

1992 Biennial Meeting

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MINUTES TO THE CONFERENCE OF THE COMMITTEE OF AGE READING EXPERTS (C.A.R.E.)

Â May 27-29, 1992 National Marine Fisheries Service Seattle, Washington

Â INTRODUCTION AND ORGANIZATION

Â Craig Kestelle, chairperson for the 1992 CARE conference, opened the meeting at 9:10 a.m. welcoming everyone to this year's conference. He began by suggesting everyone introduce him or herself and state the organization they represented. A list of the participants can be found in Appendix I.

Â For the first order of business, he directed attention to two handouts, the first being the proposed agenda (Appendix II) and the second was a memo from Mark Wilkins, current TSC chairperson (Appendix III). Mr. Kestelle had hoped to have Mr. Wilkins present for a 20-minute talk on the organizational status of the Technical Subcommittee (TSC) and the role of the CARE, but unfortunately Mr. Wilkins was on vacation. Mr. Kestelle briefly outlined the organizational network tracing CARE's lineage to the TSC whose parent organization is the Canada-US Groundfish Committee. All these groups are under the Pacific States Marine Fisheries Council (PSMFC).

Â On the proposed agenda, Mr. Kestelle asked for further suggestions and thoughts. Since these meetings have always been held in a semi-informal fashion, he suggested we feel free to include other topics of concern.

Â Shayne MacLellan (DFO) began by recalling a letter she drafted to the TSC as a result of the 1990 CARE conference. The letter listed 4 recommendations CARE felt needed investigation. TSC endorsed these 4 recommendations. Tom Jagielo, then chairperson of the TSC, circulated a letter to all participating fish ageing units outlining these recommendations (Appendix IV). Ms. MacLellan suggested that we discuss what has occurred in our different agencies and what has been implemented as a result of these recommendations. This was followed by a discussion of the other activities planned for the conference. Several speakers had presentations to give Thursday morning. Hands-on microscope work was scheduled for Thursday afternoon. Betty Goetz (AFSC) suggested that we talk about current and upcoming age validation projects. Dan Kimura (AFSC) suggested we discuss the status of the ageing manual. The manual has been in draft form since the first CARE meeting in 1983. At the last meeting Ms. MacLellan was given the responsibility of compiling the body of the manual. She brought a copy of the latest draft. Ms. MacLellan asked for input from everyone on the manual from spelling and grammar to content and structure. She felt the manual was something needed by each agency not only as a reference guide but also as a teaching tool for new age readers. She stressed the importance of good quality photos for training. The group discussed the publication of the manual. One suggestion was to print it in the form of a NOAA Technical Report. Then there was the question of incorporating pictures in the publication. The majority preferred illustrating with colored photographs because it showed better clarity and resolution but the cost would be high. Someone suggested that Ms. MacLellan or Mr. Kestelle approach the TSC for their ideas. How would TSC like to see this document handled and will TSC provide funding for publication? Ms. MacLellan said she wouldn't mind approaching TSC after we have all accepted the final draft of the manual.Â APPROVAL OF THE 1990 MINUTES

Â Going back to the agenda, Mr. Kestelle asked everyone for formal approval of the 1990 minutes. None of the past participants had anything further to add and they were approved. Â

Â OLD BUSINESS

Â Mr. Kestelle asked Ms. MacLellan to read the letter from Tom Jagielo listing the TSC endorsed recommendations from the 1990 meeting. In summary, the recommendations stated that:

Â 1. Each agency should develop a systematic precision-testing program as a routine ageing procedure. 2. Each agency has a formal training program for new fish agers. 3. Each agency document marginal growth increments for each species to determine more accurately deposition time for the annulus thereby properly assigning an age-class. 4. Adopt a single age designation system to avoid confusion during inter-agency exchanges and cooperative work.

Â Mr. Kestelle began by describing the AFSC plans for a marginal increment study analyzing 0- to 2-year-old sablefish otoliths. These fish were caught over a 2-3 year period from St. John-the-Baptist Bay in Alaska. Biologists from the NMFS Auke Bay Lab collected the otoliths for the Seattle Age Unit (AFSC) specifically for this study and included various months of the year.

Â Ms. Goetz added that age readers at AFSC have been systematically assigning an edge-type code as part of the ageing routine. Dr. Kimura pointed out, however, that this system has not been in effect very long. The data is all in the AFSC database and has yet to be analyzed.

Mr. Kastelle mentioned that the AFSC also does a systematic 20% precision testing of all otolith batches/cruises read. Usually, tester and reader are two different persons. In all cases, tester has no prior knowledge of reader age. Currently the AFSC has two designated testers.

Cal Blood (IPHC) said that the IPHC has followed suit and has implemented a similar 20% reader-tester system. Furthermore, they routinely do blind testing where two readers read the same batch without knowing the other readers estimated age. A third reader resolves any differences. Ms. MacLellan commented that different agencies probably have different methods. In their case, they used to do double readings which involved 2 different readers ageing the same batch of otoliths. Reader #2 was aware of Reader #1 ages during the second ageing. This two-reader system was too time consuming so they changed to a single reader system with 15% testing. The tester ages a minimum of 10 otoliths if there is less than 100 fish in a sample. They have not decided whether a 15% sample is sufficient. She introduced a paper by Richards, Schnute, Kronlund and Beamish titled Statistical Models for the Analysis of Aging Error, which is currently in press. This paper pointed out that error is inherent to ageing and should be taken into account. However error is something that will not go away no matter what the test sample size may be or how often a sample is read and tested. Mr. Blood asked Dr. Kimura if he (being a statistician) had any thoughts on what percentage of a given sample is necessary for valid test results. Dr. Kimura said he had some thoughts on it but cannot see doing more than 20% testing because of the volume required in production ageing.

