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Committee

REPORT OF THE WORKING GROUP ON RECRUITMENT PROCESSES FUENGIROLA, SPAIN, 23-26 JUNE 1992

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I. TERMS OF REFERENCE AND PARTICIPANTS

At the 1991 ICES Statutory Meeting, resolution 2:48 was adopted as follows:

The Working Group on Recruitment Processes (Chairman: Dr M Heath, UK) will meet in Fuengirola, Spain from 23-26 June 1992 to:

a) report on the results of the otolith microstructure intercalibration exercise to be prepared by Dr S Campana (Canada) and Dr E Moksness (Norway);

b) report on the convening of the otolith microstructure workshop organised by Dr Campana and Dr Moksness;

c) explore the experimental aspects of size-specific theory in the modelling component of recruitment processes;

d) review field and experimental evidence for a relationship between growth and mortality rates in eggs, larvae, and juvenile fish;

e) review the statistical basis for determining growth trajectories of individual fish from otolith microstructure;

f) prepare, in collaboration with the Steering Group on Cod and Climate Change, a manuscript (to be authored by Mr J Nichols, UK) on the synthesis of information contained in the checklists of spawning characteristics of cod and haddock, with a view to identifying underlying principles of recruitment variability.

The meeting was attended by the following:

J Anderson	Canada	G Laurence	USA
J Beyer	Denmark	T Linkowski	Poland
K Brander	United Kingdom	J Modin	Sweden
S Campana	Canada	E Moksness	Norway
B Ellertsen	Norway	P Munk	Denmark
P Fossum	Norway	B Rothschild	USA
J Gagne	Canada	D Schnack	Germany
A Garcia	Spain	S Tilseth	Norway
M Heath	UK (Chairman)	R Toresen	Norway

II. OVERVIEW OF THE MEETING

1. Introduction

According to the terms of reference, and from the report of the ICES/IOC Study Group Meeting on Models for Recruitment Processes (SGMRP), Paris, 1990 (Anon., 1992), the RPWG should primarily address the factors contributing to variation and stabilisation of recruitment and long term trends. Provision of direct inputs to assessments in the form of predictions was not perceived as a requirement. It was considered that the group had made a practical contribution to improving the tools for addressing such issues through its work in compiling the life history characteristics of cod from different ecosystems, and

evaluation of the methodological aspects of interpreting otolith microstructure. The ways in which these tools might be used to achieve the overall objectives had been subjected to extensive debate during previous meetings.

The extreme complexity of the issues being addressed by the group was readily apparent. The forum provided by the RPWG for discussing, for instance, the principles of modelling early life stage dynamics, was recognised as being extremely valuable. However, the group regretted that their request for a continuation of the modelling activities started by the SGMRP (CM 1990/L:96, Recommendation 2) had not been accepted by ICES. The need for theoretical and modelling progress within the field of recruitment research cannot be understated, and the dialogue started by the SGMRP needs to be supported and sustained.

There was recognition of the necessity to find ways of stimulating structured practical activity and cooperative work between members of the group during inter-sessional periods. To encourage activity outside the designated meeting times, it was decided to include more specific recommendations than in the past, concerning the requirements for members to provide data or develop analytical products for evaluation at a subsequent meeting of the group.

2. Review of the Cod and Haddock Checklist

K Brander (UK) reported on progress with the compilation of cod and haddock spawning characteristics. Few data had been submitted on haddock, but extensive information was available for almost all the known cod stocks.

Compilation work was undertaken during the meeting, drawing on the opportunity of having many of the originators of data present. It was agreed to have the information ready for submission as an ICES Cooperative Research Report by the end of 1992.

Follow-up activities related to the checklist compilation were discussed. It was decided to maintain and update a literature database.

3. Review of the Otolith Microstructure Workshop

S Campana (Canada) was able to announce that the results from the otolith intercalibration exercise described in the report of the previous RPWG meeting (Anon., 1990) had been published in the ICES Journal of Marine Science (Campana and Moksness, 1991). He also presented the report of the otolith microstructure workshop held in Norway in October 1991 (see Appendix 3).

The objective of the Workshop was to identify sources of variability in the interpretation of otolith microstructure, and recommend techniques by which accuracy and precision could be improved. Estimates of the precision of otolith age readings were obtained by controlled investigations on nine different species. The results indicated that high precision was attainable, but underlined the importance of prior experience and training. Intercalibration and regular monitoring of analyst performance should be an important part of any cooperative research programme involving otolith reading.

The participants at the Workshop considered the rationale for the practice of back-calculating body growth rates from otolith daily increment widths. The conclusion was

that the relationship between otolith growth and body growth is not sufficiently well defined for the back-calculation practice to be recommended as a routine technique. Recommendations were also made concerning the collection and preservation of larvae for otolith analyses, as well as preferred means of otolith preparation.

4. Paris Revisited

J Beyer (Denmark) presented a review of the important conclusions from the Paris meeting of the SGMRP (Anon., 1992), to remind the members of the most important conclusions.

First, it was noted that in contrast to purely physical models which are governed by well defined physical laws, biological and ecosystem modelling may be approached from a variety of starting points. Hence, the understanding of processes from first principles at the level of the individual could be considered as a main pre-requisite.

Modelling recruitment processes is an extremely difficult task, and examples in the SGMRP report demonstrated the problems. Nevertheless, a number of conclusions were drawn up by the Paris meeting. Unfortunately few of the recommendations seem to have been acted upon to date. The most important conclusions for the RPWG were that:

a) validated models of larval growth and mortality are still not available for incorporation in large scale models

b) more detailed models will be needed to elucidate the interactions between biotic and abiotic factors affecting recruitment.

c) interaction between modellers and practitioners is vital for progress towards the objective of understanding variability and stability and anticipating long term changes.

Lessons from the SGMRP are that first, there are no apparent short cuts to understanding recruitment processes. It is essential to consider processes at the individual level - the variability in rates and processes may be as important as the mean.

The modelling group recognised the great potential of otolith microstructure for revealing individual growth history information, identified as being of key importance for further progress towards studying variability. The RPWG noted that the SGMRP report contained an appendix summarising the applications of otolith microstructure data. Some aspects of this appendix were identified as requiring updating and qualification.

5. Sub-group Sessions

The terms of reference concerning the interactions of growth and mortality, size specific theory, and determination of growth trajectories from otoliths, were addressed by a series of sub-group sessions. In each session, parallel sub-groups considered the same set of questions and reported back to the meeting in plenary where a composite view was assembled. The workplan for the sub-group sessions is given in Appendix 2.

a) **What controls otolith growth?**

Up until now, the tentative basis for interpreting the width of ring increments on otoliths in terms of fish growth has been based on correlative relationships between otolith size and body size estimated from pooled samples of many otoliths. Experts in the field of otolith microstructure present at the meeting acknowledged that there were considerable uncertainties associated with this approach since there was evidence that the relationships break down under certain circumstances.

The groups attempted to set down from first principles the basic chemical and biochemical processes controlling the diel periodic growth of otoliths. Having done so, it became apparent that the basis for a sound and testable first principles model of the link between otolith growth and body growth was attainable. A further sub-group subsequently set out a conceptual model of how the development of otolith rings may be linked to body growth through metabolic rates, and why the correlations between ring increment widths and body growth rates should break down under conditions of starvation or extreme temperature.

On the basis of the conceptual model, the sub-group noted that acquisition of temperature histories of individual fish would be a pre-requisite for full interpretation of ring increments in terms of growth rates. On-going work on elemental and isotopic analysis designed to provide just such data should be given enhanced emphasis.

Some members of the group were sufficiently motivated by the results of the session to propose the development of a rigorous first principles model of otolith growth during the intersessional period. J Beyer and S Campana undertook to pursue this task, incorporating any additional information available following an impending major conference on otoliths, with a view to developing an analytical model, carrying out tests using suitable data sets, and reporting to the next meeting.

b) **Interrelationship of growth, mortality and body size**

Review papers submitted to the Working Group showed characteristic interrelations between vital rates of larval fish when data were accumulated across many species taken from a range of ecosystems and latitudes. However, the sub-groups concluded that these general relationships were not easily applicable to interpreting the responses of individual species. It was concluded that a more specific review of field evidence for a relationship between growth and mortality should be carried out. Nevertheless, there is a widespread underlying belief that differences in food concentrations may generate differences in growth, resulting in differential vulnerability to predation. Poor feeding conditions result in slow growth, longer subjection to any particular predatory field, and larger stage specific mortality.

The ratio of mortality to growth was identified by Beyer (1989) as a key rate determining the biomass of the surviving population. This ratio must be stage dependent during larval development, and there was concern that many of the reviews of growth and mortality did not compare rates over equivalent stage intervals.

In plenary session, the meeting considered the types of studies which would be necessary to detect an interrelation between growth, mortality and size, with particular reference to the questions posed by modellers to practitioners in Appendix C of the report from the

SGMRP (Anon., 1992) and the last report of the RPWG (Anon., 1990). The meeting heard descriptions of a sophisticated small scale process study recently carried out on Georges Bank (US GLOBEC), and an open sea "virtual enclosure" study on the Scotian Shelf (Canadian OPEN programme). These two studies highlighted the differences in approaches used to study physical-biological linkages within a discrete process context or within a biological population-ecosystem context. A programme such as OPEN may be sufficiently broad in scope to meet the criteria for relating growth and mortality in the field implied by the SGMRP questions, but at a very great cost. Simpler, landlocked systems might be available as alternatives. Nevertheless, the small scale process study was essential for understanding the links between physics, larvae, and their prey and predators.

The meeting identified the move from small scale process orientated understanding of growth and mortality to a population level understanding as being of key importance. Such spatial and temporal integration was identified by the SGMRP as being a major barrier separating modelling activities from field falsification. The RPWG proposed that a Study Group should be established to consider these issues, and a detailed justification for such a group is presented later in this report (Section IV.7).

c) Underlying controls of body growth

The sub-groups concluded there were few accepted models of larval (or fish) growth based on basic biological principles, and hence no models which could account for the variability in growth rates within a population.

The importance of taking into account the variability in growth was highlighted by data from cod rearing studies carried out in Norwegian ponds (Blom *et al.*, 1989). In contrasting years of high and low food availability the mean growth rate of larvae was indistinguishable. However, in the low food year cannibalistic predation drastically reduced the population at around the time of metamorphosis. Presumably, the individuals in the small tail of the cohort size distribution were small enough to be eaten by fish in the large tail of the distribution. The implication was that the variability in growth rate may have been higher in the low food year, and that this facilitated the density dependent regulation of the population size.

A number of candidate models of larval growth were expounded during brainstorming sessions in the sub-groups. The simplest growth model is one in which the rate of change in weight varies as some power function of weight itself. However, such a simple model is not sufficient to account for the dynamics of body growth under, for example, starvation and refeeding conditions. Under these conditions, additional linked equations are required describing the rate of change in other body characteristics, for example, body length. There was support in the meeting for critical evaluation of various models of growth, and the Norwegian pond data seemed to be an ideal validation data set. J Beyer (Denmark) agreed to coordinate the assembly and coding of candidate models, whilst S Tilseth (Norway) undertook to make available the raw individual based measurements on cod larvae and the associated prey and environmental data from the pond studies. Initial runs should be circulated to members for comment, and further parameter testing and evaluation work should be a task for the next Working Group meeting.

III RECOMMENDATIONS

The Working Group recommends the following:

1a) K Brander (UK) should continue assembly of the data on cod life histories solicited on behalf of the RPWG and the Study Group on Cod and Climate Change, calling on other members of the group for assistance as necessary, and aim for submission to ICES as a Cooperative Research Report by the end of 1992.

b) K Brander (UK) should coordinate an informal network of contributors to a literature data base on N Atlantic cod.

2a) S Campana (Canada) should prepare a brief state of the art summary on otolith microstructure following the symposium in South Carolina (USA) in January 1993. This should be circulated to members of the WG and submitted as a paper at the 1993 ICES Statutory Meeting.

b) J Beyer (Denmark) and S Campana (Canada) should work together to determine if sufficient information exists to develop an analytical model of otolith growth, and if so, to begin developing a model.

3a) J Beyer (Denmark) should coordinate the assembly and coding of candidate larval growth models for evaluation and testing at the next meeting.

b) S Tilseth (Norway) should make available the raw data from cod rearing experiments referred to in the document ICES CM 1989/EMEM No 1, and cooperate with J Beyer (Denmark) in the testing candidate growth models.

c) Initial simulation results based on the Norwegian data should be circulated to members prior to the next meeting, and further testing and parameterisation carried out during the meeting.

