



SLIP Ph.D. Course in Preparation, Interpretation and Analysis of Otoliths for Use in Population Studies

**University of Aarhus, Marine Research Station,
Rønbjerg, Denmark
9-15 December 2001**



1. Aim of the course

Since the early 1970s a large number of studies have examined growth, recruitment, migration, mortality and stock structure of fish using a wide variety of otolith based techniques. These studies have significantly enhanced our understanding of fish population dynamics. The aim of the course was to yield a thorough presentation, hands-on experience and discussion of the most used applications of otoliths as well as an introduction to new areas of otolith research.

2. Participants

2.1 Students

The course was attended by 16 participants representing Denmark (4 Ph.D. & 5 M.Sc. students), Germany (2 Ph.D. students & 1 Post-doc), Norway (2 Ph.D. students), Iceland (1 Ph.D. student) and Sweden (1 M.Sc. student). Three of the Danish Ph.D. students are associated with the SLIP network and five of the participants were women. The list of participants is found in Section 9.

2.2 Teachers and assistants

The teachers were Dr Arild Folkvord (Univ. of Bergen, Norway), Dr Audrey Geffen (Univ. of Liverpool, UK), Dr Peter GrønkJær (Univ. of Aarhus, Denmark, organizer) and Dr Henrik Mosegaard (Danish Institute for Fisheries Research, organizer). Furthermore, Dr Anders Koed (Danish Institute for Fisheries Research) and Jan Jensen (Univ. of Aarhus) were hired to give two seminars. Two research assistants were kindly made available free of charge from DIFRES to take care of and guide the practical aspects of fish rearing and otolith preparation. Two M.Sc. students from the University of Aarhus were in charge of logistics and food at the Marine Research Station.

3. Overall course outline

The course consisted of a combination of one-hour lectures introducing specific themes, 2-4 hr theoretical class projects, and 2-4 hr demonstrations/hands-on exercises. Furthermore, the students all signed on to a group working with specific topic at intervals during the whole workshop. Finally, four practical projects involving experiments with trout embryos and subsequent analysis of trout embryo otoliths were run alongside the scheduled program. A detailed description of the program is found in Section 6.

4. Course material

In order to give the students a common starting ground and prepare them for the topics they had chosen a selection of papers were sent out in advance. Furthermore, a binder with relevant literature was distributed at the start of the course. The list of papers is found in Section 10. Additional data files, images and software were distributed to the different groups depending on their topics. At present a CD-Rom containing all lectures, data from the practical projects and the student presentations is being prepared for distribution to all participants.

5. Topics for group work

Three groups were formed to work with a specific topic at intervals for the duration of the workshop. The subjects and rationale for them were:

"Back-calculation of fish growth: possibilities and pitfalls"

Back-calculation of previous growth by use of periodic structures in hard parts is a common procedure in fish biology. Several different procedures have been proposed with different advantages and disadvantages. In this project, a comparison of methods will be undertaken on an available data set from herring and back-calculated sizes will be compared with observed previous sizes.

"Oxygen consumption in late embryos and otolith hatch size at hatch"

Our ability to verify the link between metabolism and otolith growth in embryos, larvae and juvenile fish is dependent on our ability to correctly quantify metabolism. Oxygen consumption is the most widely used proxy for metabolism, but it is notoriously difficult to measure oxygen consumption of individual juvenile fish or fish eggs. This project will make use of an oxygen microelectrode to quantify oxygen consumption of individual salmon eggs and relate this to the otolith size at hatch.

"Revealing selection on otolith traits"

Many hypotheses concerning recruitment in freshwater and marine fish are based on the premise that some fish survive better than others due to e.g. their time of hatch, superior growth rates or large size. Otoliths contain a record of age and growth that could be used to investigate some of these hypotheses. In this project we will work with a range of statistical and graphical methods that have been devised to reveal selection, and we will look at the difficulties of setting up lab and field studies that can test the before-mentioned hypotheses using otoliths.

6. Detailed schedule

Sunday 9 Dec

Morning and afternoon

Arrival
Get to know the Marine Biological Station.