Moving to another topic, Mr. Blood asked Ms. MacLellan what her thoughts were on reader drift. Ms. MacLellan did not feel that reader drift is a problem when multiple readers are involved and have access to well documented (written and photographic) criteria. Ms. MacLellan felt that drift may occur when a reader has not read a particular species in a long time and the criteria has not been well documented.

Ms. MacLellan mentioned that Rick Stanley (DFO) once looked at their precision testing results with *S. flavidus*. Interestingly, Mr. Stanley found that percent agreement increased as the ages approached about 18- to 19-years-old then fell off again. Ms. MacLellan added that it may not be too unusual to find a novice reader underageing the older fish and overageing younger fish.

John Butler asked Ms. MacLellan what her definition was of an "expert reader". She defined an expert reader as a reader who has shown the ability to maintain a high level of precision with themselves and with others. To train a new reader they have the trainee read previously aged otoliths. Then they are tested against their own original ages and tested with an expert reader. DFO does not have designated testers. Any reader expert in the trainee's species participates in testing. To pass training they are required to achieve an acceptable percent agreement, which is arbitrary. An acceptable value depended more on the users' preference, which in most cases, is 80% +/- 1 year. This training normally takes about 2-6 months depending on previous experience.

John Butler asked if anyone ever routinely used size and weights of otoliths to help in ageing. Mr. Kastelle said that Dave Somerton at AFSC once investigated this idea. He used otolith weights and surface area measurements. Through multiple regression analysis he tried to predict age. This was quite a large study. The results showed that these measurements could predict age but with a considerable decrease in precision.

Dr. Kimura pointed out that this is an idea that is resurrected every so often but has never produced any good results. His personal feeling was that it is not sufficiently precise to be useful.

Julie Lyons (AFSC) wanted to go back and make a comment on reader drift. She believed this happens to all readers even when they have current reading experience with a particular species. Mr. Kastelle mentioned that in ageing sablefish, he tests against his original ages and when there is a between-reading bias, it usually leans toward ageing older the second time. In looking for drift, Ms. Lyons would like to see that readers reread otoliths every few years. Ms. MacLellan added that "reader drift"/"lack of precision" (there may be some confusion in the use of these terms) may also be identified simply when users complain that not enough fish were assigned to particularly strong age-classes compared to previous years. This may show up especially in flattened year-classes.

Dr. Kimura continued by adding that users do not like it either when too many fish are aged in the dominant year-classes. This was a complaint he received recently. When plugged into their models, it resulted in unreasonable bounds among the year-classes.

Dr. Kimura addressed the problem of reader bias when ageing fish knowing the year classes. To study this, he designed a test where 3 readers aged pollock otoliths previously aged 9, 10, and 11. These otoliths were taken from samples collected during 3 consecutive years from the strong 1978 year-class. Results showed that without having the year-class information, the readers still identified the dominant year-classes but with some spreading into the adjacent classes. This study was summarized into a paper currently in press titled The Effects of Year-class Strength on Age Determination by Kimura, Lyons, MacLellan and Goetz. The topic returned to the 4-point TSC recommendations on precision testing, Ms. Goetz commented that with her group they've adopted as acceptable for POP a percent agreement comparison of +/-0 age difference up to age 12, +/-1 up to age 25 and +/-2 above age 25. Ms. MacLellan felt that this might be a very useful

way to examine precision results for longer-lived species.

Mr. Kastelle commented that AFSC does not have set precision criteria for what might be considered an "expert" level. Ms. Lyons pointed out that setting levels is difficult because of the varying difficulty between otolith batches even within a given species. This variability could be due to differences in area, between cohorts, and even from one collection year to another. A poor percent agreement could reflect the difficulty of that batch in which case, it should be re-evaluated. She recommended requiring the tester to re-age the test sample without knowing his/her previous ages. The precision between the two sets of test ages should be indicative of the difficulty of the samples.

Going back to the comment on modeling and relying on the interpretations of the models, Larry Quirollo (CDFG) wanted to add a cautionary note on the effects of the commercial fisheries to these models. In California, age readers found they were ageing Dover sole younger and younger. They discovered this was not due to reader drift but was a result of the commercial fisheries targeting the younger, shallower fish, which had a better commercial appeal than the older, deeper fish. On precision testing, Mr. Quirollo added that Dover sole could be difficult and checky. Precision testing could result in poor agreements because even with himself he might come up with multiple ages for one otolith. Conflicting ages could result from reading different axes and the light casting on the otolith cross-section. Mr. Butler asked Mr. Quirollo what he does when he is confronted with multiple readings and has to assign one age. Does he take the average? Mr. Quirollo answered that he does not take an average but decides which age is the most appropriate after analyzing all the characteristics of the otolith. Then he tries to come up with that same age again. Mr. Kastelle added that for sablefish, he too does not take an average but tended to choose the oldest reasonable age. He does this because of validation studies indicating a tendency for this species to be older than previously thought.

