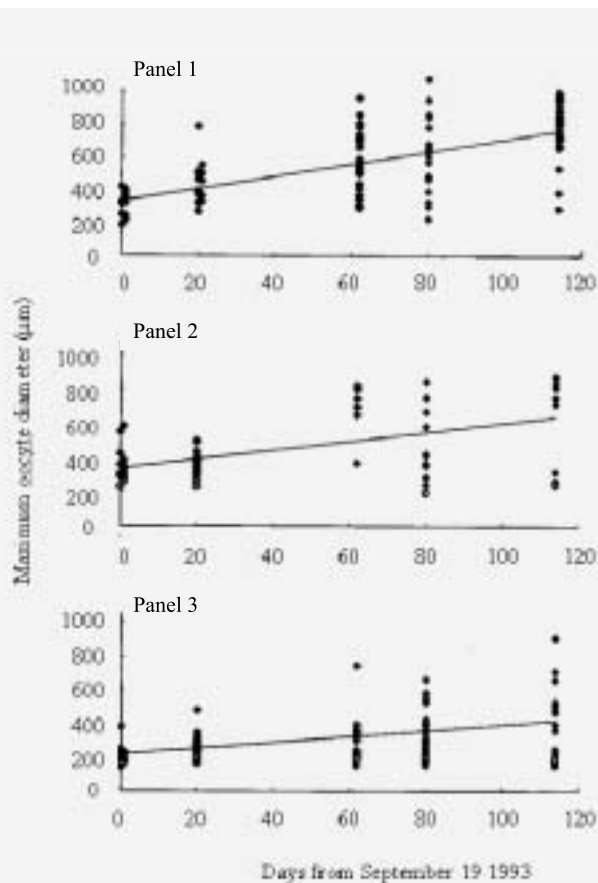
indicating their spawning was less productive than the older age groups. Some older age groups were spawning from February to June but all groups had finished by July and peak spawning months were in April and May.

The prevalence of alpha atresia was investigated in 3 year classes (3, 4 and 5+ years old) in ovaries at maturity stages 2 to 7 (Table 2) grouping them according to the size of the leading oocyte cohort (<400  $\mu\text{m}$  and > 400  $\mu\text{m}$ ) and into two monthly collection periods between February and July

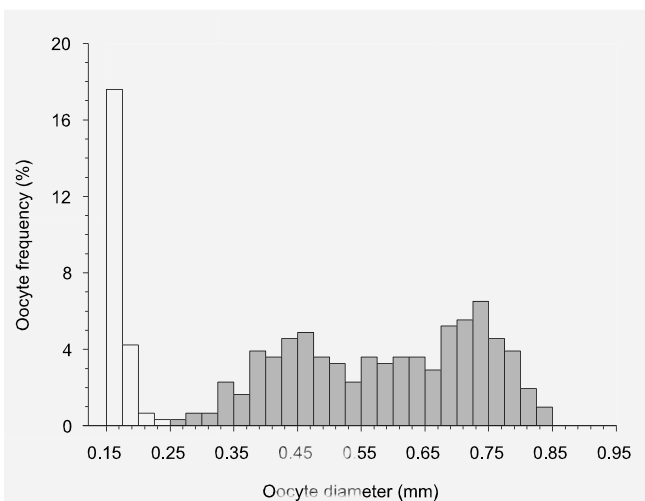
1994 (Table 1). A high proportion of three year old fish in each sample only contained oocytes smaller than 400  $\mu\text{m}$  (Table 7) which were also associated with the highest prevalence of atresia increasing to 0.33 in the last period. As there was no sign of post ovulatory follicles, indicating spawning up to 6 weeks previously (Witthames et al 2000), it is very likely that most fish in this group failed to complete maturation to spawning. In three year old fish, where oocytes had grown larger than 400  $\mu\text{m}$ , atresia prevalence was low (0.07 or less) until the last sample period when it increased to 0.73 of fish sampled. Fish of 4 years upwards mostly developed yolk granule stage oocytes and atresia prevalence was low until the final June to July sample group.