4. P Munk (Denmark) and J Modin (Sweden) should solicit data sets on size at age for larval fish from Working Group members. The data should be assembled in a standardised form together with information on temperature and prey availability at sampling sites. During the next meeting, inter-species and inter-regional variability in growth should be investigated with emphasis on the influence of temperature and prey availability.

5. The Chairman of the RPWG should coordinate the preparation of a progress report on inter-sessional activities as a document for presentation at the 1993 ICES Statutory Meeting.

6. ICES should co-sponsor with other interested organisations (eg IOC, SCOR) a Study Group on Methods of Spatial and Temporal Integration which should meet in Scotland in 1993 (Chairman: Professor W Gurney), with the following terms of reference:

a) consider and report on methods of statistically characterising the temporal and spatial variability in populations of larval fish and their prey and predators.

b) to consider and report on the feasibility of integrating temporally and spatially variable abundance and vital rates over population time and space scales.

c) to consider how sub-grid scale temporal and spatial variability in abundance and rates may be represented at the grid scale in marine ecosystem models.

d) to consider methods of determining the most appropriate temporal and spatial grid resolution for models of fish recruitment.

7. The next meeting of the RPWG should take place in Lysekil, Sweden, between 14 and 17 June 1994, with the following terms of reference.

a) to review ongoing work in connection with the cod and haddock checklist (K Brander, UK);

b) to assemble and analyse data on the inter-species and inter-regional variability in growth of larval fish, under the coordination of P Munk (Denmark) and J Modin (Sweden).

c) to critically review and refine the performance of a candidate set of larval growth models assembled by J Beyer (Denmark), with reference to data on larval cod to be supplied by S Tilseth (Norway).

d) to review progress in the development of an analytical model of otolith increment formation (J Beyer (Denmark) and S Campana (Canada)).

e) to review recent progress on interpreting temperature histories of larvae from otolith elemental and isotopic analysis.

f) to review results of studies examining the relationships between larval size, growth, and mortality rates.

g) to consider the implications of the report of the Study Group on Methods of Spatial and Temporal Integration for the design and conduct of field investigations of recruitment processes.

IV REPORTS ON INDIVIDUAL DISCUSSION TOPICS

1. Synthesis of Information on Cod

The group was asked to produce a synthesis from the information provided in the checklists of spawning characteristics of cod and haddock and the material supplied to the Steering Group on Cod and Climate, with a view to publishing this as a Cooperative Research Report. Information was available for 11 NW Atlantic and 12 NE Atlantic cod stocks and the contributors are listed in Table 1. Insufficient information was supplied for haddock to warrant inclusion.

The stock contributions were reviewed and edited during the meeting by individual members of the group. Data for the synthesis were extracted as consistently as possible, after definitions, units and the format of a series of summary tables had been agreed.

The headings for the main synthesis tables are given in Table 2. Considerable progress was made, but the task was not completed during the meeting, because for some areas there was no participant at the meeting with sufficient knowledge of the stock to carry out the editing. Also in some cases members needed to refer to recent studies and publications which were not available. These additional details will be incorporated by correspondence over the next few months. Partly completed examples of a table and a figure are given (Table 3, Fig. 1). The aim remains to complete the editing and publication of the Cooperative Research Report this year.

The study highlights some of the difficulties of defining terms (eg duration of the pelagic stage) and shows up the gaps in our knowledge for particular areas. Taken together with the very extensive reference lists which were supplied, it should be a useful starting point for researchers who wish to locate information for a range of stocks with which they are not familiar. The group recognised the value of keeping the reference information up to date, using a common list of key words to index them (Table 4) and proposed that an informal network of area contributors should pool information about publications for their area on an annual basis.

2. Review of the Otolith Microstructure Workshop

Following the recommendation of the 1990 meeting of the RPWG, S Campana and E Moksness convened an Otolith Microstructure Workshop in Norway in November 1991. The objective of the workshop was to identify sources of variability in the interpretation of otolith microstructure and recommend techniques through which accuracy and precision could be improved. The workshop was attended by 19 participants representing 11 countries. The complete workshop report is presented in Appendix 3.

Using both round-table discussion and microscopic examination of otoliths provided by participants, the workshop focused on the influence of otolith interpretation error on age estimation, growth back-calculation and other applications. Numerous recommendations were made concerning project design, sample preservation, technical considerations, and protocols necessary for collaborative studies. The workshop results reaffirmed the power and accuracy of the technique as a source of data for many different types of studies. However, it also underlined the role of prior experience and training in the accurate interpretation of otolith microstructure features. The workshop participants strongly recommended initial training and calibration, as well as regular monitoring of accuracy in any cooperative research programme involving otoliths.

The workshop carefully considered the evidence for a relationship between the width of otolith daily increments and body growth. The participants concluded that the relationship between otolith growth and somatic growth was complex and that growth back-calculation based on otolith increment widths could not be recommended until the relationship between the two was better understood.

3. Revision of the Study Group on Models of Recruitment Processes Appendices on the Use of Otolith Data

The SGMRP included two appendices in its report (Anon., 1992) outlining the use of otolith microstructure data in recruitment research. The RPWG found the appendices to be a useful statement, but identified a number of aspects which required updating or revision. Refined version of the SGMRP appendices are given below:

a) Replacement for Appendix A on page 37 of Anon., 1992

LARVAL OTOLITH MICROSTRUCTURE and THE GROWTH TRAJECTORIES OF INDIVIDUAL LARVAE IN THE SEA

It is well known that the microstructure of larval fish otoliths may be interpreted in terms of the age of individuals with a resolution of one day. In general, after some early stage corresponding approximately to the time of hatching, growth increments are deposited on the otolith with diel frequency, and are visible by light microscopy. The number of rings in an individual otolith, therefore, indicates the age since hatching minus the age at first ring deposition (Pannella, 1971; Campana and Neilson, 1985).

In principle, considerably more information on the past history of the individual larvae may be obtained from otolith microstructure. On average, otolith width is linearly related to larval standard length for most species. Hence, the radial distance of each ring from the otolith centre is a direct record of the growth trajectory of that individual. This realisation has given rise to several approaches which have potential to give insight into early life survival processes, and provide vital data for modelling studies.

The first approach is designed to estimate the temporal variations in relative mortality within an annual spawning season for a population. The principle is to sample the surviving recruit population (metamorphosed individuals), and to estimate the proportion of the survivor population originating from each hatching date during the season from otolith microstructure. After adjusting for cumulative mortality differences across the age range of survivors, the difference between the proportion of survivors derived from each hatching date, and the actual contribution of that hatching date to the total annual production of larvae (estimated from ichthyoplankton sampling) is then a measure of the mortality of those hatchlings relative to larvae hatched on other days during the season. The survivor-birthdate approach was developed to study the seasonal pattern of survival of northern anchovy (*Engraulis mordax*) in relation to mesoscale oceanographic features. Periods of strong upwelling were found to be correlated with low relative survival of larvae (Methot, 1983). The approach has subsequently been successfully employed in a number of regions to establish the important mesoscale processes having the most significant influence on survival.

The second valuable application of otolith microstructure involves the evaluation of size-dependent mortality in a population. As before, otoliths are collected from samples of the surviving metamorphosed population, but in this case the objective is to determine what the length distribution of the survivors was on some date prior to sampling, eg when the population was still in the larval phase. Instead of back-calculating the age at a particular size (hatching) from individual otoliths, the size at a particular age is determined from ring radius measurements. Any discrepancy between the back-calculated length distribution of the population and that measured at the time in the field is then a measure of the relative size-specific mortality. In general, where this approach has been applied, the data indicate higher mortality of the smaller individuals in the population relative to the larger individuals (ie the mean back-calculated length of the survivors is shifted towards the larger sizes relative to the original mean length) (Post and Prankevicius, 1987).

The third approach takes advantage of the otolith's sensitivity to growth perturbations. Stressful events, such as life history transitions (eg metamorphosis) are often reflected in the otolith by growth increments of altered appearance, or by formation of checks (periods of unusually narrow rings). Since rings can be assigned both ages and dates of formation, the age and size of various life-history transitions can be determined. Examples include metamorphosis in flatfish (Campana, 1984), and settlement from the plankton in coral reef fishes (Victor, 1982).

All these approaches rely critically upon unbiased sampling of the survivor population to obtain otoliths. Further, they assume an exact correspondence between fish and otolith growth which, for physiological and statistical reasons, may not always be present (Campana, 1990). Nevertheless, if carefully performed, the methods provide unique and powerful opportunities to study the interactions of growth and mortality at the population level in the field, and their full potential for evaluating models of survival processes has yet to be realised.

b) Replacement for Appendix B on page 38 of Anon., 1992

The Working Group did not fully understand the original Appendix in the SGMRP report, and therefore requested its author to supply an explanation. R A Myers (Canada) kindly supplied the following text after the meeting.

ESTIMATION PROBLEMS OF LARVAL FISH GROWTH

Consider the sampling and analysis required to estimate the growth and mortality of a year class of larvae in which growth has been estimated using daily growth rings. The key difficulty is that there is size-selective natural mortality and size-selective sampling with selective gear. These two will be confounded, and independent estimates of the size-selective sampling should be carried out. Even if the size-selectivity of the gear is known, variation in growth among larvae may create large biases. A statistical approach to solving this problem is to consider the distribution of trajectories of individuals. The growth trajectory of each larvae can be viewed as a single realisation of the stochastic process generating the distribution of growth trajectories. By sampling the distribution of larvae at several times, it will be possible to infer the process responsible for variation in growth and mortality. A maximum likelihood method should be used to estimate the distribution of egg production over time, the variation of growth among larvae, and mortality.

We will assume that there are several representative samples during the larval and early juvenile stages. The primary goal is to estimate the environmental conditions responsible for the variability in survival and growth in the year class.

First, consider the sources of variation in the numbers of larvae at any time:

1. Average mortality.
2. Size-dependent mortality.
3. Environment-dependent mortality.
4. The distribution of eggs released over time.

Next, consider the causes of variation in growth rate:

1. Phenotypic differences among individuals.
2. Seasonal differences.
3. Environmental variation.
4. Age effects.
5. Non-systematic differences not explained by the above effects.

Finally, consider the sampling variability:

1. Measuring error of length.
2. Aging error.
3. Net avoidance, which will be size selective, (rapidly growing fish may be undersampled. Net avoidance may also depend upon time of day, towing speed and temperature, ie burst speed increases with temperature).
4. Sampling error in estimating abundance (representative sample of a population even if net avoidance can be eliminated may be very difficult. Roughly the same number of sample sites may be needed as is required for a trawl sample survey. If the larvae are more clustered than adult fish, then more sample sites may be required).

The statistical problems of estimation in such a situation are difficult. If at all possible independent examination of as many of the model parameters should be obtained from independent measurements, eg a trawl survey might be used to estimate the distribution of egg production over time. It is crucial that net avoidance be quantified if accurate mortality rates are to be estimated. The model structure would model the distribution of egg production over time, and as many factors affecting growth and survival as can be inferred from the data. It is important to consider individual variation in growth, because the unit of sampling is the growth rings on an individual otolith. A maximum likelihood model which combines the above factors should be used.

4. Study Group Session I - What Controls Otolith Growth?

On average, the relationship between otolith and fish growth is well defined. In theory, the relationship can be used to estimate a previous size at age, through the simple measurement of otolith radius (or a daily increment width) at some previous age. The latter can be determined from the sequence of daily increments, each of which can be assigned an age and date of formation. However, otolith growth is known to vary both among and within populations. There are numerous reports in the literature of uncoupling between otolith and fish growth, whereby otolith growth in slow-growing fish occurs more rapidly than would be expected based on the fish-otolith regression. For reasons that are not entirely clear, the presence/absence of this uncoupling is not always predictable, highlighting our lack of knowledge of the mechanism linking otolith growth to fish growth.

Most of the Working Group felt that it was not appropriate to back-calculate previous growth under the assumption that the otolith:fish growth relationship was invariant. Knowledge of the mechanism relating fish and otolith growth was considered to be an important prerequisite to our ability to accurately estimate previous sizes and growth rates from the otolith. This was highlighted by the fact that tests of existing back-calculation models have demonstrated that they were capable of only moderate accuracy at best, and were occasionally capable of gross errors.