Mr. Kastelle directed discussions to item four on the 4-point TSC recommendations. Ms. MacLellan clarified this item by explaining that some agencies reported their results in year-classes such as fish "born in 1950" whereas other agencies reported in age-classes such as a "50-year-old fish". This can result in some confusion. Mr. Quirollo said their computer programs required them to input data in year-classes but they converted to age-classes when reporting otolith ages. Mr. Mello (CDFG) said he did the opposite by starting with age-classes and converted his results to year-classes. Someone pointed out that the Tiburon lab also reported in year-classes. Ms. MacLellan cautioned everyone about the potential of year-class biasing that might occur when using the year-class system. All the other participants conformed to age-classes.

At that point Mr. Kastelle declared that aside from item three, to be further discussed later, the other three points of the TSC recommendations were considered dealt with.

A BREAK was called at 10:15 a.m.

The meeting was reconvened at 10:50 a.m.

First business was the updating of the species list aged by the various West Coast age reading units (Appendix V). Sandra Rosenfield (WDF) requested that Mr. Kastelle quickly read through the list and members were to call out revisions to their agency list when necessary.

The next item on the agenda was the status of the ageing manual. Members received photocopies of the current form of the manual. Ms. MacLellan explained why she had revised it to its current format. The manual was separated into three sections, the first was a general ageing procedure applicable to all species. The second part was an ageing procedure for rockfish and the final part was an ageing procedure for sablefish. If published as is, she foresaw it as a simple matter to include other species in the form of future addenda without further revisions to the format. Mr. Blood suggested adding a section on flatfish. Ms. Goetz recommended that perhaps we should have something written up on all basic species groups before publication. Ms. MacLellan thought that might delay publication for years considering how long it has already taken to get this far on just two species groups. However, if some groups felt comfortable with their ageing criteria for the other species and wrote it up within a time frame before publication, it could be included in the manual. Ms. MacLellan and Mr. Kastelle suggested organizing a small committee to prepare the manual for publication. Dr. Kimura thought it might be difficult to have this committee meet because of the distances involved between the different agencies. He suggested assigning an "editor-in-chief" who would have the overall responsibility for the final draft of the manual. The editor would make sure each agency reviewed the final form. Dr. Kimura suggested that Ms. MacLellan be the "editor-in-chief" since she has already contributed so much time to it. Ms. MacLellan accepted.

Ms. MacLellan commented that she wanted to see more figures/photos incorporated in the manual and asked for good quality photos from other members. A discussion began on the expense of including the photos, especially for high quality results. However, the group agreed that if this manual was to be a good teaching tool we could not compromise good quality photos. Ms. MacLellan agreed to talk to Guy Thornburgh of the Pacific Marine States Fisheries Commission over the possibility of funding.

Â NEW BUSINESS

Â Returning to the agenda, Mr. Kastle introduced the first of the new business topics. He commented on the TSC's support in encouraging marginal increment analysis or edge-type studies. Mr. Blood said that the IPHC has been working with the treaty Indian tribes on an ad hoc basis. The tribes have been collecting halibut otoliths for the IPHC. The problem is that the commercial fishing occurs from May to September and interpretation of marginal increment formation in May is always difficult because this is believed to be the beginning of the growing season.

Â Ms. MacLellan felt that we may never get clear answers to the problem of interpreting marginal growth formation. Numerous factors could affect annulus formation even in a given species. Variations could occur between cohorts, location, sex, age-classes, environmental factors, etc.

Â Mr. Blood questioned Dr. Kimura about the Observer Program and all the otoliths collected over various months throughout the years. He asked if AFSC has done any historical studies on this problem using those otoliths. Dr. Kimura said that they haven't really done anything historical but are just beginning a systematic study on marginal increment as mentioned earlier. Mr. Kastle pointed out that the collections from the Observer Program were dependent upon the fishery openings, often for limited time spans once or twice a year.

Â Someone asked how the AFSC did their edge-type determination. Ms. Goetz explained that it was a relatively subjective system where each reader assigns a code ranging from 0-5 for each otolith read. Zero was an indication of a clear, strong annulus on the edge. Assignment of 1-4 would represent the increasing growth increment beyond the last annulus and 5 would be the indistinct formation of a new annulus on the edge.

Â Ms. MacLellan mentioned that DFO also has a similar system on a scale of 1-4. She emphasized that she thought this problem would always be a continuing nuisance. Part of the problem is our use of the break-and-burn technique, which may leave a dark mark at the edge of the otoliths. Is the mark really an annulus, a check or a result of the burning process?

Â Mr. Butler summarized the discussion by commenting that to undertake this study properly we needed to have samples collected at least quarterly to identify the incremental growth and annulus formation. This led him to voice a concern about our current system where we have users who request these ages. They may not be aware of the problems of ageing but want "real" ages to make their stock assessments. Then we have the researchers who study the problems of ageing but do not generate the ages for the users. Then there are the production age readers who are aware of the ageing problems but are normally not in the position of studying these problems. He questions this current system and wants to see production readers, who directly affect the ages for stock assessment calculations, more involved in studying some of these problems.

Â Â Â Â Â Â Â Â Â Â The morning session was adjourned for lunch at 11:55.

Â The afternoon conference session was reconvened at 1:06.