#### Morphology of ovaries close to spawning

Cryostat sections of ovaries from fish at maturity stage 5 and 6 (Fig. 5A –C) show the eye-side ovary is approximately twice the size of the blind-side ovary. In both ovaries the cross-sectional area increases from the tail forward to where they form a common oviduct leading into the cloaca behind the operculum (Fig. 5C). Prior to a spawning hydrating oocytes in the stage 5 ovary (Fig 5A) appear to occur at random throughout the cross-section and this provides a homogenous distribution for sub-sampling to estimate batch fecundity. Following ovulation the hydrated



**Figure 3.** Change in maximum oocyte diameter with time. A linear regression was fitted to the data after removing the  $x$  percentile of each month's observations (hollow circles) where  $x$  is the percentage of fish containing advanced oocytes < 400  $\mu\text{m}$  in March and April.  
 5+ year old  $O = 332 + 3.76d, n=94 P < 0.0001$   
 4 year old  $O = 338 + 2.66d, n=63 P < 0.0001$   
 3 years old  $O = 221 + 1.76d, n=95 P < 0.0001$   
 where  $O$  = oocyte diameter ( $\mu\text{m}$ ) and  $d$  = days after September 19.

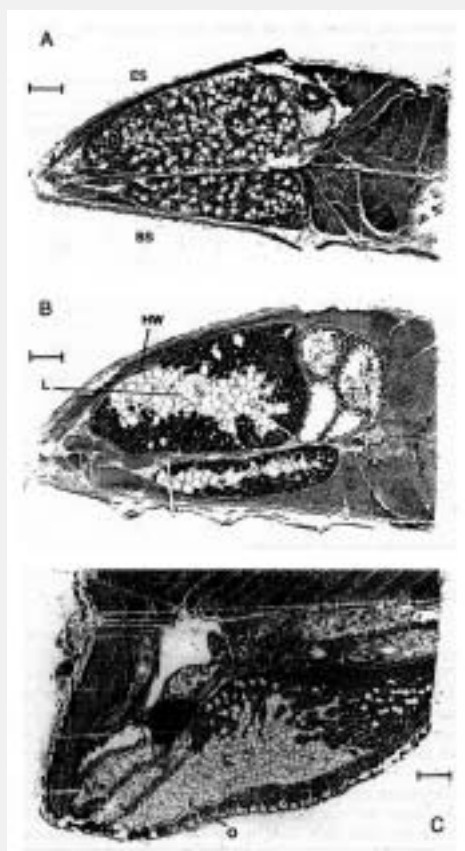


**Figure 4.** Previtellogenic (clear bars) and vitellogenic oocyte (dark bars) frequency distribution found in a sole collected during March 1999. The total number of oocytes measured was 307.

**Table 6.** Prevalence of fish observed with hydrated oocytes in their ovaries by month.

Age (years)	Month (during 1994)						
	January	February	March	April	May	June	July
3	0	0	0	0.13	0.16	0.04	0
4	0	0.03	0.07	0.35	0.24	0.14	0
5	0	0.10	0.7	0.43	0.42	0.6	0
6+	0.03	0.14	0.03	0.50	0.44	0.07	0

eggs become concentrated in the lumen. The eggs can now be extruded from the ovary and the ovary cross section (Fig. 5B) show they lie in a band stretching from the outside ovary wall, appearing as a transparent window inwards to the centre of the ovary. In longitudinal section (Fig 5C) the mass of hyaline eggs stretches down the length of the ovary. In conclusion the stage 6 ovary contains a very heterogeneous distribution of oocytes and should not be used for fecundity studies.



**Figure 5.** Cryostat sections (25  $\mu$ m) of whole ovaries from spawning sole, maturity stage 5 (A) and 6 (B+C), stained with haematoxylin and eosin. The scale bar represents 1 cm on the section. A, a transverse section showing a larger eye side ovary (ES) above the smaller blind side (BS) ovary and widely dispersed hydrated oocytes. B, a transverse ovary section showing the lumen (L) packed with ovulated eggs which stretches to the edge of the ovary wall forming a hyaline window (HW). C, a longitudinal section of the anterior end of the ovary showing an accumulation of ovulated eggs in the lumen and in the oviduct (O) leading to the cloaca.