The factors which are known to influence otolith growth are well documented, and include food quantity, food quality, temperature, photoperiod, endogenous factors (eg-genetics), ontogeny, metamorphosis and other life history transitions. In addition, the width of individual otolith increments can be influenced by the form of the otolith:fish relationship, the position of the increment on the otolith and the presence/absence of accessory primordia. Given that many of these same factors have a similar influence on somatic growth rate, knowledge of their influence is not necessarily relevant to growth back-calculation unless they affect otolith growth and somatic growth differently.

Our understanding of the mechanisms underlying otolith growth is incomplete. However, we do know that the otolith floats in a fluid-filled sac, and that the outer (growing) surface of the otolith is covered by an otolithic membrane. Otolith growth apparently proceeds through formation of a protein mesh on the outer surface, followed by calcification. The protein-calcification cycle is repeated each day. The calcium carbonate is crystallised from the fluid bathing the otolith, which in turn is supplied from calcium in the blood plasma. Plasma calcium appears to originate mainly via uptake from the gills (ATPase-mediated), and to a lesser extent, from the diet. Significant biological regulation of calcium concentration occurs at several different levels. Note that the otolith is not made of bone, but is essentially a crystalline limestone structure. It is completely acellular.

Decoupling of fish and otolith growth indicates that different mechanisms control each. The mechanisms controlling bone and otolith growth almost certainly differ. However, a common feature of somatic and otolith growth is the synthesis of protein. Using the known linkage between protein synthesis and metabolic rate as a basis, the Working Group prepared a conceptual framework for otolith growth which appears to account for empirical relationships between fish and otolith growth, as well as decoupling under conditions of starvation and high temperature. The framework is both preliminary and untested. However, each of its components is testable.

The conceptual framework begins with the hypothesis that a protein mesh is deposited on the growing surface of the otolith, with the amount of protein proportional to metabolic rate. Protein synthesis is proportional to metabolic rate elsewhere in the body, and there is no reason to expect that relationship to differ in the inner ear. Next, we hypothesise that calcification occurs so as to fill in all but the innermost portion of the protein mesh. Calcification using protein as a template and regulator is consistent with a number of biomineralisation studies, and would explain why calcium deposition would be expected to cease after the protein mesh was filled. Finally, we hypothesise that protein catabolism (breakdown) in the inner ear is reduced or non-existent relative to the rest of the body, in keeping with the primary function of the otolith as a balance organ. Since the balance function of the otolith requires a specific size and orientation, and given that a fish without balance would probably die very quickly, it is reasonable to assume that the inner ear would be largely protected from protein and/or calcium resorption in times of stress.

The specific hypotheses which arise from the conceptual framework are as follows:

H1: The amount of protein deposited within a given daily increment is proportional to metabolic rate.

H2: The amount of calcium deposited within a given daily increment is determined by the size of the protein mesh which has been produced.

H3: The rate of protein catabolism is less on the otolith than elsewhere in the body of the fish.

H4: Somatic growth is not necessarily proportional to metabolic rate.

All of these hypotheses are testable. Refinement of the conceptual framework requires input from physiologists familiar with metabolic processes and protein synthesis, and from biomineralogists familiar with crystallisation processes and the formation of protein templates.

The framework described above has a number of attractive features. First of all, under normal growth conditions, one would expect that a given metabolic rate would result in similar rates of protein synthesis in both the body and the otolith. As a result, the framework predicts that "normal growth" should result in proportionality between fish and otolith growth. This is usually the case. Secondly, under starvation conditions, somatic growth would stop as the rate of protein catabolism reached or exceeded the rate of synthesis. On the other hand, otolith growth would continue in proportion to basal metabolic rate, taking advantage of circulating amino acids present even in starving fish. This prediction is consistent with observations of continued daily increment formation in starved fish. Finally, under superoptimal temperatures, otolith growth should become decoupled from somatic growth as a greater proportion of somatic metabolic expenditures go into maintenance rather than growth. This is consistent with the experimental results of Mosegaard *et al.* (1988).

If the conceptual framework described above holds, it indicates that otolith increment width is proportional to metabolic rate. Both growth rate and metabolic rate are complex functions of food, temperature, body size and other variables. However, it should be possible to improve growth back-calculation accuracy by making use of data on both increment width (= metabolic rate) and temperature at the time of increment formation. Back-calculations based only on measurements of increment width would approximate to somatic growth only under "reasonable" growth conditions. Unfortunately, it is not immediately obvious how "reasonable growth conditions" can be defined a priori.

It is now technically possible (or almost so) to estimate the temperature at otolith increment formation based on elemental or isotopic composition. These technologies are advancing rapidly. Therefore, it seems possible that improved back-calculations of previous size and growth rate, based on increment width and temperature measurements, will be possible in the near future. This, of course, assumes that the hypotheses mentioned earlier cannot be falsified.

References to "otolith growth" in the conceptual framework were intentionally left undefined. The Working Group noted that various measurements of increment size were possible, including linear width, area and volume. The preferred form of measurement is yet to be determined.

5. Sub-group Session II. Interrelation of Growth, Mortality and Size, and Relationship Between Small and Large Scale Studies

The discussions in the sub-groups were designed to address the terms of reference:

- Explore experimental aspects of size-specific theory in the modelling of recruitment process.
- Review field and experimental evidence for a relationship between growth and mortality rates of eggs, larvae and juvenile fish.

The WG had received a number of documents for consideration which provided experimental data to indicate that size was an important factor in determining mortality rates in predator-prey systems, but not always in the way suggested by the theory outlined in the 1990 RPWG report. More work needs to be carried out on the theoretical aspects before further evaluation can be made. The consequences of non-linearity in the ecosystem size distribution, and temporal changes in the size distribution need to be addressed by modellers.

The theory that links growth and mortality was briefly reviewed (Anon., 1990), together with the recent work of Houde (1989), Miller *et al.* (1988) Pepin (1991) and Pepin and Myers (1991). Such studies were considered useful steps in evaluating general characteristics of the early life stages of fish species in a broad range of environments. However, it was agreed that they provided little direct improvement in understanding of the processes linking growth and survival for a single population. There seems to be little conclusive evidence from field studies that faster growth is correlated with higher survival for individual species. It was agreed that much of the difficulty revolves around the problem associated with obtaining precise measures of individual growth and mortality. This arises from present limitations in sampling and, more generally, from a poor understanding of the spatial and temporal scales of variability important to the processes controlling growth and mortality of larval fishes.

In attempting to simultaneously measure growth and mortality it was agreed that two approaches were necessary, operating at very different scales. First, it is necessary to measure at the scale of an individual larva, to determine the variability in parameters determining the predator-prey interaction. Second, it is necessary to integrate from the scale of an individual fish larva to that of the population. Although measurements in the field at the scale of an individual are in principle possible, there is presently no method available for determining how variability in these processes contributes to the performance of the population as a whole. The problem lies in the integration from very small scales to the meso- and large scale. It was felt that this move could only be made with the aid of modelling studies that base population processes on individual behaviour and parameterised variability. While many of the parameters will be unknown it was felt that modelling is a pre-requisite to defining the field measurements that must be made.

The 1990 SGMRP identified five questions that related directly to the problem of scaling and integration over space and time (Anon., 1992; Appendix C, Section 2, Growth and Mortality and Their Interaction). Recent and planned field work in the NW Atlantic as part of the US GLOBEC, and Canadian OPEN programmes was considered to determine the extent to which the conditions implied by these questions were met. In general, it appeared that the two programmes addressed different problems - the GLOBEC study was focused on small scale processes, whilst the OPEN programme attempted to delineate and track an entire patch of larvae by means of real-time interactive hydrodynamic modelling. These two studies highlighted the problems associated with studies at the two extremes of the space-time spectrum, and also the lack of facility for relating one to the other. As a result of this discussion, the WG resolved to request that ICES sponsor a Study Group to review and address these particular problems.

6. Sub-group Session III. Controls of Fish Growth

The RPWG noted that the study of the actual life-histories of individual fish was encouraged by the SGMRP. This was because the vital rates in most population models

refer to the individual level and constitute the fundamental basis for the quantification of stage-specific survivorship.

Larval fish show considerable individual variation in growth rate. Even within a batch of larvae of initially the same size and living in the same food environment, the body size distribution will quickly become spread out. This phenomenon has been observed to begin at first feeding. Individual larvae may differ by more than a factor of two in length, and cannibalism may occur, after only a few weeks. To ignore the size structure in a cohort of larvae may be equivalent to merging prey organisms and predators into one functional group thereby masking completely the underlying dynamics. The effect of the heterogeneous composition of a cohort on its dynamics was illustrated by Norwegian cod rearing studies (Blom *et al.*, 1988). It was reported that completely different food situations could lead to the same mean growth rate. The underlying dynamics were first revealed by considering the variability in individual growth rates. When alternative prey were scarce, the larvae from the small tail of the size distribution apparently served as a food supply for the larvae in the large tail of the distribution (cannibalism). Hence, the mean growth of the survivors under low food conditions was raised to the same level as that of the survivors under high food conditions when cannibalism did not occur. Other examples showed the occurrence of multi-peak length distributions, indicating that the treatment of cohort size distribution cannot be limited entirely to simple distributions such as the log-normal type.

It has been demonstrated by stochastic simulation models that the variability in individual growth rates can be explained by a random element in the encounters between larvae and prey. It is not known to what degree genetic differences may play a role. Unfortunately, no general model of larval fish growth has yet been accepted, and it therefore seems that there is a need for development of mechanistic individual based growth models, derived from first principles rather than purely empirical based approaches. A simple allometric growth model is actually capable of explaining most of what seems to be known about the mean growth and mortality of larval fish in the sea, but not the variability in these rates. A change of emphasis is needed to obtain simultaneous data at the level of the individual (eg length, weight, otolith reading, stomach content) in order to address the dynamics of size specific growth and test individual growth models. In particular, it would be useful to obtain individual growth trajectories from mesocosm studies (applying for example, chemical or genetic marking methods).

The requirement for size specific data on larval fish also extends to prey and predators. Data on the small scale (spatial and temporal) distributions of prey and predators need to be collected in order to move forward the quantification of size-specific trophodynamics.

7. The Need for a Study Group on Spatial and Temporal Integration

The experience of recruitment studies carried out over almost a century is that the environment supporting early life stages of fish is extremely variable, and that somehow, this variability is reflected in recruitment itself. Attempts at finding large- and mesoscale environmental correlates of recruitment for predictive purposes have invariably failed. There seems to be no alternative but to accept that the variability originates at smaller scales, and re-focus on the lowest common denominator in the system - the individual. However, it is unrealistic to expect to be able to explain all the variability in recruitment. Thus, the variability at the individual level must somehow be caricatured at a lower

resolution. In modelling terms, this may be referred to as representing "sub-grid scale" events at the "grid scale". This problem is at the very heart, not just of fish recruitment research, but of all ecosystem research and modelling.

The SGMRP recognised the scaling-up problem described above as being of fundamental importance to recruitment research, but was completely unable to address the issue at its meeting in Paris in 1990 on account of its extreme difficulty. The members of the RPWG endorsed the emphasis placed on the problem by their modelling counterparts, and considered a solution to be a pre-requisite for "breaking new ground" in the field. The meeting considered that to start addressing the problem, it would be necessary to assemble the necessary cross-disciplinary expertise to:

a) consider methods of statistically characterising the temporal and spatial variability in populations of larval fish and their prey and predators;

b) to consider and report on the feasibility of integrating temporally and spatially variable abundance and vital rates over population time and space scales;

c) to consider how sub-grid scale temporal and spatial variability in abundance and rates may be represented at the grid scale in marine ecosystem models;

d) to consider methods of determining the most appropriate temporal and spatial grid resolution for models of fish recruitment.

The RPWG strongly recommends that ICES should support the establishment of a Study Group with the above tasks as terms of reference. The Group should be convened from a sufficiently wide research base to encompass the physical, chemical and biological science disciplines. For this reason, the WG urges ICES to seek co-sponsorship for such a SG with organisations having the potential to provide financial assistance to academics, and other scientists normally outside the ICES geographical area or sphere of activities, to support their attendance. The chairman for such a Study Group should have long-standing experience in the fields of individual and ecological modelling, and should have wide contacts with other scientists in the necessary disciplines. The RPWG propose Professor W.Gurney (University of Strathclyde, UK) as a suitable chairman.

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VI. APPENDICES

Appendix 1

Agenda for the Meeting

Tuesday 23 June

am
- Introductions, acceptance of agenda and establishment of objectives and workplan for the meeting.

pm
- Cod and haddock check list (K Brander). Formation of sub-groups to work on manuscript preparation during the meeting.
- Otolith microstructure intercalibration and workshop report (S Campana/ E Moksness).
- Case study - a fish growth model as the basis for a model of a fish population in the North Sea (M Heath).