Â The first topic was a continuation of the discussion on marginal increment validation. Mr. Kastle talked about the proposed project he mentioned earlier this morning about the juvenile sablefish otolith collected by the Auke Bay lab for studying marginal increments. He hoped to do a thorough study leading towards publication.

Â Ms. MacLellan talked about being involved in an English sole OTC validation study, which led her to believe that growth is affected by the season of reproduction. She began the study on the premise that otolith growth begins around June and full growth is seen in September. However, she found that in some fish she identified growth from February to June. The principle researchers were pleased to learn this because it conformed to their thoughts on growth occurring after reproduction, which was in the fall. So another possibility in the scheme of otolith growth is to consider younger fish growing from June to September and older fish possibly delaying somatic growth until after reproduction. Of course this could mean different growth periods between males and females and between different species.

Â Mr. Butler added that he too felt that post-reproduction is strongly linked to somatic growth.

Â Ms. MacLellan noted that for most groundfish species, if we considered gonad development beginning in September with spawning resulting around January to March, it made sense that somatic growth should occur afterwards. This could explain why she sometimes noticed very little growth occurring as late as June or July and suddenly saw large amounts of growth in August. However, this is difficult to assess because at their lab, reader ageing assignments jumps from species to species, month to month, and year to year.

Â Mr. Kastle directed the discussion back to the memo from Mark Wilkins where it referred to age validation of rockfish and thornyheads. He asked John Butler if he had knowledge of other studies on thornyheads. Mr. Butler said he knew of a graduate student at the Moss Landing Lab who is doing validation studies of longspine and some shortspine thornyheads as part of her master's thesis due in June. He added that with his work on them, there were still a lot of

questions in his mind about the correct ageing criteria for these fish. He also had some questions concerning the choice of ageing structure.

Ms. Goetz pointed out that Vicki Poage, a former AFSC age reader, studied several possible ageing structures and eventually eliminated the use of opercles and fin rays. This brought it back to the otolith. Mr. Butler said that the otolith may be the structure of choice but a valid set of criteria for thornyheads needed to be identified.

Ms.

Ms. Goetz continued by presenting a project just begun at AFSC in conjunction with Dan Ito and Julie Pearce of the AFSC rockfish working group. They plan to compare scales and otoliths of young shortspine thornyheads to try to identify characteristics which might lead to some ageing criteria for the younger fish.

Mr. Kastelle added that Mr. Ito had obtained funding for a radiochemical validation study for shortspine thornyheads and other rockfish species.

This led to a discussion over the importance of radiochemical validation studies and the possibilities of a NMFS sponsored lab for the sole purpose of age validation. The idea was to create a central lab where all West Coast ageing units could send samples for age verification of their species of interest. Mr. Butler wanted to emphasize the importance that this lab functions solely for age validation and working only with the necessary isotopes to reduce the possibility of contamination. The working levels of these isotopes are so minute that cleanliness is crucial. As an example, Mr. Kastelle pointed out there was probably a higher level of radium in the soles of our shoes than in 1 gram of otoliths.

Mr. Kastelle mentioned that in searching for a lab to do the radioisotope studies for Dan Ito, they discovered a lab within NOAA's Building 3. This lab is not in use currently and already housed some of the necessary equipment. A request was made through proper channels for the use of this lab. But the request was denied for various reasons.

The discussion turned to the cost of such a set-up. Mr. Kastelle and Mr. Butler estimated that it would be approximately \$30,000 - \$40,000. Someone pointed out this is less than the cost of a SEM and less than the cost to fund a survey cruise. The discussion was concluded by adding this to the list of recommendations to TSC.

Returning to a second point from Mark Wilkins' memo, Mr. Kastelle opened discussion on FDA concerns over the use of OTC in tagged fish studies. The problem was in the possibility of an injected fish ending up in the market place. Mr. Blood began by saying that before he did his work with OTC fish injections he did a literature research on the historical use of OTC. He found some work done in aquaculture where OTC treatment was allowed with the requirement that these fish could not be harvested earlier than 21 days after treatment. So when IPHC did their tagging studies they made sure they released their fish in areas away from possibly entering the sport fishery at least 21 days before a commercial opening in the vicinity.

To explain the FDA's concern over this matter, Mr. Butler thought this could be traced to the problems in the poultry industry where OTC was used in treating chickens. Later, it was discovered this resulted in OTC resistant strains of the Salmonella bacteria. Mr. Butler further pointed out there are other dyes that could be used in place of OTC. These dyes were proven to be just as useful, and may require less approval requirements with the FDA. He mentioned the use of strontium chloride (absorbed instantaneously), calcine and alizarine compounds. John Gunn from the CSIRO lab in Hobart, Tasmania did some work with strontium chloride and is due to publish a paper on this soon. Tsukamoto (1988) reported successful use of alizarine and illustrated this with colored pictures.

Someone asked how OTC became the dye of choice. Mr. Blood thought perhaps it's history goes back to Kobayashi's paper in the 1960s which described the potential use of OTC in otolith marking. Everyone has used it since. This discussion concluded with a suggestion for a recommendation to TSC on the use of other dye products for future tagging studies to alleviate the problems with the FDA.

The final item on the Mark Wilkins' memo was age validation of Dover sole and arrowtooth flounder. But, as pointed out in the memo, this was put on hold due to the cancellation of the 1992 West Coast flatfish survey. This concluded the discussion on the Mark Wilkins' memo.