Evening

A kick-start presentation – “Otoliths – blind recorders or physiological interpreters?” (Geffen)
Presentation of practical projects and group forming

Monday 10 Dec – Otolith formation

08.30 – 09.30

Lecture: Otolith chemistry & biochemistry (Audrey)

Otoliths incorporate a wide range of elements in its protein calcium structure. The elemental composition of the otolith reflects the concentration of the elements in the habitat of the fish as well as exogenous factors such as the ambient temperature. Therefore, the elemental composition of the otolith is increasingly used for e.g. discriminating between marine and freshwater populations and life stages, assessing

population structure in marine fish and determining temperature history of individual fish. This lecture addressed these topics and introduced the various methods to determine whole otolith and well as transect or point sampling of elemental composition. It furthermore introduced the datasets available for the following class project.

- 09.30 – 12.00 **Class project: “Otolith microchemistry data: the acquisition is easy but what does it mean?”**
The aim of this class project was to work with otolith elemental composition datasets in order to learn the various analytical and statistical methods used for discriminating between populations of fish based on their otolith elemental composition.
- 13.30 – 17.00 **Class project: “Otolith microchemistry data: the acquisition is easy but what does it mean?”**
- 17.00 – 18.00 **Lecture: Back-calculation of otolith growth - assumptions and methods (Folkvord)**
Back-calculation of previous growth by use of periodic structures in hard parts is a common procedure in fish biology. Several different procedures have been proposed with different advantages and disadvantages. In this lecture these procedures and their assumptions were presented and discussed.
- 20.00 – ? **Demonstrations/projects/try-outs: Rounding up the class project**
The results and conclusions regarding the use of otolith elemental composition were presented and discussed.
Later, equipment incl. microscopes, computers and software was available for try-outs and use with own material.

Tuesday 11 Dec – Otolith analysis

- 08.30 – 11.00 **Group projects introduction**
The three groups were introduced to their specific subjects and made up a work plan for the rest of the course
- 11.00 – 12.00 **Lecture: Environmental and ontogenetic influence on otolith growth - covering temperature and growth effects on larval otoliths (Folkvord)**
Otolith growth is generally believed to mirror the somatic growth of the fish. However, several studies have shown that the relationship between fish growth and otolith growth may change due to e.g. temperature and the growth rate of the fish. These phenomena will have profound effect on the validity of simple back-calculations and the use of otolith growth to compare the growth of different cohorts of fish. In this lecture these issues were addressed in order to extend on the simplistic view of otolith and fish growth
- 13.30 – 17.00 **Demonstration and hands-on: Preparation**
The various methods used to prepare otoliths for examination were demonstrated. This included mounting, cutting and polishing. The participants primarily used their own material and displayed their own favourite methods.
- 17.00 – 18.00 **Lecture: Anatomy and function of the inner ear in fishes (Jan Jensen, AU)**
The otolith is an integral part of the inner ear in fishes. However, the physiological role of the otoliths is often not appreciated by fisheries biologists for whom the otoliths just are convenient tools. This lecture gave a zoo-physiologists view on the inner ear of fishes and explained the “normal” role of the otolith. This may be very useful for fisheries biologists when interpreting the growth patterns of the otoliths.
- 20.00 – ? **Demonstrations/projects/try-outs: Computer enhancement of otolith images**
The use of different computer software for enhancement of otolith images were

demonstrated and the participants could use their own material and compare and discuss the different methods.

Wednesday 12 Dec - Otolith data

- 08.30 – 09.30 **Lecture: Interpretation of increments and ageing (Henrik)**
The interpretation of otolith structures both on an annual and daily basis is at the heart of most otolith-based techniques. The structures that are visible in juvenile fish otoliths include increments formed with daily and sub-daily periodicity, checks laid down in response to lunar and tidal cycles, checks formed at hatch, first-feeding and metamorphosis. Similarly, the formation of increments in adult fishes is influenced by growth pattern of the fish as well as exogenous factors such as temperatures. This lecture introduced a conceptual model for the formation of otolith increments and in doing so addressed the various pit-falls with regard to interpretation of the temporal meaning of the increments.
- 09.30 – 12.00 **Demonstration and hands-on: Ageing known and unknown age larvae, juveniles and adults, determining otolith growth and secondary growth structures**
During this hands-on exercise otoliths from known age fish were used to compare and validate the different interpretations of the otolith structures. Furthermore, many participants had brought their own material in order to get a second opinion on their specific otolith interpretation.
- 13.30 – 16.00 **Group projects**
The groups worked on their specific projects.
- 16.00 – 18.00 **Demonstration and hands-on: SEM - preparing otoliths for Scanning Electron Microscopy**
Scanning Electron Microscopy is often used to verify the presence of increments that are too narrow for detection in light microscopes. However, the preparation of otoliths for SEM analyses is very different from the usual preparation for light microscopy and hence a special demonstration was scheduled for this subject
- 20.00 – ? **Demonstrations/projects/try-outs**
The participants were free to choose whatever subject they would work with.