Wednesday 24 June

am
- Reminder of discussions at the 1990 ICES/IOC Workshop on Models of recruitment (J Beyer)
- Review of papers at the 1991 ICES Statutory meeting Theme Session on Models of Recruitment (M Heath)

pm
- Formation of sub-groups
- Plenary discussion - field and experimental data requirements for progress in modelling growth - discussion leader - J Beyer
  Topics
  - individual data
  - population data
    - spatial integration
    - temporal integration
  - physiology and otolith growth
  - relationships between growth and mortality
- Sub-group discussions and writing

Thursday 25 June

am
- Sub-groups continue writing

pm
- Plenary presentations by sub-groups
- Plenary discussion - future activities of the PRWG
- Report writing

Friday 26 June

am
- Assembly of report, recommendations and conclusions.
- Terms of reference and location for next meeting

pm
- Free presentations of recent research by participants.
- Meeting close

Appendix 2

Work plan for sub-group activities during the meeting

OBJECTIVES to be achieved

1. Re-write and flesh out the Appendices on otolith applications in the Paris modelling group report

2. Outline the principles of body growth in fish larvae and the links with otolith growth

3. Outline the current state of knowledge concerning the relationship between growth, mortality and body size.

4. Produce terms of reference for next meeting, including change of name if necessary, and a specific work plan for the interim.

SUB GROUP TASKS

Sub-groups work in parallel, results being summarised following plenary presentations.

Session 1

Topic - What controls otolith growth?

- What work has been done, and what were the limitations of each attempt.
- Brainstorm the underlying principles of otolith growth
- What is possible, what has been done, what is important to do
- What expertise and new approaches are needed to make progress.

PLENARY SYNTHESIS - review of what has been done
group view on principles of otolith growth
statement of what needs to be done
statement of what expertise is needed
action plan for RPWG

Session 2

Topic - linkage between growth, mortality and body size

- Review evidence for relationship between growth and mortality from lab, field and mesocosms - papers available from Pepin and references therein, especially Houde.
- How can otolith microstructure be utilised to investigate these relationships.
  - flesh out the Paris appendix.

PLENARY SYNTHESIS - combine sub-group reviews into group report
consensus rewrite of Paris appendices
statement of future action for RPWG

Session 3

Topic - underlying controls of body growth

- Brainstorm concepts and principles underlying the control of body growth in fish larvae
- Attempt to produce a conceptual model of growth
- What methods are available to study the underlying basis of growth control
- What is feasible to study in the lab, field and mesocosm

PLENARY SYNTHESIS - compare and contrast sub-group "models"
group summary of methods for studying growth
statement of future action for RPWG

Appendix 3

Report of the ICES Otolith Microstructure Workshop, Arendal, Norway, 26-28 November 1991

Table of Contents

I. Terms of reference and participants

At the 1990 meeting of the Recruitment Processes Working Group of ICES, the following recommendation was made:

> "... that an otolith microstructure workshop should be convened by S. Campana and E. Moksness within the next two years. The workshop should examine sources of variability in otolith interpretation, and recommend techniques by which accuracy and precision can be improved. Workshop attendance should be restricted to individuals experienced in otolith microstructure techniques who are also members of the Working Group (or their proxies at the same laboratory) and/or participants in the 1990 Otolith Microstructure Intercalibration Exercise."

The meeting was attended by the following:

Name	Country
Steve Campana	Canada
Lindsay Cargill	U.K.
Edgar Dalley	Canada
Petter Fossum	Norway
Jacques A. Gagné	Canada
Alberto Garcia	Spain
Audrey Geffen	U.K.
Inger Henriksen	Norway
Gunnar Joakimsson	Germany
Françoise Lagardère	France
Raymonde Lecomte	France
Tomasz B. Linkowski	Poland
Johan Modin	Sweden
Erlend Moksness	Norway
Henrik Mosegaard	Sweden
Peter Munk	Denmark
John Nichols	U.K.
Pedro Ré	Portugal
David Secor	USA

II. Overview of the workshop

The objective of the workshop was to identify sources of variability in the interpretation of otolith microstructure, and recommend techniques by which accuracy and precision could be improved. The influence of otolith interpretation error on age estimation, growth back-calculation and other applications was the primary focus of discussion. Numerous recommendations were made concerning project design, sample preservation, technical considerations, and protocols necessary for collaborative studies. The workshop format was one of round-table discussion and microscopic examination of the otoliths of a number of species.

Sample fixation can affect both otolith preservation and the reliability of any correction for shrinkage due to death and fixation. All fixatives induce some shrinkage, but the degree of shrinkage is variable. Larval dry weight is the most useful measure of fish size.

The decoupling of otolith growth from somatic growth is well documented. In general, slow-growing larvae have larger otoliths than fast-growing larvae of the same size. This phenomenon can sometimes introduce significant error into growth back-calculations based on the fish-otolith size relationship. Otolith size can be used to estimate live fish size only when the fish-otolith regressions of all of the relevant samples have been demonstrated to be not significantly different.

Otolith microstructure examination is now an accepted, and often preferred tool, for many early life history studies. There are significant differences in the accuracy and precision of the various otolith-based procedures, and these in turn influence the accuracy and precision of the end product. The major applications of otolith-based data were assessed in terms of the power and sensitivity of the underlying procedures.

Microscopic examination of several otoliths from each of nine species demonstrated that precise age estimates were possible for most of the species. The role of prior experience and training was particularly important in the case of species with many subdaily increments and/or complex nuclei. Several sources of potential interpretation error were identified. A high level of ageing precision seems possible for all of the species examined after appropriate experience is obtained.

Workshop participants reviewed commonly-used technical procedures associated with otolith preparation and interpretation, and recommended some preferred procedures.

Collaborative studies have the potential to introduce more error than would occur if conducted by a single investigator. On the other hand, well-designed collaborative programs can be more rigorous than single-investigator studies. Daily increment validation based on mesocosm-reared fish, and continual monitoring of ageing accuracy through "seeding" protocols are important components of both single-investigator and collaborative otolith studies.

III. Review of the otolith microstructure intercalibration exercise

The results of the 1990 Otolith Microstructure Intercalibration Exercise indicated that both the accuracy and precision of daily increment counts varied significantly among investigators. Much of the variability appeared to lie with differences in reader experience, otolith interpretation, and equipment used. The results were discussed in detail at the 1990 meeting of the Recruitment Processes Working Group, at which time a report was also submitted. Since that time, S. Campana and E. Moksness carried out the recommendation of the Working Group and submitted an enhanced version of the report for publication in the ICES Journal of Marine Science (Campana and Moksness, 1991). The manuscript, which

has now been accepted for publication, includes additional analyses suggested by the Working Group, as well as a full discussion of the implications of the study. A number of recommendations concerning the strengths and limitations of otolith microstructure studies were made. All participants in the Intercalibration Exercise were sent drafts of the manuscript for comment before the manuscript was submitted for publication.

During a review and discussion of the most recent analysis of the 1990 Exercise data, workshop participants made two significant comments concerning the implications of the study. Firstly, it was noted that the study report defined age underestimation as days post-hatch. In some situations, days after first feeding might have been a more useful definition of larval age. In such a case, the magnitude of age under-estimation would have been somewhat less than what was reported. Secondly, participants in the Exercise did not necessarily interpret the herring otolith hatch check in the same way; some measured the diameter of the first increment, while others measured the diameter of the first prominent check. As a result, the finding that there were significant differences in hatch check diameter among participants was confounded by differences in the definition of the hatch check. This possibility was mentioned in both the Exercise report and the subsequent manuscript. However, the discussion at the Workshop made clear that calibration errors were not necessarily responsible for the reported differences in hatch check diameter.

IV. Review of the different types of preservatives and their impact on back-calculated growth rate

The following review of larval shrinkage was prepared as an overview of the topic by E. Moksness. References associated with the review are presented in the Appendix:

Gear treatment and death processes

Considerable shrinkage in length caused either by gear (net) treatment or death processes has been reported in the literature. Depending on the duration of the gear treatment, delay before fixation and larval size, shrinkage between 4% and 43% has been reported (Appendix Table 1). Additional shrinkage (4-6%) has been reported for those larvae fixed in formaldehyde and between 0-5% for those larvae fixed in alcohol. As a consequence, shrinkage caused by gear treatment, death processes and sample preservation could make it impossible to back-calculate to the true live length of field caught fish larvae.

Only one study (McGurk, 1985) reports on the effect of gear treatment on the dry weight of fish larvae. He reported significant loss of dry weight for herring larvae up to an average size of 12.4 mm; however, the results (see Table 1, McGurk, 1985) indicate that the loss is most significant for the yolk-sac larvae. This shrinkage may have been caused by the loss of body fluids,

particularly those associated with the yolk-sac, or the net-indu ed loss of the yolk sac itself. The results indicate that post yolk-sac larvae probably do not have as much loss in dry weight, although this has to be examined more closely in the future.

Overall, considering the impact of gear treatment and death processes on fish larvae, dry weight may be a more accurate measurement of the size of field caught post yolk-sac larvae, and will most likely give the best estimate of true live size.

Shrinkage caused by fixation only

In formaldehyde, shrinkage is reported to be greatest in the early-stage larvae, decreasing with larval size. However the degree of shrinkage varies both with the concentration of formaldehyde and salinity, as well as the species used (Appendix Table 2). The shrinkage is reported to be between 4 - 10% in length and 19 - 36% in dry weight. For those larvae fixed in alcohol, shrinkage in both length and dry weight are dependent on alcohol concentration, size of the fish and species. Shrinkage in length varies between 0 - 20% and in dry weight between 12 - 60%. For frozen larvae, 7-8% shrinkage in length has been reported for herring.

In both formaldehyde and alcohol, most of the shrinkage takes place within the three first weeks after fixation.

After discussion of the above and related issues, the workshop participants made the following recommendations:

1. In general, fish larvae requiring preservation should be stored in 95% ethanol so as to avoid otolith dissolution. Buffered formalin (pH 8.5-9.0) may also be a suitable preservative, although the pH has to be carefully monitored so as to ensure otolith integrity. Use of formalin leaves the larva in a condition more suitable for histological examination. Larvae can be safely freeze- or air- dried if only the otoliths are required.

2. All larvae shrink in length at the time of death. Subsequent shrinkage of the larvae, both in length and dry weight, takes place both in alcohol and formalin. A larger and more variable shrinkage has been reported for both dried and frozen larvae. Where possible, live larvae from the collection should be measured before and after fixation in order to determine a shrinkage correction factor.

3. Dry weight is probably the best measurement of larval size, since it is unaffected by either the death process or air drying. Air dried larvae are the best source of dry weight data. However, length measurements are still useful if only for comparability to most of the published studies.

4. Shrinkage, either in terms of length or dry weight, is not necessarily a problem if all samples are fixed in a similar fashion

and the hypothesis is one of differences among samples. However, shrinkage is a problem when growth rate is to be back-calculated.

5. Developmental stage is a reliable measure of fish size for the early stage of some fish species. However, it is too coarse and/or variable a measure for most species and the late larval-juvenile stage.

6. When measuring the dry weight of preserved larvae, the condition of the larvae should be considered, since it may have a significant impact on the estimate of the magnitude of shrinkage.

V. Review of the evidence for decoupling of otolith growth and somatic growth

Besides random error, various factors may influence the otolith - fish size relationship. Many studies have shown a growth rate effect, whereby slow growing fish have relatively large otoliths in relation to fish size. Temperature has also been shown to increase otolith size in relation to fish size, especially at superoptimal temperatures. Since the temperature optimum is food-level dependent, there is reason to expect a temperature-growth rate interaction effect. The dynamics of changes in growth rate over time must also be considered, since it will influence both otolith size directly and the back-calculated recent growth history of the fish. This is particularly evident in starved fish, in which otolith growth rate is proportional to fish size.

If the rate of $CaCO_3$ deposition is influenced by otolith size, otolith size alone will influence incremental width. In addition, the recent back-calculated growth history may be coupled to otolith growth through a time lag.

There was a general consensus that slow growing fish have relatively large otoliths. However, the university of the phenomenon was challenged with two samples of field-collected herring larvae in which no growth rate-relative otolith size relationship was observed. The workshop participants could not agree on the significance of these latest observations, but the problem deserves further research.