### Spawning and realised fecundity

Samples collected in 1997 from a more northerly population (Table 1) were used to study the dynamics of egg production at the individual level from the start to almost the end of the population spawning season (Fig 6). Proportions of fish either hydrating a batch of oocytes or containing post ovulatory follicles rose rapidly from the date of the first sample. The logistic regression model (Equation 2) predicted that 50% of the population had ovulated at least once by day 92 (April 2). After a period from day 92 until day 140 (9 May) when a high proportion of fish were hydrating a batch of eggs their proportion dropped sharply over a week to less than 0.5 in the last two samples. The proportion of post ovulatory follicles never dropped below 1 after 110 days suggesting this stage has a life lasting several weeks at the prevailing water temperatures (10°C) expected in this area of the Irish Sea during the last two weeks of May. Atretic oocyte condition was high in the first 4 samples reaching a maximum on the 9 April and then fell to low levels for 4 samples taken over the next 33 days but increasing again in the last sample. Both the standing stock of fecundity and batch fecundity condition fell following the average start of spawning in the population with predicted numbers of each oocyte class falling from 303169 to 39745 and 27397 to 5445 between days 92 to 156 respectively (Table 8) in a 35-cm fish.

### DISCUSSION

#### Selected methodology

Ovaries were preserved by freezing for the cryostat study of ovary morphology, and with buffered formaldehyde for the other studies. Initial experiments to develop protocols for preserving ovary tissue by freezing showed that cellular morphology was very damaged when blast frozen (to -30°C). Immersion in iso pentane cooled to -150 °C gave better results but this approach would not be practical for routine preservation of ovary tissue for population fecundity studies. Previously Gilson fixative (Simpson, 1951) has been used to preserve and separate oocytes from ovary tissue but all delicate cellular structure like POFs are probably lost and the survival of atretic oocytes is open to question. Gilson fixative also contains mercuric chloride, a very poison substance, and the costs of disposal and risks to laboratory workers mean its use should be discontin-

**Table 7.** Prevalence of atretic oocytes in ovaries with either a leading oocyte cohort of cortical alveoli stage oocytes (<400µm) or with advanced yolk granule oocytes (>400µm) during three periods of two months in 1994. The total number of fish is appended in brackets (ID = insufficient data; 5 or less fish in the group).

Oocyte size µm	Age (years)	months		
		February & March	April & May	June and July
<400	3	0.16 (32)	0.28 (29)	0.33 (48)
>400		0.07 (28)	0.03 (33)	0.73 (11)
<400	4	ID (3)	ID (4)	60 (10)
>400		0.16 (19)	0.15 (13)	ID (4)
<400	5	-- (0)	ID (5)	0.44 (9)
>400		0 (20)	0.11 (9)	ID (4)
<400	6+	-- (0)	ID (2)	0.31 (16)
>400		0.13 (16)	0.13 (16)	ID (2)

ued. The buffered formaldehyde is also toxic but incurs much smaller disposal costs and preserves structure for histology and gravimetric fecundity analysis (Hunter and Mazewicz, 1985b, Hunter et al., 1989). For block preparation hydroxy methyl methacrylate was preferred to paraffin wax because the quality of sections is superior for resolving different stages of follicular development. It is also possible to cut large ovary cross sections (up to 50 mm x 50 mm) so the stereological method can be applied to a wider size range of ovaries and the change in dimensions on drying resin sections has been evaluated (Hansted and Gerrits, 1983).

The stereological method (Emerson *et al.*, 1990) has been evaluated (Kjesbu *et al.*, 1998, Armstrong *et al.*, 2001) in cod and sole respectively. In the first case the fecundity estimate was 1.035 CV=26,7 %, (n=27), greater compared to the gravimetric fecundity analysis. The CV was high because cod ovaries are too large to prepare whole ovary cross sections and the stereological technique was adapted to estimate the number of oocytes in a weighed piece of tissue. Fragments of ovary have a much more irregular oocyte packing density compared to when the ovary was fixed whole because the outer ovary tunica constrains the tissue from opening out. In the case of sole the stereometric estimate of fecundity was 8% higher (n=8) than fecundity determined by sub-sampling a suspension of oocytes separated by digestion in Gilson fixative (Walsh et al., 1990). However, Gilson fixative induces a large amount of shrinkage in oocyte diameter (Witthames & Greer Walker, 1987) and it is therefore difficult to assess the lower size limit of vitellogenic oocytes identified by histology. In summary the advantages of the stereometric method is that a section provides a means to quantify oocytes of all classes following their morphological identification and to determine spawning status by presence of markers such as POFs. However, in species with ovaries having a cross section diameter