The next topic was OTC use and application. Mr. Kastelle voiced regrets that the two members who wanted most to address this topic, Bill Barss (ODFG) and Don Pearson (SWFC-Tiburón), were not able to attend this conference. However, Mr. Kastelle and Mr. Blood were able to put together an impromptu demonstration on the equipment and techniques used in OTC tagging work. Mr. Blood's equipment included an 18-gauge needle connected to a dark bottle by a flexible surgical tubing used on large halibut. He stressed the importance of housing the OTC solution in a dark bottle. He pointed out the difficulties and potential dangers involved in using this needle on a large, flopping fish aboard a moving vessel at sea. It is important to inject the OTC into the peritoneal cavity of the fish because of the large volume necessary (it actually causes a distention of the abdominal region). Also, OTC injected into the flesh region causes discoloration of the fish meat, an unappealing quality should it end up in the food stores.

Mr. Kastelle demonstrated a similar set-up used for sablefish. The bottle and tubing were smaller but the needle was bigger. He pointed out that the needle size must be sufficiently large due to the highly viscous nature of the OTC solution.

It was emphasized that the time of year for doing the injections was thought to be crucial. You wanted it available during the period of fast growth allowing it to be incorporated into the otolith. Mr. Blood said that for halibut it would be between July to September.

Mr. Blood pointed out that as far as reading the OTC marked otoliths, there are some problems with otoliths autofluorescing. This could lead to misreading the OTC mark. Ms. MacLellan concurred and described studies where some people believed OTC did not lay down marks on salmon scales. To verify this, Ms. MacLellan was asked to identify the OTC mark on a mixture of tagged and untagged (control) salmon otoliths, fins and scales. She was able to discern the OTC mark on scales with difficulty, but it was possible if one knew what kind of mark to look for. It is important that experienced people be involved with OTC mark studies.

The next agenda item was the selection of a new chairperson and vice-chairperson. The next TSC meeting will be in California so it was suggested that one of the newly elected persons be from California. It was also suggested that the other elected person be from Washington. CARE meetings were traditionally held in Seattle because of its central location on the West Coast and is the site of 3 member agencies, AFSC, WDF, IPHC. A person from Washington could facilitate the preparation for the next meeting. Mr. Kastelle called for volunteers. Mr. Butler and Mr. Blood volunteered. Others wanted to think about it so Mr. Kastelle suspended further discussion on this matter until Thursday or Friday.

Pete Hagen (ADFG) asked if anyone has ever addressed validation work studying the El Nino influence on growth increment. Ms. MacLellan said she had done some literature research and otolith studies on this question with Mark Saunders (DFO) on hake. Ms. Goetz confirmed the presence of a natural mark in the 1977 hake year-class. In addition, she noted that since a strong year-class of 3- to 4-year-olds (in 1991) is coming into the fishery, it will be interesting to observe possible impacts of the current strong El Nino event on growth patterns of these fish. Ms. MacLellan added that she sees a growing interest in documenting otolith and scale growth patterns leading to the use of digitizing and computerized image analysis equipment. She pointed out the usefulness of these image analysis systems in storing the data.

This evolved into a technical discussion on the usefulness of these computer systems and the variety of hardware and software packages now available. Many fisheries agencies along the West Coast already had these systems on line or will be getting them in the near future. Mr. Butler felt that we hear so much talk about these systems, but few fully understand the extent of their usefulness or understand the differences between them. He proposed CARE set up a special study of these systems. He was concerned that some people not familiar with ageing methods think that these machines could replace humans in production ageing. Mr. Kastelle suggested that these systems are useful as a data-gathering tool, but will always require a skilled age reader to oversee any attempt at "ageing".

Mr. Kastelle directed attention to last year's TSC recommendations for CARE, reading from page 113 of the 1991 TSC minutes. In summary TSC encouraged CARE to solicit participation from the SWFC La Jolla lab. This resulted in John Butler's participation at this meeting. TSC recommended more hands-on and regional workshops. It encouraged more communication between agencies on validation work and looking into biostatistical methods, Pb-210/Ra-226 studies and lipofuscin. Mr. Butler enlightened everyone on the definition of lipofuscin. These are spots that develop on the body as a result of growing old. This happens to humans as well as fish. There was a study which suggested an ageing method for euphausiids by measuring their lipid spots. Some people thought the same could be done for fish, but subsequent studies showed inconclusive results.

A break was called at 3:20 p.m.

The afternoon session was reconvened about 3:40 p.m. The discussion returned to the ageing manual. Ms. MacLellan had photos spread out on the table and encouraged everyone to review them because xerox copies would not replicate the quality of the photos. These photos were selected to illustrate the points outlined in the draft of the ageing manual. Again she asked for group input on the manual such as suggestions on which direction the group wished to pursue as far as the final form of the manual, what kind of publication to gear toward, at what quantities, and quality of photo reproductions.

The first conference day was called to conclusion at 4:20 p.m.

Thursday, May 28, 1992

PRESENTATIONS

Â The second day of the conference was called to order by Mr. Kastle at 8:20 a.m. The morning was scheduled for presentations.