There are two major potential applications of fish-otolith size data: 1) estimation of live fish size in preserved fish through use of measured otolith sizes in the preserved fish in conjunction with a fish-otolith size relationship derived from live fish, and 2) back-calculation of previous sizes-at-age and growth rates through measurement of daily increment widths. There was extensive discussion of each of these items.

There was a consensus that the fish size - otolith size relationship from a sample could be used to reduce the variability of the estimated mean fish sizes from the otoliths of the same sample. The size distribution should be reestablished using the error distribution. Sample pooling would be appropriate if no statistical differences among samples could be shown, given sufficient statistical power. However, there was no support for the suggestion that a fish size - otolith size relationship from one sample

could be used to estimate fish size from the otoliths of a second sample in the absence of a comparison of the fish-otolith regressions of the two samples.

Growth backcalculation was discussed at length. Backcalculation is complicated and the biological and theoretical basis is poorly understood. Bias is easily introduced by the various effects discussed earlier. Backcalculating growth rate from increment widths was discussed, but could not be recommended without greater knowledge of fish-otolith decoupling effects. The difference in area between adjacent increments (rather than linear distance) may be a better measure of daily otolith growth, although such a measurement remains technically difficult.

Measurement of the most recently-deposited growth increments as an index of instantaneous growth rate was discussed. However the rationale was unclear and the application of the method considered premature. Increment width is also highly dependent upon the size of the fish.

Temperature and growth effects on the fish size-otolith size relationship could be a matter of concern in field samples. A good relationship between otolith size and fish size is not a sufficient precondition for using incremental widths to backcalculate daily growth rates.

Studies involving back-calculation based on fish-otolith size relationships and otolith measurements require validation that the resulting fish size estimates are accurate.

VI. Review of current applications of otolith microstructure examination in the context of accuracy and precision

Otolith microstructure examination is now an accepted, and in many cases, a preferred tool for the study of young fish. The incrasing importance of the technique is reflected in the number of primary publications reporting its use (Fig. 1). The number of published papers has increased almost exponentially since 1978, and now totals more than 300 papers. Field applications now make up most of the published studies.

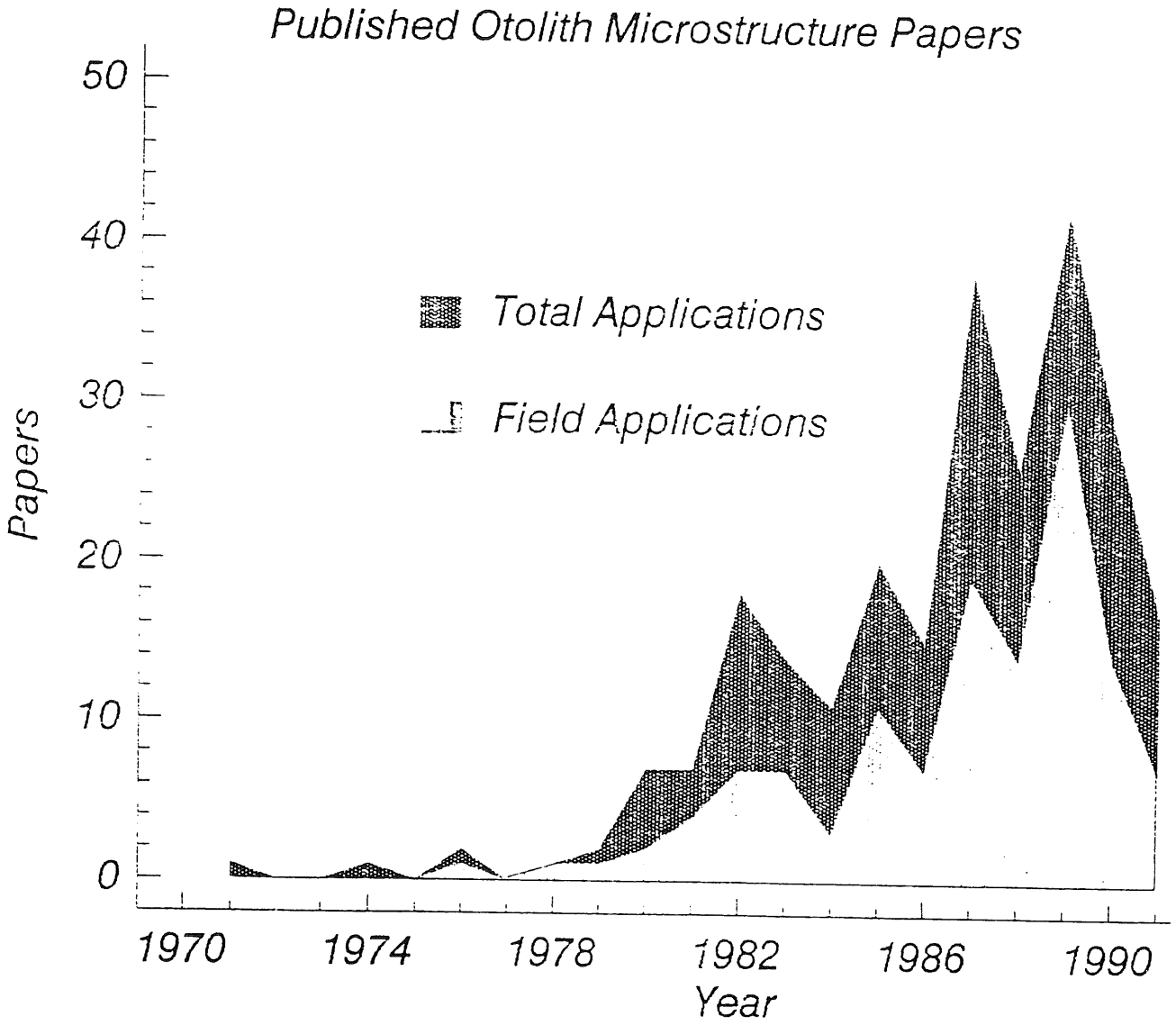

Figure 1. Published otolith microstructure papers (D. Secor)

Applications of information derived from otolith microstructure can be classified in several ways, one of which is in terms of age-specific, date-specific, size-specific, and source-specific studies. Since there is considerable overlap between these categories, the following table lists the major applications themselves, each of which can subsume one or more specific objectives. Some of these objectives were discussed at the workshop, but they were too numerous to present in a comprehensive table. For example, the "mortality" application could include studies of size-selective mortality, changes in age-structured abundance, predation, starvation, competition and changes in hatch date distributions, among others.

The sensitivity and power of each of the listed applications depends on the accuracy and precision of the techniques and a clear understanding of the underlying prosesses. Both accuracy (bias) and precision (sensitivity) can be influenced at the sampling stage, as well as by the physiological processes which control otolith growth. Otolith preparation, interpretation, and instrumentation also affect accuracy and precision.

As a guide to the power and sensitivity of otolith microstructure-derived data, the following table lists the major applications along with the generic otolith microstructure procedures required to complete the application (**xx** indicates that the application is heavily dependent on that procedure; **x** indicates that the application involves that procedure). The text following the table summarizes the strengths and weaknesses of each procedure. Through cross-reference between a given application and the strengths/weaknesses of the associated procedure(s), some idea of the power and sensitivity of the application can be developed.

Note that the table does not address the power of alternative, non-otolith based techniques. Therefore, while most of the applications listed are best conducted with the aid of otolith-based information, there are undoubtedly specific applications which are best addressed through other means.

Table 1. Summary of the otolith microstructure procedures underlying each of the major applications.

Application	Daily Aging	Back-calc Increment Width.	Micr Feature (Checks)	Chemical Marking	Elemental Analyses
Age Determination: Spawn/Hatch Date	xx			x	
Growth Rate: Size@Age/ Back-calculated	xx	xx	x	x	
Mortality: Starvation/Predation	xx	x	x	x	
Life History Dynamics	x	x	xx	x	x
Migratory/Dispersal rates	xx		xx	x	x
Migratory History					xx
Environmental history	x	xx	xx		xx
Origin: Population, Site, Time, Nursery, Parents			xx	xx	xx
Competition				xx	
Abundance			x	xx	
Condition	x	xx	xx		
Taxonomy			xx		

There are five generic otolith microstructure procedures which underlie each of the above applications. A brief summary of these procedures, along with major advantages and constraints of each, follows:

Daily Aging

> Relatively accurate, precise method; Accuracy and precision varies with species; Requires validation for each species

Growth Back-calculation and Increment Width Measurements

> Good potential for investigating size-specific processes; Biological basis of method poorly understood; Little validation reported; Bias can be easily introduced and difficult to detect

Microstructural Features (Natural and induced checks and shifts in microstructural appearance, Shape, Primordia, Nuclei)

> Relatively accurate and precise in terms of identification, but correspondence to life history and environmental events or effects requires validation

Chemical Marking

> Very accurate; Some methods may mark more than one increment; Method requires experimental trials prior to application to determine marking efficiency, marking effects, and retention of the mark.

Elemental Analysis

> Excellent potential for studying environmental history; Relationship between otolith composition and ambient water and diet is poorly understood; An emerging technology

VII. Interspecies comparison of precision of daily increment counts

The Otolith Microstructure Intercalibration Exercise identified highly significant age estimation differences among otolith readers. These differences may have been due to differences in increment interpretational skill, differences in equipment quality, or preparation differences. In order to determine the relative importance of the interpretation component of the variability, an otolith reading exercise was held at the workshop. The exercise had two objectives: 1) assess the counting variability associated with individual age readers in an experiment in which the effects of inter-otolith variability, equipment, and preparation were controlled, and 2) determine the factors which contribute to interspecific differences in counting precision (coefficient of variation - CV).

Workshop participants were invited to provide a minimum of 5 prepared otoliths of a species with which they were familiar. Otoliths from field-

collected fish were preferred, but participants were permitted to bring laboratory-reared fish as long as their otolith microstructure was reasonably representative of that of wild fish. Nine species were selected at random for the experiment: herring (*Clupea harengus*), sprat (*Sprattus sprattus*), sole (*Solea vulgaris*), sardine (*Sardina pilchardus*), brown trout (*Salmo trutta*), striped bass (*Morone saxatilis*), sculpin (*Myoxocephalus thompsoni*), capelin (*Mallotus villosus* Müller), and a myctophid (*Loweina rara*). Species were assigned to one of nine microscopes, half of which were equipped with video systems. Five prepared slides, spanning a variety of ages/sizes, were available for each species, and all were read at the same microscope by each participant. All participants attempted to read each otolith of each species. Unless specific guidelines as to a starting point (eg- hatch check) were provided with the slide, all participants counted what they considered to be the complete sequence of daily increments. No advance guidance as to the identification of daily increments was provided with any of the species, and participants were instructed not to compare increment counts among themselves.

The precision of the increment counts for each slide and species was calculated by applying Chang's (1982) coefficient of variation (CV). The increment counts of all workshop participants were pooled in order to determine the counting precision of a given slide. Precision measures were subsequently segregated on the basis of prior experience with the otoliths of the species in question: each participant self-coded their prior experience into either a familiar or unfamiliar category.

With a few exceptions, counting precision was similiar across all slides of a given species within a given experience category (Table 2).