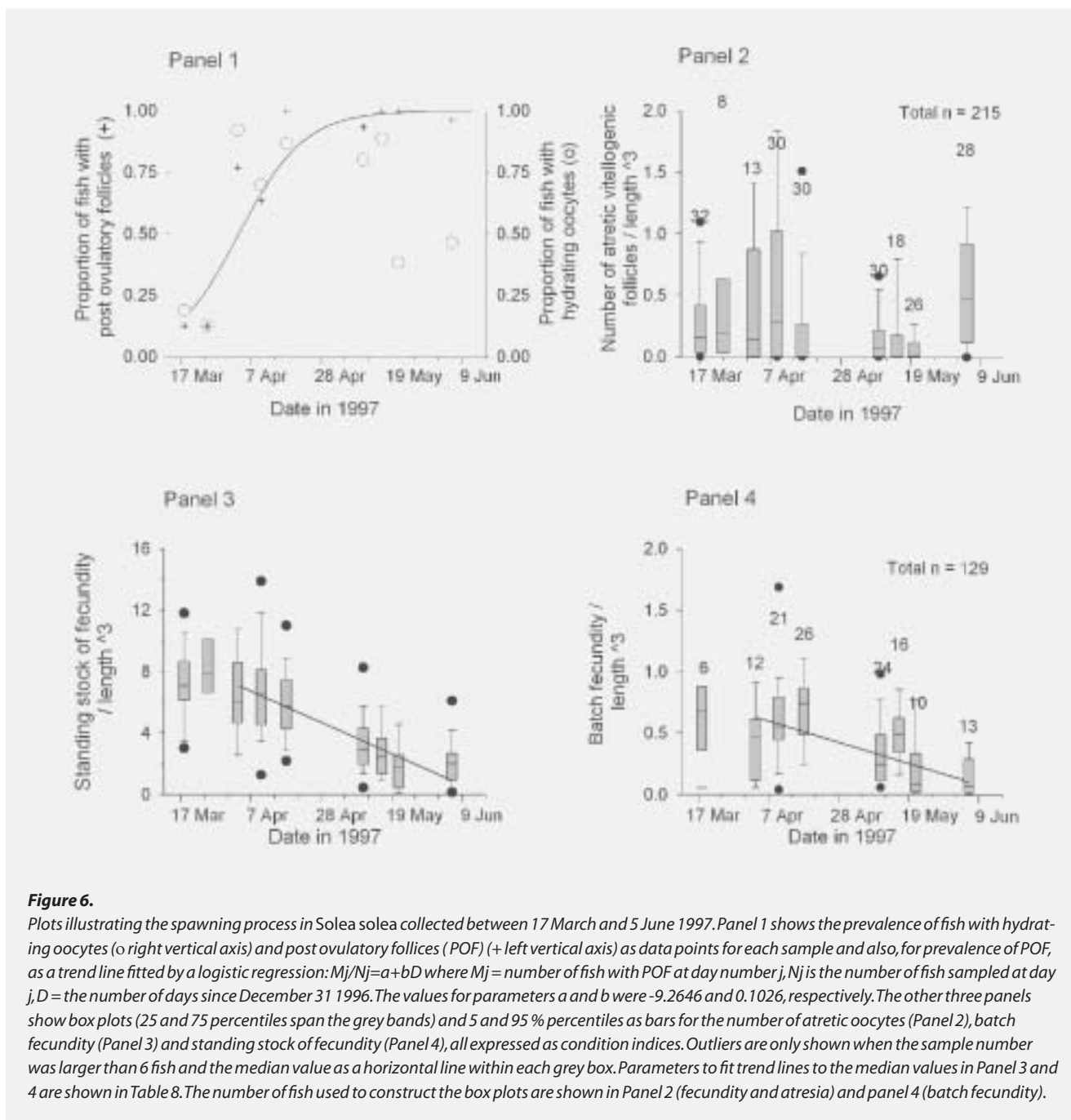
larger than 50mm (tunas, halibut and cod etc.) a sub-sample of tissue must be processed and more fields analysed to reduce the CV to acceptable proportions. The gravimetric method (Hunter et al., 1989) based on sub-sampling formaldehyde fixed ovaries is very effective for estimating potential and batch fecundity, providing it is supported by analysis to show the oocyte density is uniform (Nichol and Acuna 2001). If criteria can be developed, either through selective staining or morphology to distinguish spawning markers and atretic from vitellogenic follicles in the course of gravimetric analysis of formaldehyde fixed tissue this would also remove the need for expensive histology. An indirect method (Thorsen and Kjesbu, 2001) to estimate fecundity would also benefit in this context.