Thursday 13 Dec - Analysis of otolith data

- 08.30 – 11.00 **Demonstration and hands-on: Back-calculation of growth**
Back-calculation of growth was demonstrated and all participants were given a dataset from which they should back-calculate fish sizes using the methods described in the lecture on Monday.
- 11.00 – 12.00 **Lecture: Ontogenetic and size effects on otolith growth - Fluctuating asymmetry in fish otoliths (Folkvord)**
This lecture elaborated on the effect of ontogeny and size on otolith growth. Furthermore, developmental stability expressed as fluctuating asymmetry was discussed in relation to nutritional and other stress imposed on the fish. The potential use of otoliths to assess the level of stress based on FA was discussed.
- 13.30 – 17.00 **Group projects**
The groups worked on their specific projects.
- 17.00 – 18.00 **Lecture: Validation & quality control of otolith data (Grønkjær)**
Validation and quality control of otoliths data should be an integral part of any study

using otoliths. Even so, this is very seldom performed. In this lecture different methods for validating age and carrying out quality control of otolith data were presented.

20.00 – ? **Demonstrations/projects/try-outs**
The participants were free to choose whatever subject they would work with.

Friday 14 Dec - Otolith data and complex models

08.30 – 11.00 **Practical projects**
The groups worked on their specific projects.

11.00 – 12.00 **Lecture: Linking metabolism, otolith and somatic growth (Mosegaard)**
The decoupling often observed between fish and otolith growth has led several authors to suggest that the growth of otoliths is more closely coupled to metabolism than to fish growth. This lecture explored the link between metabolism, otolith and somatic growth based on a spreadsheet model made by Dr. Mosegaard. The bioenergetic model that uses temperature and fish growth as input parameters is able to closely mimic the increment pattern seen in adult cod. The implications of the link between metabolism, otolith and somatic growth for interpreting increments in adult fishes and back-calculation of growth were discussed.

13.30 – 14.30 **Lecture: Selection revealed by analysis of otolith traits (Grønkjær)**
Many hypotheses concerning recruitment in freshwater and marine fish are based on the premise that some fish survive better than other due to e.g. their time of hatch, superior growth rates or large size. This lecture presented ideas and examples on how these hypotheses could be tested using age and growth data from otoliths.

14.30 – 17.00 **Projects**
Presentation and discussion of group and practical projects

Saturday 14 Dec - Rounding up, evaluation, packing and leaving

7. The organizers summary of the course based on the evaluation submitted by the participants

The participants overall opinion of the course was very positive. The content and the structure of the course were generally evaluated as good; hence in case of a future course this structure will probably be reused. The topics were adequately covered; however an increased focus on adult fish otoliths would have increased the relevance of the course to some of the participants. The lectures were very well received and made up a very important part of the course. The material sent out in advance and distributed during the course was also well received. The participants were satisfied with the logistics at the field station. In order to make this blend of lectures, exercises and laboratory work, a field station or similar facilities are necessary. Finally the students judged this course to be very relevant for their future careers in fisheries science.

8. Evaluation of the Ph.D. Course in Preparation, interpretation and analysis of otoliths for use in population studies

Please give us your opinion on to the following questions. Do not hesitate to include supplementary comments that you may not think relevant. Do not write your name anywhere, it is an anonymous questionnaire.

Overall evaluation

What is your overall opinion about the course?

Poor.....	0
Not very good.....	0
OK.....	1
Good.....	11
Excellent.....	5

Course content

Did the course provide a reasonable overview of the subject?

Yes.....	16
To some extent.....	1
No, important items were missing.....	0

Comments (e.g. missing topics)

- ?? Too bad the intro to using microscopes had to be cancelled. An intro to the use of related software would have been nice too.
- ?? Annual structures and age validation methods got too little attention.
- ?? Different preparation methods shown more systematically
- ?? More on adult fish next time – annual structures.

Were the topics included adequately covered?