Table 2. Average count, SD, and CV for each slide and species. For species 1 and 4 all the slides (5) have been included and and for species 9, all but one of the slides has been included. N = number of readings. Exp. (experience) : 1 = the reader is not familiar with the otolith of that species; 3 = the reader is familiar with the otolith of that species. Oto = otolith no.

species	oto	exp.	average	sd	N	CV
Brown trout	1->5	1	86,0	28,6	56	0,33
	1->5	3	111,9	11,8	15	0,11
Capelin	1	1	44,5	8,7	14	0,20
	1	3	50,7	2,1	3	0,04
	2	1	31,3	4,6	14	0,15
	2	3	30,7	4,7	3	0,15
	3	1	57,9	9,4	14	0,16
	3	3	64,3	7,6	3	0,12
	4	1	53,6	8,5	14	0,16
	4	3	54,3	8,0	3	0,15
	5	1	61,6	11,1	12	0,18
	5	3	58,0	2,6	3	0,05
Herring	1	1	53,0	4,2	4	0,08
	1	3	69,9	14,5	13	0,21
	2	1	46,0	4,6	3	0,10

	2	3	58,5	13,3	14	0,23
	3	3	63,5	9,9	6	0,16
	5	1	44,7	17,9	3	0,40
	5	3	63,1	6,7	14	0,11
	6	1	43,7	8,5	3	0,19
	6	3	51,2	6,3	12	0,12
Loweina rara	1->5	1	49,9	9,1	80	0,18
	1->5	3	54,2	7,9	5	0,15
Sardine	1	1	22,1	8,3	13	0,38
	1	3	16,4	6,4	5	0,39
	2	1	14,1	1,9	13	0,14
	2	3	13,6	3,4	5	0,25
	3	1	22,2	7,0	13	0,32
	3	3	19,6	3,6	5	0,19
	4	1	17,0	7,5	12	0,44
	4	3	15,8	4,3	4	0,28
	5	1	16,1	4,1	11	0,25
	5	3	14,6	2,9	5	0,20
Sculpin	1	1	55,6	2,1	13	0,04
	2	1	27,2	7,4	13	0,27
	3	1	33,0	7,6	13	0,23
	4	1	45,1	4,1	12	0,09
	5	1	43,4	13,6	11	0,31
Sole	1	1	30,3	8,2	15	0,27
	1	3	32,0	3,5	3	0,11
	2	1	19,1	3,3	15	0,17
	2	3	21,7	5,5	3	0,25
	3	1	20,3	4,2	15	0,21
	3	3	22,0	2,0	3	0,09
	4	1	17,7	5,1	15	0,29
	4	3	20,3	4,7	3	0,23
	5	1	20,1	7,3	14	0,36
	5	3	19,7	2,5	3	0,13
sprat	1	1	20,9	3,7	9	0,18
	1	3	17,5	1,5	8	0,09
	2	1	21,2	2,5	10	0,12
	2	3	20,5	2,4	8	0,12
	3	1	16,3	2,3	10	0,14
	3	3	17,0	1,3	8	0,08
	4	1	21,7	11,0	10	0,51
	4	3	29,0	4,0	8	0,14
	5	1	44,3	7,7	10	0,17
	5	3	49,5	8,6	8	0,17
Striped bass	2->5	1	42.6	26.1	47	0.61
	2->5	3	31.9	3.9	8	0.12

The precision of otolith readers who were familiar with a given species was substantially better than those who were unfamiliar with a species. This result was not surprising in light of the broad range of experience levels present at the workshop. It also confirms that advance training is particularly important in this field.

The precision of the unfamiliar otolith readers varied substantially among species, probably in response to the very different microstructural patterns evident in some species. In particular, the otoliths of brown trout and striped bass proved to be difficult to interpret by those unfamiliar with these species. On the other hand, the precision estimates of those familiar with the species varied relatively little among species, indicating that prior experience is an important factor when considering relative precision.

No trend in CV with age (mean increment count) was evident within any of the species. Therefore, the average CV for each species was calculated for all species with sufficient observations within each experience category.

Table 3. Average CV for those species with more than two readers on each slide. Exp. : 1 = the reader is not familiar with the otolith of that species; 3 = the reader is familiar with the otolith of that species.

species	exp.	average CV
Capelin	1	0,17
	3	0,10
Herring	1	0,19
	3	0,16
Sardine	1	0,30
	3	0,26
Sculpin	1	0,19
Sole	1	0,26
	3	0,16
sprat	1	0,22
	3	0,12

Mean species CV ranged between 10-26% for the experienced readers, and between 17-30% for the inexperienced readers. With the exception of the sculpin otoliths, which all readers found to be difficult to interpret, the CV's of all of the species read by experienced readers were 16% or less. The CV's of individual experienced readers was undoubtedly less, indicating that, overall, otolith microstructure examination can result in very precise age estimates over a broad cross-section of species.

The precision levels associated with the herring otolith increment counts were very similar to those reported for the Intercalibration Exercise. Since the precision measure used in the Intercalibration Exercise included inter-reader differences in terms of equipment used and sample preparation, it appears that most of the variability associated with individual readers is due to differences in interpretational skill.

Discussion of the above results by the workshop participants centred around the sources of error and uncertainty associated with interpreting the various otoliths. There was a consensus that the otoliths of certain species were relatively easy to interpret (eg- sole, sardine, anchovy, sprat), even with no prior experience, while others proved difficult

without prior experience (eg- brown trout, striped bass). The following features were considered to be important modifiers of precision:

Otolith shape:	Circular or symmetrical otoliths tended to be easier to interpret. Apparent shape was also a function of the preparation method, e.g. - frontal sections of the striped badss otoliths were less symmetrical than sagittal sections.
Subdaily increments:	Species with many subdaily increments were confusing to those who were unfamiliar with the species. This was particularly evident in the case of striped bass. In contrast, those who were familiar with the species apparently had little difficulty differentiating daily from subdaily increments, as evidenced by their low CV's.
Narrow increments:	While increment width varied among otoliths and species, it did not appear to influence counting precision unless the width approached the resolving power of the microscope. This was particularly evident in the case of the sculpin otoliths, where it was difficult to see extended sequences of increments. Long secquences of narrow increments were also difficult to count with precision.
Nuclear structure:	The presence of a complex nucleus, consisting of multiple primordia or an irregular shape, complicated increment interpretation by inexperienced readers. Brown trout typified this type of nuclear structure. However, experienced readers apparently had little difficulty in recognizing nuclear boundaries.
Increment regularity:	Otoliths characterized by relatively constant visual contrast and smooth transitions in width were easier to interpret than those with abrupt changes. For instance, the otolith microstructure of the myctophid was unfamiliar to all but one participant, but was considered among the easiest to interpret due to its long regular sequences of increments.

Two additional sources of variance were identified. Despite the fact that many of the microscopes were identical models, there were noticeable differences in the optics, and hence the visibility of some increments, among microscopes. These differences in optical quality or alignment may have contributed some variance to the otolith readings. Secondly, large otoliths, particularly those with extended featureless increment sequences, were occasionally difficult to shift among fields of view (where a single field of view was too small to include the entire counting axis). This was one instance in which most readers appreciated the ability to count from a video monitor.

This exercise was designed as an examination of factors affecting precision, not accuracy. Nevertheless, there were three species provided

for which the true ages were known. In these species (brown trout, striped bass, sole), the mean estimated ages, pooled across all participants, were close to the true ages.

In summary, the precision exercise highlighted the following observations:

1. Differences in increment counts among otolith readers is attributable more to differences in interpretational technique or skill than to differences in equipment or mode of sample preparation. The effect of sample preparation was not examined.

2. Experience or training is an important component of reliable age estimation from otolith microstructure.

3. Experience or training is more important in some species than in others. Experience is most valuable for differentiating daily from subdaily increments and in interpreting the nuclear boundaries.

4. On average, a high level of ageing precision (at the daily level) is possible using the otolith microstructure.

VIII. Recommended procedures for the preparation and interpretation of otoliths at the daily level

The discussion began with a description of the procedures in routine use for preparing otolith samples, viewing samples, interpreting increments, and recording results. Otolith preparation and interpretation clearly varied among species and investigators. There was insufficient time to discuss all methodological aspects. Nevertheless, the following items were raised for discussion:

Preparation
1. preservation of larvae*
2. extracting otoliths
3. mounting otoliths*
4. durability/storage
5. grinding/polishing*
6. etching

Viewing, counting and interpretation
1. type of equipment*
2. magnification*
3. light level
4. criteria for acceptance or rejection of increments*
5. definition of starting point
6. selection of otolith type*
7. verification with SEM
8. number of counts*
9. measurement of increment width

*points which generated discussion

Most workshop participants fixed and preserved larvae in 95% ethanol.

Problems which came up in discussion were the dissolution of otoliths of some species and larval shrinkage. The question of shrinkage is discussed elsewhere in this report. Nail varnish, permount, superglue, heat-cured resins, and thermoplastic resins are used by many people for mounting. Problems with mounting media included dissolution in alcohol or oil, warping, insufficient hardness for grinding, and/or long curing times (>1hr). No one used coverslips to cover the preparations on a regular basis. Crystalbond, superglue, and nail varnish preparations are durable for several years. Many people working with otoliths of small larvae do not grind or polish routinely. The issue of comparability of marginal increment width measurements between polished and whole mount preparations was raised, but there was no data upon which to base any conclusions or recommendations.

Approximately one half of the workshop participants used a high resolution video camera system, and counted increments off the video screen. The remainder counted directly through the microscope, although measurements were made with a video system. Several image analysis systems were decribed and discussed. Magnifications used for counting ranged from 400x-1250x, depending on increment width and clarity. There was much discussion concerning criteria used to distinguish daily increments. Most of the participants relied on experience to discriminate increments and that experience led to two different criteria being used. One group relied on the "integrity" of the increment eg- whether or not it could be split into more than one increment by changing the focal plane. The other group relied on the width of the increment being consistent with the widths of surrounding increments, rejecting increments that would cause sudden changes in increment width. It was evident that the major interpretational problems being discussed were caused by subdaily increments. Concern was expressed that disregard of narrow increments because they seemed "out of place" would mask real events which were of interest, but the opinion was also expressed that larvae which were large enough to be producing subdaily increments would not respond immediately to poor feeding conditions. Temperature changes could produce immediate changes in increment widths, regardless of larval size. Most participants had serious reservations about increment interpolation when faced with sequences of narrow or low contrast increments, but there was little time for discussion.

The following methodological recommendations were made. Note that the list may be incomplete due to time constraints:

1. Fixation and preservation in 95% ethanol is preferable, but the solution may require changing after 24 hr. Formalin (4-5%) may give comparable results if buffered (and maintained) at a pH of 8.5-9.0 with borax. Freezing, air drying, and freeze drying also result in good otolith preservation, but pose other problems with respect to variable larval shrinkage.

2. There are a variety of acceptable mounting media. Coverslips have the advantage of protecting the otolith surface from clearing due to immersion oil, but may crack the otolith after repeated

examination. Any medium which is sufficiently rigid to permit polishing (if applicable) or has the desired clearing action (if required) will be appropriate. Acidic media must be avoided.

3. The effect of otolith polishing and different sectioning planes on the accuracy and precision of increment counts and measurements should be assessed prior to routine sample processing.

4. High resolution video can improve operator efficiency and increment contrast, but will not necessarily improve counting accuracy. It is highly recommended for otolith measurements.

5. Image analysis systems can provide image enhancement at levels beyond that possible with high resolution video.

6. Choice of magnification and use of immersion oil should be selected on the basis of increment clarity and width. In general, magnifications of greater than 400x are recommended for all but the fastest growing species.

7. The measurement axis should be standarized. In many (but not all) species, the longest axis will be most useful. Area-based measurements deserve more research.

8. The preferred otolith type (eg- sagitta, lapillus) varies among species. However, the asteriscus is not appropriate for most applications.

9. SEM use may be warranted in cases where the accuracy of daily increment interpretation is questionable.

10. Standard counting procedure requires a measure of precision. Counting precision can be determined most accurately when otoliths are blind-coded and read at least twice at different times. It may be preferable to count increments alternately, from the outside in, and the inside out.

11. Objective criteria should be established to determine when an otolith should be rejected.

IX. Recommended protocol for the conduct of collaborative otolith microstructure studies

Large scale recruitment programs, involving collaborative research at both the national and international level, are becoming increasingly common. Current programs which involve a component dealing with otolith microstructure include SARP (Sardine Anchovy Recruitment Program - Europe), OPEN (Ocean Production Enhancement Network - Canada), FOCI (Fisheries Oceanography Coordinated Investigations - U.S.), SABRE (South Atlantic Bight Recruitment Experiment - U.S.), Cod and Climate (multinational), as well as others. Accordingly, the Workshop participants prepared a list of recommendations which may prove useful

in the design of a collaborative otolith research program.

A general principle guiding the design of collaborative otolith research is the practise of continual monitoring so as to ensure a constant level of both accuracy and precision throughout the lifetime of the project. This principle is easiest to implement if all of the otolith interpretation is conducted at a single laboratory; the variance of the otolith readings is likely to be minimized if restricted to a single laboratory, rather than split among several laboratories. However, we recognize that practical considerations must also be taken into account; training requirements, data flow, sample sizes, and political realities must all be given considerable weight. In addition, there are probably long-term advantages if otolith interpretation expertise is divided among multiple sites. Nevertheless the following recommendations have greater implications for collaborations involving multiple laboratories than for multiple readers within a laboratory.

1. Age validation is a mandatory component of a collaborative study. Since known-age otoliths are involved, the validation study can be used to determine both the accuracy and precision of increment counts and/or measurements. Validation by area is recommended even if increment formation has been validated elsewhere for the species under study; interpretive errors can be substantial and will vary significantly among age readers.

2. The validation study is best conducted under natural or quasi-natural (eg- mesocosm) conditions, selected so as to most closely represent the population and environmental conditions expected to be encountered in the field. Known-age otoliths derived from other conditions may or may not prove useful in determining the accuracy and precision of increment counts in field-collected samples.