### Maturity assessment

The analysis of maximum oocyte size, atresia and the incidence of spawning indicated by the presence of hydrated oocytes in relation to fish age and the annual maturation cycle showed that April was the optimum period to assess sole maturity. Many 3-year old fish appeared to only partially mature with their ovaries containing small cortical

**Table 8.** Details of the regression parameters and statistics after fitting median values of either the standing stock of fecundity or batch fecundity condition as values of  $y$  in a linear model  $y = ax + b$  where  $x =$  days after December 31 1996. The start day (92) was taken when 50% of the population sample contained post ovulatory follicles calculated from a logistic equation (Equation 2) until the final observation (156 days  $n=7$ ).

Case	a (se)	b (se)	r <sup>2</sup>	p
Standing stock fecundity condition	-0.096 (0.013)	15.903 (1.594)	0.918	0.0007
Batch fecundity condition	-0.008 (0.003)	1.375 (0.377)	0.590	0.04



alveoli stage vitellogenic oocytes, which were also associated with a high prevalence of follicular atresia. The oocyte growth rates also appeared to be relatively slow in this group and it was therefore unlikely that they could produce viable oocytes before the end of the spawning season. This group could easily be confused with spent fish stage 7 using macroscopic features. Thus some objective criteria is required so that partially mature fish are not included with the spawning stock biomass when assigning maturity stage by macroscopic assessment (Table 2). If this key was modified so that the stage 4 ovary, the most advanced pre spawning stage, must contain yolk oocytes visible to the unaided eye (about 0.5 mm diameter) this would probably prevent such error. Ovaries from 3-year old fish with oocytes larger than 400  $\mu\text{m}$  appeared to behave more

like the older fish with generally low prevalence of atresia until the end of spawning in June and a higher incidence of spawning. Samples in this study were collected from an area characterised by both high stock abundance and high egg production and to obtain a population maturity assessment the total geographic distribution of the stock should be taken into account. In this context flatfish maturity (plaice *Pleuronectes platessa*) at age varies spatially by depth (Rijnsdorp, 1989) and over relatively short distances (150 km) in the same depth band (Nash et al., 2000). To integrate this variability over the distribution range it is necessary to weight the results according to the abundance of mature / immature fish by the area of biologically relevant strata (Armstrong et al., 2001) and include the variance determined by replicate samples.

### Estimation of realised fecundity for application in fishery independent stock assessment

The study of fecundity production in wild sole found that the standing stock of vitellogenic oocytes accumulate rapidly at the start of maturation and the process was completed early in the maturation cycle up to 100 days before any fish were found with hydrated oocytes. This finding is supported by laboratory studies investigating fecundity regulation in trout *Oncorhynchus mykiss* and cod where the change in egg size and fecundity was observed following unilateral ovariectomy (Tyler et al., 1994; Andersen et al., 1999). In both experiments fecundity compensation depended on the maturity of the ovary when it was removed and if it, in the case of trout, contained advanced oocytes the remaining ovary produced larger rather than more eggs. Just prior to spawning low numbers of oocytes were found (Figure 4) in wild sole at the interface between pre-vitellogenic and vitellogenic follicle populations suggesting no further enhancement of fecundity during spawning. This conclusion is supported by a study using traditional Gilson fixed tissue (Horwood and Greer Walker, 1990) and more recently with stereometric methods (Witthames and Greer Walker, 1995). In both instances low to zero numbers of oocytes were found in several size classes at the interface between pre-vitellogenic and vitellogenic oocyte populations and the gap between the two populations continued to expand prior to spawning. Contra indications to this conclusion have been made (Urban, 1991), but this maybe due to regional differences asserted by the author or non representative sampling. Only 6 small fish at the end of the spawning season were used in the Urban study and the histograms presenting the oocyte frequency data had a low resolution of 0.1 mm per class interval. The size and morphology of atretic oocytes, when they are most abundant at the beginning of spawning, suggest they originate mostly, but not always, from the smallest vitellogenic oocytes (Witthames and Greer Walker, 1995), so that potential fecundity is reduced rather than enhanced at this time. In conclusion it is possible to apply the annual egg production method without the uncertainty that potential fecundity will be enhanced by further recruitment of vitellogenic oocytes during spawning, but significant reduction might occur through atresia. It is therefore necessary to interpret the instantaneous values of atresia shown in Figure 6 in terms of lost potential fecundity. This has been done in two assessments of sole (Horwood, 1993 and Armstrong et al., 2001) using Equation 3 below.