Â The first presentation was by John Mello on the results of an age validation study on chilipepper rockfish. The study is summarized in a paper titled Validation of Otolith Annuli for Use in Age Determination of Chilipepper *Sebastes goodei*. This study involved monthly collections of chilipepper otoliths in 1987. All the otoliths were pooled together and a reader recorded the outer edge growth as either opaque, translucent, or unknown for each otolith. To avoid biasing the reader, he was not given any information on the fish or the month caught. The data resulted in a histogram showing the frequency of occurrence of opaque edges (rapid growth period) is over 50% from May through December. The study suggested the seasonality of opaque and translucent zone formation for chilipepper rockfish and that they are deposited once a year.Â Mr. Mello believed he could identify the 82-83 El Nino effect on the otolith pattern which produced a distinctively darker annulus. The following year's growth increment also appeared wider. An apparent effect of El Nino in the fishery seemed to indicate fewer new fish in 1983 and a year class increase in 1984.

Â The second speaker was Joan Forsberg from the IPHC. Her study is summarized in a paper titled Estimating Sex of Pacific Halibut (*Hippoglossus stenolepis*) Using Fourier Shape Analysis being reviewed for publication. The study was attempted because otoliths sampled from the commercial fisheries arrived at the IPHC labs without sex information. Fish sex information is needed for proper stock management. Age readers given otoliths with sex information observed some differences between female and male otoliths of the same age-class. There were size and weight differences where the females were generally bigger than the males. The shape of the male otolith tended to be thicker and more elongated while the female shape was broader towards the posterior lobe. Also in older males, the annuli tended to be more closely spaced toward the edge. Simple visual examination of otoliths to predict sex was inadequate for use in current stock models, but gave promising results. The researchers hoped that better classification rates could be achieved using a mathematical technique, which could pick up differences too subtle for the human eye to detect. The technique used was Fourier shape analysis; a method that converts a shape outline (computer-digitized otolith outlines in this case) into a series of coefficients that can be compared numerically. For this preliminary study, a single age group (11-year-olds) of halibut otoliths was used. One hundred forty four left side sagittal otoliths (74 male, 70 female) were digitized and the data was divided into "training" and "test" sets for linear discriminant function analysis. The training set used the shape information from all the otoliths of the 2 sexes and generated 2 discriminant functions; one for each sex. The test data set used the discriminant functions to classify by sex. Successful classification rates ranged between 79% to 91% for the training set and 63% to 65% for the test set. When otolith weights were incorporated into the analysis, successful classification rates range increased to 82% -87% for the training sets and 71% - 73% for the test sets. From these results, it was concluded that otolith shape alone was not a reliable enough indicator to predict sex in the Pacific halibut.

Â The third speaker was Craig Kastle who presented a radioisotope age validation study on sablefish, which was part of the work required for his mastersâ€™ thesis. It is summarized in a paper titled Using Pb-210/Ra-226 Disequilibrium for Sablefish (*Anoplopoma fimbria*) Age Validation in review for publication. This study was based on the fact that when fish take in calcium for bone growth, radium accompanies calcium in the process. This is because radium is a calcium analog, which is naturally occurring in seawater. Three assumptions in this study were that radium: (1) is selectively incorporated in the bone; (2) locked in a closed-system structure along with its daughter products and; (3) its input rate is constant in the parts of the bone analyzed.

Â Mr. Kastle began his discussion with a brief summary of the decay chain from Ra-226 to Pb-210. The ratio of Pb/Ra is a function of time and is known. Therefore, it is possible to use the Pb:Ra ratio measured in the otolith core (the section of the otolith found inside the first year annulus) with the known function to estimate the age of the fish. This study involved measuring levels of the isotopes Ra-226 and Pb-210 in the core of fish otoliths. To do this, Mr. Kastle needed a minimum of one gram of otolith material. To obtain this much material with the samples he had available, he pooled the otoliths into 4 age groups. The first group consisted of all 1-year-olds, the second group consisted of 9- to 11-year-olds, the third group consisted of 14- to 23-years-old, and the fourth group consisted of fish 24- to 34-years-old. Fish in the first and second group were aged by only one reader but fish from groups 3 and 4 were aged by 3 experienced age readers and an average age per reader was taken. Mr. Kastle said he used the ratio of Pb-210:Ra-226 because this was appropriate for the lifespan of sablefish. Other isotope pair ratios may be more appropriate for other species, especially the shorter-lived fish.

Â Â Â Â Â Â Â Â Â A break was called at 10:35 a.m.

Â The next speaker was John Butler who presented results from an Age Determination of Shortspine Thornyhead, *Sebastobus alascanus*, Using Otolith Sections and 210Pb:226Ra Ratios. His results will be written up into a paper upon his return to La Jolla. Mr. Butler began this study because of growing commercial importance of the thornyheads in California. To manage the fisheries properly, users needed to know how old these fish get. Mr. Butler was assigned the task of finding this out. Mr. Butler started his ageing experience by looking at various fish structures including opercles and scales but decided to use the otoliths. He began by counting all the reasonable bands. This resulted in ages up to 150-years-old. Then he went back, using the same otoliths, and decided to do more banding. This resulted in another set of data ranging in ages up to around 80 years. To validate his ages he did a radioisotope study with the otoliths. The

resulting ages appeared to indicate the second criteria (leaning toward younger ages) might be more appropriate to ageing this species. However, he believed these to be preliminary results and needs more work especially in the study of radium and the use of ratios. In addition to his results, Mr. Butler briefly talked about another radioisotope age validation study on *Sebastes rufus*. A graduate student as part of her masters' thesis is doing this work at the Moss Landing lab. Results such as these led Mr. Butler to believe in the usefulness of radiochemistry for age verification. He would like to see NMFS support such a lab. He reiterated the importance of having a lab solely for fish age validation to minimize contamination from unwanted isotopes. He pointed out another advantage to one central lab is by having methods and equipment standardized for all agencies.