3. The use of multiple age readers, either within or among laboratories, will help minimize bias and the possibility of a drift in accuracy. Each otolith need not be read by each reader. However, randomly-selected otoliths should be read independently by all readers at frequent and periodic intervals.

4. For each of the otoliths read in replicate, the coefficient of variation (CV) or some other measure of precision should be calculated so as to ensure that inter-reader interpretation differences remain within acceptable bounds.

5. The first step in the program is the conduct of a training and calibration sesssion which brings together all of the otolith readers. This session has five objectives:

a) to evaluate the possible otolith preparation/interpretation techniques and to select those which are most appropriate,
b) using both known-age otoliths and those derived from field collections, quantify accuracy and precision for each of the selected techniques.
c) select the technique which provides levels of accuracy and

precision which are sufficient for the intended application,

d) standardize the preparation and interpretation skills of the various otolith readers,

e) ensure that all otolith readers meet a minimum specified level of accuracy and precision consistent with the objectives of the program.

6. Where a joint calibration session is not possible, a single otolith reader should visit each otolith reader with a collection of known-age otoliths in order to meet the objectives in #5.

7. A single otolith preparation procedure should be used by all otolith readers.

8. The equipment used by each of the readers should be calibrated so as to ensure increment count and measurement comparability among all readers.

9. If otolith samples are to be distributed among laboratories, each sample is best split among laboratories, so as to avoid laboratory bias.

10. Accuracy and precision of both increment counts and otolith measurements must be monitored through the duration of the study, since "drifting" is a common problem. Acceptable levels of accuracy and precision were set in the initial training and calibration session. Current levels can be determined through periodic seeding of otoliths used in the initial session into regular samples. Seeded otoliths should not be identifiable as such by the otolith reader. Seeding periodicity can be in terms of either time or number of otoliths.

11. Where seeding has identified unacceptable levels of accuracy and precision, all readings by the affected reader should be stopped until all have once again been standardized.

12. Where seeding is not possible, a training set of otoliths should be distributed at periodic intervals among all otolith readers so as to ensure acceptable levels of accuracy and precision. However, this approach is unlikely to provide the same drift detection capabilities as seeding.

X. References (Including those from Appendices)

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XI. Appendices

ICES - OTOLITH MICROSTRUCTURE WORKSHOP

26 - 28 NOVEMBER 1991, Arendal, Norway

Agenda: (Lunch every day between 1200 - 1300 Hrs)

Tuesday 26. November 1991

0900. Welcome by J. Gjøsæter, Leader at Flødevigen Marine Research Station

0915 - 1100 Review the the Otolith Microstructure Intercalibration Exercise
Introduction by S. Campana

1100 - 1500. Otolith examination by Group 1
Group 2 prepare Task 2

1500 - 1800. Otolith examination by Group 2
Group 1 prepare Task 1

Wednesday 27. November 1991

0900 - 1000. Review different types of preservations and their impact on backcalculated growth rate
Introduction: E. Moksness

1000 - 1200 Review evidence for decoupling of otolith growth and somatic growth
Introduction: H. Mosegaard

1300 - 1500. Visit to Flødevigen Marine Research Station

1600 - 1800. Review the applications to which otolith microstructure examination can be put. Is the technique suffiently accurate and precise to warrant applications in all cases
Introduction: D. Secor

2000 Dinner at Hotel Phønix

Thursday 28. November 1991

0900 - 1100 On the basis of the analysis of the Tuesday exercise, evaluate and rank the sources of counting variance. Add recommendations to list of recommended procedures as appropriate

1100 - 1400 Develop a list of recommended procedures for preparation and interpretation of otoliths at the daily level
Introduction: Group 1

1400 - 1600 Establish a recommended protocol for the conduct of collaborative programs using otolith microstructure preparations.
Introduction: Group 2

ICES - OTOLITH MICROSTRUCTURE WORKSHOP

26 - 28 NOVEMBER 1991, Arendal, Norway

The participants are divided into two groups, each with the following task:

Group 1. Develop a list of recommended procedures for preparation and interpretation of otoliths at the daily level.

Group 2. Establish a recommended protocol for the conduct of collaborative programs using otolith microstructure preparations.

The results from the discussions will be discussed on Friday.

As one group prepare their list of recommendation, the other group will examine the otoliths which have been brought by the participants. The objectives will be to quantify:

a) counting variability associated with individual investigators; how does it compare with the variance levels reported in the Intercalibration Exercise where some variance was due to differences in equipment and sample preparation among investigators

b) interspecific differences in CV; what factors contribute to the differences (eg - relative increment width, growth rate, etc.) ?

Group 1	Group 2
L. Cargill	S. Campana
E. Dalley	P. Fossum
A. Garcia	J. Gagné
A. Geffen	G. Joakimsson
I. Henriksen	F. Lecomte
F. Lagardere	T. Linkowski
E. Moksness	J. Modin
H. Mosegaard	P. Ré
P. Munk	D. Secor
J. Nichols	

Appendix table 1. Effect of gear treatment on length and weight of fish. L = length, TL = total length, SL = standard length, DW = dry weight, FORM4 = 4% formaldehyde, FORMB5 = buffered 5% formaldehyde, AL80 = 80% alcohol.

Species	Body part	Gear treatment Shrinkage (%)	Preservation Type	Preservation Shrinkage (%)	Comments	Reference
Herring	L	- 20	FORM4	- (1-4) - 6	after 1 wk after 3 mnd	Blaxter, 1971
Engraulis mordax	L	- 19	FORMB5 AL80	- 3 - 5	(3.7 - 21.6 mm) Additional shrinkage	Theilacker, 1980
Herring	L	- (13-17) - (34-43)*			*: If preservation was delayed 15 min.	Hay, 1981
Herring	L	- 13* - 4$	FORM2		7-13 mm *: yolk-sac larvae $: post yolk-sac larvae	McGurk, 1985
	DW	- 35* +17 - - 7$			100-500 µg	
Cod	TL	- (30-40)* - 40$	AL95	< - 0.2	3.6 - 50 mm *: within 15 min $: overall (newly hatched)	Radtke, 1989
Pacific hake	L	- 17* - 40$ - (9-20)§			First-feeding larvae *: 9 min. delay preservation $: 29 min. delay preservation § general shrinkage in net	Bailey, 1982

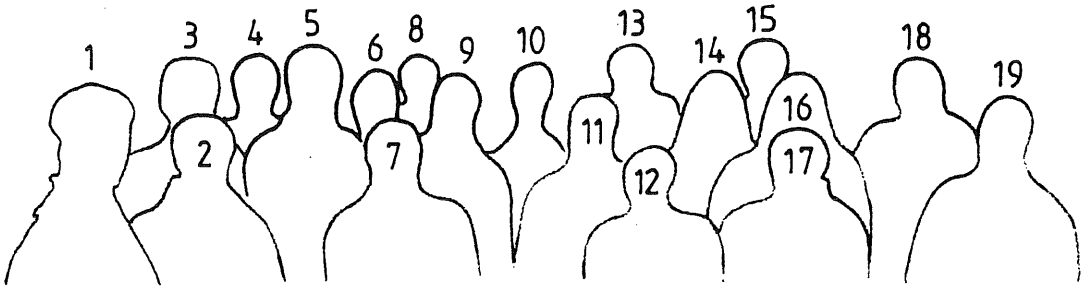
Appendix table 2. Effect of different fixatives on body shrinkage.

Species	Body part	Preservation Type	Preservation Shrinkage (%)	Comments	Reference
Pilchard Anchovy		FORMB5		Microstructure unaffected up to 2 yrs	Re, 1986
Pimphales notatus	SL	FORM4 AL60	- 4.1 - 4.9	26.5 mm	Leslie and More, 1986
Silver Hake	L	FORM4*	- 4.3	*: 337 d, 31-32 ‰, largest reduction the first 15 d (3.7-14.4 mm)	Fowler and Smith, 1983
		AL95	- 7 - (0-20)	205 d, (3.2 - 16.8 mm)	
		Freeze	- 1.4 (+26.5 - 29.9)	(4.6 - 41.0 mm)	
Pacific hake	L	AL80 FORM3	- 3.6 - 8.9	First-feeding larvae	Bailey, 1982
	DW	AL80 FORM3	- 57.8 - 24.1		
Macruronus novaezelandiae	TL	AL95	- 5		Thresher et al., 1988
Mackerel	L DW	AL95	- 4 - 23	after 7d. (0-group)	D'Amours and Landry, 1989
Larval (Stage 1) and juvenile (stage2) bass	L	FORMB4	- 7* - 4$	Most shrinkage the first 6 days.	Jennings, 1991
		AL70	- 7* - 4$	*: stage1 (7.5 ± 0.4 mm) $: stage2 (31.4 ± 1.4 mm)	

Species	Body part	Preservation Type	Preservation Shrinkage (%)	Comments	Reference
Capelin	TL/SL	FORM4 AL95	- 4 - 12	after 6 weeks, (4.4 - 22.9 mm)	Kruse and Dalley, 1990
Herring	TL	Freeze	- (7-8)	(27-45 mm)	Townsend and Graham, 1981
Herring	L	FORM2-5 FORM20-30	- 2* - 10$ - 3* - 5$	*: low salinity $: high salinity (8 - 26 mm)	Hay, 1982
Herring	L	FORM4	- (4-5)		Schnack and Rosenthal, 1978
Herring	DW	FORM*	- (19-36)*	*Depending on formaldehyde and salinity concentration	Hay, 1984
Herring	SL DW	AL80 AL95 FORMB4 AL80 AL95	0 0 - (12-17)* - (30-36)$ - (28-36)$	Yolk-sac larvae (9-11mm) *: increase w/starvation $: decrease w/starvation	Moksness, unpubl data
Herring	SL DW	FORMB4 AL80 AL95 FORMB4 AL80 AL95	- 6 - 4 - 4 - 28 - 42 - 47	(20 - 33 mm)	Moksness, unpubl data

Appendix table 3. Mounting media

Trade name	Description	Source	Name
Lake Side	Thermoplastic Cement	Buhler Ltd. from Micron AB Sweden	Henrik Mosegaard
Crystal bond	Thermoacrylic cement	Aremco Products P.O.Box 429 Ossining, NY 10562	Edgar Dalley
Bison Super glue	Cyanoacrylate superglue	Local hardware stores - readily available	John Nichols
Eukitt	Xylene diluted resin	Bie and Berntsen Copenhagen	Peter Munk
"Charlie"	Clear nail polish	Available at most good chemists	Lindsay Cargill
Permount	Mounting medium	Chemical calatog	Francoise Lagardère
Euparal	"		
Super glue	glue		
Lake Side	Thermoplastic	Buehler	
Eukitt	Xylene diluted resina		Alberto Garcia
DePex	Mounting medium	Chemical calalogs	Pedro Ré
Nail polish	Acetone diluted		Gunnar Joakimsso
Promodentaine	Epoxy resin (rapid polymerization)	Promodentaire catalog (resin used by dentists)	R. Lecomte
Krazy Glue	Cyanoacrylate glue	General	Steve Campana
Cutex	Clear nail polish	General	Erlend Moksness



THE ICES OTOLITH MICROSTRUCTURE WORKSHOP

Institute of Marine Research, Flødevigen Marine Research Station
Arendal, Norway - 26-28 November 1991

1. Inger Henriksen
2. Audrey Geffen
3. David Secor
4. John Nichols
5. Petter Fossum
6. Jacques A. Gagné
7. Pedro Ré
8. Peter Munk
9. Tomasz B. Linkowski
10. Steve Campana
11. Gunnar Joakimsson
12. Françoise Lagardère
13. Alberto Garcia
14. Lindsay Cargill
15. Erlend Moksness
16. Henrik Mosegaard
17. Raymonde Lecomte
18. Edgar Dalley
19. Johan Modin

Appendix 4

List of documents supplied to the Working Group

1. Report of the ICES Otolith Microstructure Workshop, Arendal, Norway, 26-28 November 1991.

2. Report of the ICES-IOC Study Group Meeting on Models of Recruitment Processes, Paris, 1990. ICES Cooperative Research Report 185.

3. ICES Recruitment Working Group Discussion Document, M Heath, Marine Laboratory, Aberdeen, UK.

4. Temperature and maternal effects forming a mechanism for adaptive larval production in Arcto-Norwegian cod: an overview. Solemdal, P. and Kjesbu, O.S., Institute of Marine Research, Bergen, Norway.