Yes.....	16
To some extent.....	1
No.....	0

Comments (e.g. topics receiving insufficient/too much attention)

Lectures

How was the level of the lectures?

Too low..... 0
Adequate..... 14
Too high..... 0

Comments

- ?? Very good lectures
- ?? Several lectures prepared by the teachers were impressive
- ?? The lectures are mostly covering very recent development in otolith analysis, and gave ideas to come up with new research projects.

Were the lectures well prepared and easy to understand?

Yes..... 14
To some extent..... 2
No..... 0

Comments

- ?? Concerning otolith microchemistry some parts are rather hard to understand. This could be because my knowledge in chemistry is poor.

Practicals

Were the exercises useful for understanding and getting hands-on experience with the subjects?

Yes..... 14
To some extent..... 3
No..... 0

Comments

- ?? Good mix of practical and theoretical subjects.
- ?? Very good

Course material

Were the distributed papers and other material sufficient?

Yes..... 14
To some extent..... 2
No..... 0

Comments (e.g. suggestions for additional papers, handouts etc.)

- ?? Great collection of articles in the course ringbinder

Logistics

How was the physical framework - accommodation, lecture room, food etc.?

Poor.....	0
Not very good.....	0
OK.....	3
Good.....	11
Excellent.....	3

Comments

- ?? Some time was spend on finding things for materials
- ?? Food was excellent
- ?? Lecture room not comfortable
- ?? Accommodation and food was fine, labs could have been better equipped (more computers & image software)

Overall balance and timing

Was the balance between lectures and exercises right?

Yes.....	15
To some extent.....	0
No.....	1

Comments

- ?? It was a good idea to spread out the lectures, so we didn't sit and listen for more than an hour at a time
- ?? Too much time spent on exercises.
- ?? Good that time provided to look at own samples and problems.

Was the time assigned to each subject appropriate?

Too short.....	2
Yes.....	11
Too long.....	1

Comments (e.g. which subjects were given too much or too little time?)

- ?? Too short on tasks requiring special software, image analysis.
- ?? The practical aspects connected to the subjects allowed us time to concentrate on the area we wanted to most. I feel this reduced any unbalances here.

Was the overall duration of the course appropriate?

Too short.....	0
Yes.....	14
Too long.....	1

Comments

Relevance

Do you think that the course will be relevant for your above career in fisheries science?

Yes.....	13
Don't know.....	2

Probably not..... 0
Not at all 0

Comments

- ?? A really good opportunity to get a thorough introduction to otolith work for someone who knew nothing about it before the course.
- ?? Thanks for the great opportunity. This course just came at the right time and will be very important for my Ph.D.
- ?? Even if I don't work in this field in the future, I have gained a greater understanding of the possible uses, biases and future possibilities of using otoliths.

9. Participants

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10. Literature

General papers (all)

- Kalish, J.M., Beamish, R.J., Brothers, E.B., Casselman, J.M., Francis, R.I.C.C., Mosegaard, H., Panfili, J., Prince, E.D., Thresher, R.E., Wilson C.A., and Wright, P.J. (1995): Glossary for otolith studies. In: Recent Developments in Fish Otolith Research, edited by D.H. Secor, et al, pp. 723-729. Univ. of South Carolina Press, Columbia.
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Back-calculation project (Line, Kaare, Hanne, Groa, Thomas, Christian, Mukhlis)

- Campana, S.E. (1990): How reliable are growth back-calculations based on otoliths? Canadian Journal of Fisheries and Aquatic Sciences 47: 2219-2227.

Selection project (Vivian, Jonathan, Michele, Karin, Rune, Claus S)

- Chambers, R.C. and Miller, T.J. (1995): Evaluating fish growth by means of otolith increment analysis: special properties of individual-level longitudinal data. In: Recent Developments in Fish Otolith Research, edited by D.H. Secor, et al, pp. 155-175. University of South Carolina Press, Columbia.
- Anderson, C.S., Dean, J., and Campana, S.E. (1995): Calculating size-dependent relative survival from samples taken before and after selection. In: Recent developments in Fish Otolith Research, edited by D.S. Secor, pp. 455-465. University of South Carolina Press, Columbia, SC.

Oxygen measuring (Anders, Jens -Peter)

- Wright, P.J. (1991): The influence of metabolic rate on otolith increment width in Atlantic salmon parr, *Salmo salar* L. Journal of Fish Biology 38: 929-933.