The final presentation was from Larry Quirollo on the Dover sole ageing workshop between CDFG and ODFG. Mr. Quirollo regretfully mentioned that his talk required slides, which the Oregon group was supposed to bring. Since they could not attend, he passed out copies of a memo to himself from Bob Mikus (ODFG) summarizing the meeting (Appendix VI). The subject title of the memo was Report on CDFG/ODFW Dover Sole Aging Workshop 04-14-92 through 04-16-92.

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Â IMAGE (VIDEO) STORAGE SYSTEMS

After the presentations concluded, discussion began on the use of cameras and video recorders for storing images. Mr. Kastle asked each agency what equipment they used. Ms. Munk (ADFG) said they have a video printer for printing images. DFO uses a simple set-up with a 35mm camera mounted on a photo tube over a microscope. ODFG was thought to have a computerized system with a high-resolution camera mounted on a photo tube. No one recalled the name of the system. AFSC ageing lab uses a computerized Wild Photoautomat with a 35mm camera on a photo tube. The AFSC larval fish group has a high-resolution video camera and recorder. This was followed by discussions on the more sophisticated, computerized image analysis systems. Mr. Butler pointed out there is a choice between IBM and Macintosh hardware. Multiple kinds of software are available for each of the two types of hardware. He happened to be using McIntosh mainly because the software is available and free. He suggested that CARE evaluate the utility, resolution, and the ease of communicating between agencies using different systems. Â

Â AGE VALIDATION AND OTHER SPECIAL PROJECTS

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Ms. Goetz introduced the topic of current and future age validation projects. Julie Pearce began by outlining a project, in the beginning stages, involving the AFSC rockfish working group and the age lab. The project is devoted to age validation of rockfish species. The rockfish working group have collected scales, fin rays, otoliths and opercles from 79 specimens of shortspine thornyheads with an average length of 23 cm. Ms. Pearce asked for group suggestions that may be incorporated into their strategy. Mr. Butler suggested they weigh the otoliths. He pointed out that for the California and Oregon samples, they found it sometimes difficult to separate the shortspine and longspine thornyheads just by physically examining the fish. However, they found that there exists a distinct difference in the otolith weight to fish length relationship between the species. He didn't know how far north the longspine thornyhead species extends up the coast and he cautioned that this relationship for the Alaskan stocks should first be verified.

Ms. Goetz added it is their intention to compare scales against the otoliths of the shortspine thornyhead to try to interpret the pattern in the younger years.

Ms. Goetz discussed another study the AFSC age lab would like to pursue involving the verification of some 0- to 1-year-old Pollock. A problem occurred when readers aged a certain group of fish 0 taking into consideration the time of year and no visible annulus. Some users complained because the reported length of the fish indicated to them that these fish should be 1-year-olds. To verify the age, Ms. Goetz planned to do a daily growth study with the help of the larval fish group who is experienced in doing daily increment ageing. Using an eyepiece micrometer, AFSC is currently measuring the size of the first year on clear otolith surfaces of fish aged 2, which were collected the following year. These measurements will help identify the size range of 0- to 1-year-old Pollock otoliths.

Ms. MacLellan said that from what Pollock she had seen, there had been a variety of sizes of first year growth. Ms. Goetz commented that sometimes it is difficult to convince users of this. She further added that data exists documenting a very wide range of spawning dates (up to as late as September/October) in the Bering Sea.

Mr. Kastle spoke of the upcoming study at AFSC documenting marginal increments on the juvenile sablefish collected by the Auke Bay lab mentioned earlier in the meeting. There is also a radioisotope study planned for the various rockfish species.

Kent Scott (AFSC) outlined a study investigating alternate ageing methods for rex sole. The otolith break-and-burn is difficult to interpret due to faint annuli formation so he is exploring other methods of preparing the otoliths to define the

annuli more clearly. One method involved the use of EDTA to etch out the opaque zones thereby highlighting the translucent zones. Other possibilities include looking at thin sections and various dye treatments.

Delsa Anderl (AFSC) discussed a validation study on atka mackerel. There is currently an ageing practice which users would like verified involving the deposition of the first clear annulus on the otolith. It was documented in old Russian literature that atka mackerel are late spawners, leading to the belief that young fish do not lay down an annulus during their first winter. AFSC age readers report to the users ages that only account for the number of annuli they see on the otolith. The users, for their stock assessment calculations, add a year to the reported ages to account for the late spawning. The first attempt at verification had been to prepare otolith thin sections from supposed 1-year-old fish to count daily growth increments up to the first annulus using a compound microscope. If necessary, using the SEM was planned. In the spirit of inter-agency assistance, Mr. Butler said that he currently has a technician preparing Dover sole otoliths for the SEM. He did not foresee a problem of adding in an atka mackerel otolith. Ms. Anderl said she appreciated the offer and would surely take him up on it.