5. Ecosystem- and taxa-specific dynamic and energetic properties of fish larvae assemblages. Houde, E.D. and Zastrow, C.E. Contributions in Science, Los Angeles County Museum (in press).

6. Field evidence that mortality of fish larvae is independent of size. Pepin, P. Department of Fisheries and Oceans, St John's Newfoundland, Canada.

7. Application of empirical size-dependent models of larval fish vital rates to the study of production: accuracy and association with adult stock dynamics in a comparison between species. Pepin, P. Department of Fisheries and Oceans, St John's Newfoundland, Canada.

8. Significance of body size to the interaction between a larval fish (*Mallotus villosus*) and a vertebrate predator (*Gasterosteus aculeatus*). Pepin, P., Shears, T.H. and de Lafontaine, Y. (1992). *Marine Ecology Progress Series*, **81**, 1-12.

9. Effects of temperature and size on development, mortality, and survival rates of the pelagic early life history stages of marine fish. Pepin, P. (1991). *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 503-518.

10. Significance of egg and larval size to recruitment variability of temperate marine fish. Pepin, P. and Myers. R.A. (1991). *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 1820-1828.

11. Estimating relative survival rate for two groups of larval fishes from field data: do older larvae survive better than young?. Hoenig, J.M., Pepin, P. and Lawing, W.D. (1990). *Fishery Bulletin US*, **88**, 485-491.

12. Relationship between production of zooplankton and production of cod fry (*Gadus morhua* L.) - modelled from studies in a marine semi- enclosed ecosystem. Blom, G., Kristiansen, T.S., Ottera, H. and Svasand, T. (1989). ICES CM 1989/EMEM Paper 1. 32pp.

13. Larval size and recruitment mechanisms in fishes: towards a conceptual framework. Miller, T.J., Crowder, L.B., Rice, J.A. and Marschall, E.A. (1988). *Canadian Journal of Fisheries and Aquatic Sciences*, **45**, 1657-1670.

TABLE 1

Contributors to checklists for cod

Code	Stock	Area	Contributors
W1	W Greenland	1	Hovgaard, Buch
W2	Labrador/Grand Bank	2J3KL	Anderson
W3	Flemish Cap	3M	Anderson
W4	S Grand Bank	3NO	Anderson
W5	N Gulf of St Lawrence	3Pn4RS	Gagne, Frechet
W6	St Pierre Bank	3PS	Anderson
W7	S Gulf of St Lawrence	4TVn	Cagne, Chouinard, Hanson, Delafontaine
W8	Banquereau and Sable	4VsW	Campana, Fanning
W9	W Scotian Shelf	4X	Campana, Lambert
W10	Gulf of Maine	5Y	Laurence, Lough, Serchuk, Berrien
W11	Georges Bank	5Z+6	Laurence, Lough, Serchuk, Berrien, Cohen
E1	E Greenland	14	Hovgaard, Buch
E2	White Sea	1E	Serebryakov
E3	NE Arctic	1&2	Ellertsen, Sundby, Sunnana, Solemdal, Skreslet
E4	Iceland	11	Asthorsson
E5	Faroe Plateau	5A	Hansen, Kristiansen
E6	Faroe Bank	5B	Hansen, Kristiansen
E7	W Scotland	6	
E8	North Sea	4	Munk, Heesen, Nichols
E9	Celtic Sea	7F&G	Brander
E10	Irish Sea	7A	Brander
E11	English Channel	7D&E	Brander
E12	Skagerrak	3A	Moksness
E13	W Baltic (22 & 24)	3D	Modin, Schnack, Larson, Pliksh
E14	Baltic (25-32)	3D	Modin, schnack, Larson, Pliksh

TABLE 2

Headings for the main synthesis tables in the cod spawning survey

Larval stage data summary

Size at			Growth	Mortality	Date at			Distance from spawn to settle
hatch	metamorph	settle			hatch	metamorph	settle	

Adult stage - stock and biological data summary

Mean latitude	Recruits (age 3)	Spawning biomass	Landings	Maturity		Fecundity
				50% age	50% length	

Factors affecting survival (hypotheses being tested)

Starvation	Predation	Advection	Disease	Other

TABLE 3

Spawning and egg stage data summary

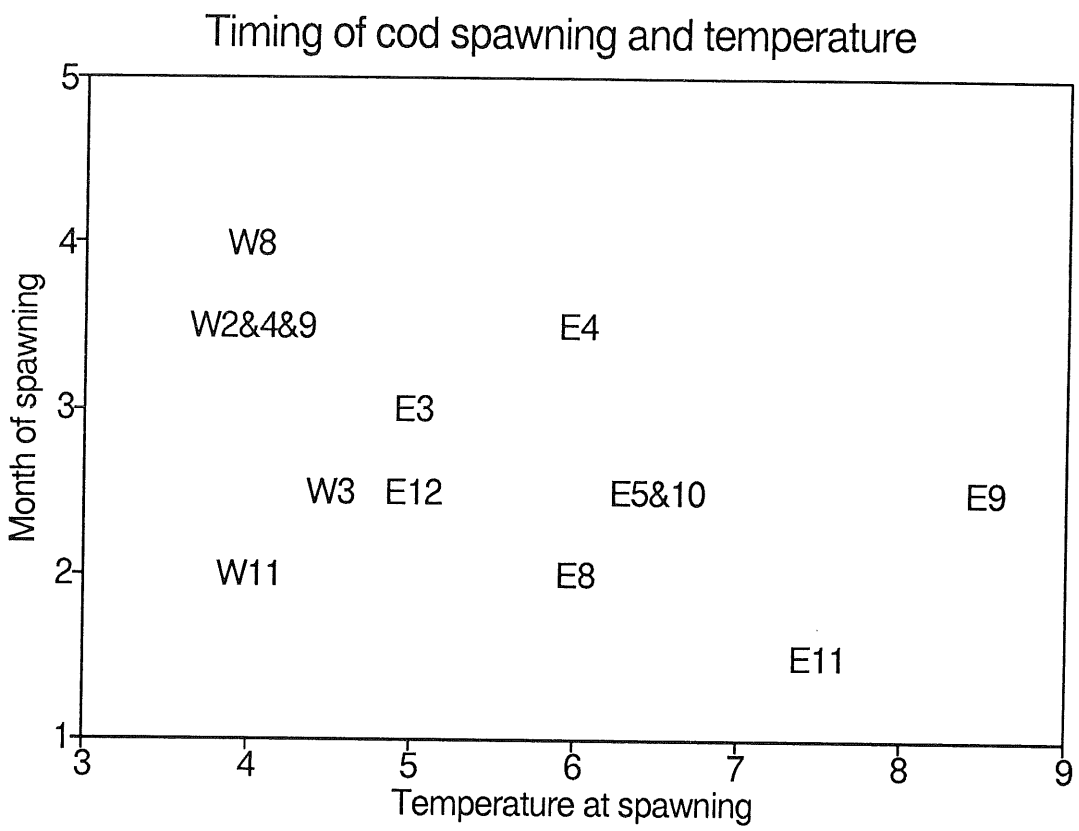
Code	Stock	Area	Dates		Depth	Temperature		Egg				
			Mean	Duration		Adults	Eggs	Size	Density	Conc	Mortality	Total prod
W1	W Greenland	1	April	Feb-Jul			0.5-4			(Hansen**)		
W2	Labrador/Grand Bank	2J3KL	Apr	Mar-Jun	300-600	3-5	<1	1.2-1.6				
W3	Flemish Cap	3M	Mar	Feb-May	200-400	3-6	3-5	1.2-1.6				
W4	S Grand Bank	3NO	Apr	Mar-May	200-400	3-5	2-5	1.2-1.6				
W5	N Gulf of St Lawrence	3Pn4RS		Apr-Jun								
W6	St Pierre Bank	3PS	May	Apr-Jun	200-400							
W7	S Gulf of St Lawrence	4TVn	Jun									
W8	Banquereau and Sable	4VsW	Apr-May	Mar-Jun	40-60	4	4-6			1		
W9	W Scotian Shelf	4X	Apr[c]	Feb-May[c]	100	4	4-6	1.5		2	0.2	1E+14
W10	Gulf of Maine	5Y										X*1E12
W11	Georges Bank	5Z+6	Feb-Mar	Nov-May	60-90	3-5	3-7	1.5	25-26	100-300	0.025-0.041	40-110E12
E1	E Greenland	14	Apr	Mar-Jul	170-400		3.2-5.2					
E2	White Sea	1E	Apr	Mar-May	15-100		1.4-1.6					
E3	NE Arctic	1&2	1 Apr	60-70 days	60-150	4-6	1.3-3.6	1.2-1.6	23.5-26	400	0.1	4E+13
E4	Iceland	11	Apr	Mar-May	30-100	5-7	5-7	1.3-1.5		10		
E5	Faroe Plateau	5A	Mar	Feb-May	80-180	6-7	6-7					
E6	Faroe Bank	5B	Mar	Feb-May								
E7	W Scotland	6										
E8	North Sea	4	Feb-Mar	Jan-Apr[c]	30-?	5-7	5-7	1.4		1-10	0.027-0.404	
E9	Celtic Sea	7F&G	Mar	Feb-Apr	70	8-9	8-9			1-10		
E10	Irish Sea	7A	Mar	Feb-Apr	20-50	6-7	6-7			1-10		
E11	English Channel	7D&E	Feb	Jan-Mar	20-50	7-8	7-7			1		
E12	Skagerrak	3A	Mar	Feb-Apr		4-6	4-6	1.1-1.7	23.8-27.1			
E13	W Baltic (22 & 24)	3D	Mar	Feb-Apr								
E14	Baltic (25-32)	3D	May	Mar-Aug	60-80		3-7	1.4-2.0		100		

TABLE 4

Key words used for indexing literature information on cod life histories

age at maturity
Arctic
Baltic
Barents Sea
Barents Sea
batch spawning
Browns
Calanus finmarchicus
cannibalism
catch per unit effort
climate
cod
cohort
competition
condition factor
copepod
correlation with VPA
daily growth increment
demography
density
density dependent growth
diel
diet
diurnal rhythm
DNA
egg bouyancy
egg density (ie no m^{-3})
egg density (ie specific grav)
egg development
egg distribution
egg identification
egg incubation
egg mortality
egg production
egg size
egg stages
egg surveys
egg weight
eggs
electrophoresis
enclosure
energetic model
English Channel
estuary
Faroe
fecundity
feeding
feeding behaviour
feeding incidence
feeding ration
fertilisation rate
field study
first feeding
fishing mortality
front
gear avoidance
genetics
Georges
Greenland
growth
growth backcalculation
growth curve
growth model
growth rate
Gulf of St Lawrence
Gulf Stream
haddock
haemoglobin
haemoglobin polymorphism
hatch
hatch check
hatch date
hatching rate
ice index
Iceland
image analysis
increment width
inshore
instantaneous growth rate
intertidal
Irish Sea
juveniles
larvae
larvae bouyancy
larvae density (nm^{-3})
larval development
larval distribution
larval drift
length at maturity
length frequency analysis
length-weight
light
lipid
Lafoten
mark-recapture
match:mismatch
meristics
mesh selection
mesocosms
metabolic rate
metamorphosis
migration
mismatch
morphometrics
mortality
mortality rate
mtDNA
natural mortality
North Sea
Norwegian Sea
nursery
nursery areas
0-group abundance
0-group distribution
otolith growth
parasite
patch
phytoplankton
point of no return
population dynamics
predation
productive cycle
races
recruitment
restrocking
retention
RNA
RNA:DNA
Rockall
salinity
Scotian Shelf
search volume
simulation
size-selective mortality
spawning
spawning area
spawning behaviour
spawning biomass
spawning curve
spawning migration
spawning period
spawning strategy
spawning temperature
spawning time
species succession
specific fecundity
spring bloom
stage
stage duration
starvation
statistical analysis
stock identification
stock recruitment relationship
stock structure
stomach-content
survey
swimming behaviour
tagging
temperature
thermoicline
tide
time series
transferrin alleles
trophic
turbulence
upwelling
variability
vertebrae
vertebral number
vertical distribution
vertical migration
von Bertalanffy
waves
wind
year class strength
young fish survey
young-of-the-year
zooplankton

Figure 1


Timing of cod spawning and temperature
Month of spawning
Temperature at spawning
5
4
3
2
1
3
4
5
6
7
8
9
W8
W2&4&9
E4
E3
W3
E12
E5&10
E9
W11
E8
E11