$$R = F_p - (F_a \cdot P \cdot D / A_d)$$

Equation 3

where

R = realised or spawned relative fecundity ( $g^{-1}$  female)

$F_p$  = potential relative fecundity ( $g^{-1}$  female)

$F_a$  = Geometric mean intensity of atresia  $g^{-1}$  female excluding fish with no atresia

P = Prevalence of early alpha atresia in the spawning population

D = the average spawning period of an individual female (Horwood, 1993) or the duration when data was collected for the atresia assessment (Armstrong 2001).

$A_d$  = duration in days that an early alpha stage atretic follicle persists in the ovary

It has been assumed that atretic follicles persist in the ovary for 9 days by extrapolation from data on anchovy (average 9 days with a range of <3 to 20 days at 16°C, Hunter and Macevic, 1985a) and cod (10 days at 8°C Kjesbu et al., 1991). In the two assessments above it was estimated that 40 and 28 % respectively of the annual potential fecundity was lost through atresia. Parameters  $F_a$  and D will interact because individuals with high atresia will very likely spawn for a shorter period than on average and there will be a temperature effect on atretic follicle duration. In plaice (Nash et al., 2000) atresia is much less common, but all species probably regulate their realised fecundity output to some extent depending on their environment either through natural causes (Kurita and Kjesbu and also Hardardottir *et al.*, this volume) or in response to pollution (Johnson, 1998). More work is therefore required to develop a more objective way of assessing atresia and to investigate the dynamics of the process during the annual cycle. In mammals the process has been associated with apoptosis or programmed cell death and this has also been investigated in fish (Wood and Van der Kraak, 1999, Witthames et al., 1999). In each case the studies failed to find elevated signs of the apoptosis markers (characteristic oligonucleotides or apoptotic nuclei) in association with high levels of atresia.

The standing stock of fecundity declined significantly from the average start of the spawning process (Fig. 6) and by fulfilling this basic requirement it could be the Fecundity Reduction Method (Lo et al., 1992), would provide some advantages over the Annual Egg Production Method (Lockwood et al., 1981). Working over a selected period during the annual egg production cycle would provide a means to avoid the highest period of atretic fecundity loss and there would be no requirement to estimate spawning duration. The Daily Egg Production Method (Parker, 1980) would not be effected by atresia and at least one of the parameters, batch fecundity, is easy to determine. The method used to provide the data in Figure 6 has not previously been applied to batch fecundity determination but the results were very similar to that reported previously (Urban, 1991). Spawning fraction however is more difficult and would require research on post ovulatory follicle duration and would be

more sensitive to aggregation of the population in different parts of the water column (e.g., trawl effects on cod reproductive parameters: Armstrong et al., 2001).

Samples for this study were collected in the Eastern Channel and from the Irish Sea but from previous work the production and regulation of sole fecundity follows a similar pattern (Horwood 1993, Witthames et al., 1995). These surveys both indicated that fecundity varies significantly between areas and probably between years as in other species such as plaice (Horwood et al., 1986) or cod (Kjesbu et al., 1998). It must be strongly advised that maturity and fecundity data is collected at the relevant situation until there is a sufficient body of data to show the likely error in using a mean value.

In conclusion the paper describes the methods and their application to assess maturity and realised fecundity and the issues needing further research to reduce the bias in fecundity and maturity estimates. The need for this work is quite apparent because reproduction underpins fishery independent stock assessment based on egg production methods where the data collection is totally under scientific control. Further work is required to understand the reasons underlying the large disparity of 2 to nearly 5 fold between fishery dependent (agebased population assessment) and fishery independent methods (Horwood 1993, Armstrong et al., 2001).

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