Julie Lyons mentioned that users want age data from arrowtooth flounder but this is a difficult species to age using the otoliths. So, the age unit is looking into other structures for ageing. Kristen Munk mentioned two studies she would like to pursue in the future. One study is to correlate environmental variables such as El Nino cycles or sea surface temperatures to the strong patterns evident in some Southeast Alaska Yelloweye (*S. ruberrimus*) stocks. Another study is to look at possible stock separation using these patterns. This idea evolved from a recent observation of markedly different patterns between 2 separate otolith batches. She later discovered there had been a break in the time between sampling of the two batches corresponding to two very different areas. Digitizing the patterns (using image-processing software) and microprobe analysis of the Sr:Ca ratio may be used to evaluate patterns in both studies.

This brought the discussion back to the various hardware and software set-ups in image analysis equipment and the potential problems of transmitting images for exchange work between agencies using different systems. The group felt that perhaps a standard imaging system should be investigated and it was reiterated that this be a recommendation to TSC.

The morning session was adjourned for lunch at 12:05 p.m.

HANDS-ON SESSION

The afternoon session began at 1:45. Microscopes were available and the afternoon was devoted to hands-on work. The following list of species was available for viewing and ageing techniques were discussed between agency members.

1. Dover sole - provided by CDFG
2. Rougheye rockfish - provided by AFSC
3. Sablefish - provided by AFSC
4. Jack mackerel - provided by SWFC
5. SS Thornyheads - provided by SWFC and AFSC
6. LS Thornyheads - provided by SWFC
7. Chilipepper rockfish - provided by CDFG
8. Ling cod - provided by ADFG
9. Pollock - Polish samples aged by Poland, DFO, AFSC

Prior to the conference, AFSC compiled 22 sablefish otoliths from a batch (currently being aged) collected along the Aleutian Islands. In this batch were some otoliths with patterns common to this collection but uncommon to otoliths seen from other areas. They tended to be more difficult to interpret. These otoliths were exchanged between 4 different agencies (AFSC, SWFC, ADFG and DFO) and aged by 5 experienced readers. Resolution of the discrepancies between the age readers present at this conference took place at this time.

Kristen Munk received a request from ADF&G Groundfish Management to age lingcod. She will compare ageing potentials between lingcod otoliths and fin spines to decide which is the better structure for these northern stocks. Prior to this conference a preliminary evaluation of three Alaska stock lingcod otoliths indicated that these might be reliable structures to age.

John Butler presented a collection of shortspine thornyhead otoliths prepared into thin sections. He had never seen break-and-burn otoliths from this species before. AFSC had some stored from previous ageing attempts and showed them to John for comparison. With the break-and-burns he was able to identify the patterns he used in ageing with thin sections. Because it is simpler and faster, John was considering using the break-and-burn as a future ageing method.

With the chilipepper rockfish otoliths John Mello showed members how he had analyzed for edge growth and also pointed out the annuli, which he believed were affected by the 82-83 El Nino.

The members began dispersing around 5:00 p.m.

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Everyone was present around 8:15 and informal, small group discussions were occurring. Mr. Kastle produced a draft form of the four recommendations so far discussed at this conference to be sent to TSC. He asked it be passed around to the different members for comments and revisions. Hands-on microscope work also took place with the fore-mentioned samples.

Mr. Kastle called the meeting to order at 9:50 a.m.

APPROVAL OF NEW CHAIRPERSON AND VICE-CHAIRPERSON

Mr. Kastle announced that John Butler volunteered to chair the next CARE meeting and Betty Goetz volunteered to vice-chair. The group approved this.

CARE RECOMMENDATIONS TO TSC

Mr. Kastle directed attention to the list of recommendations, which had various comments, added to it. With group input, he made further revisions. A summary of the recommendations is in Appendix VII.

The group approved a recommendation that CARE hold a meeting in the spring of 1993 solely for comparing the different image analysis systems (hardware and software) currently available. Ms. MacLellan suggested that we as age readers should have more interactions on a regular basis, or as needed, to maintain inter-agency calibration. We needed more sharing of ideas especially among the key species. Someone suggested that this be a recommendation of CARE to itself.

FINAL COMMENTS

Mr. Blood suggested that CARE consider meeting annually as opposed to the current schedule of meeting every two years. He found these three days highly informative and worthwhile. He felt members might benefit more from annual meetings. Some members indicated agreement. Dr. Kimura reserved the right to think about it. His concern was the time required preparing for these meetings. It can cut into production schedules.

Mr. Butler asked about funding sources for travel to these meetings. Mr. Kastle answered that PSMFC does allow funds for one member of each agency, except DFO, to attend.

Wrapping up discussions on the ageing manual, Mr. Kastle called for volunteers to represent their agencies in finishing the document within the next few months. Ms. MacLellan had already been appointed editor. Volunteers included: Betty Goetz (AFSC), Larry Quirollo (CDFG), Cal Blood (IPHC), Kristen Munk (ADFG), and John Butler (SWFC). Ms. Goetz mentioned that Bill Barss, who was not present, might want to participate, as he was involved with the manual since the first CARE meeting. Mr. Quirollo said he would talk to Mr. Barss about it when he meets him in June.

The 1992 CARE conference was called to officially close at 11:00 a.m.