# CSIRO DIVISION OF FISHERIES RESEARCH FINAL REPORT FOR FIRC

Direct otolithic ageing of southern bluefin tuna, *Thunnus maccoyii* (Castlenau), exploited by the Australian fishery.

#### FIRTA 84/71

This two year project commenced in January 1985 with the following objectives:

To validate otolithic ageing techniques developed by CSIRO for southern bluefin tuna. To determine the timing and periodicity of otolithic check formation and hence the growth season of southern bluefin tuna at different ages and in different geographical areas.

To satisfy these objectives it was proposed to collect otoliths from fish caught in the three areas of the Australian fishery, i.e., Western Australia, South Australia and New South Wales. In the first year of the project otoliths had been collected from 689 fish from South Australia and 529 fish from Western Australia. These samples represent a complete time series fro the 1984/85 and 1985/86 fishing seasons. No otoliths were collected from New South Wales due to the lack of tuna caught there. A key part of the study was the collection of otoliths from recaptured fish that had been marked with oxytetracycline during a previous tagging program carried out by CSIRO. A total of 31 fish were recovered that had previously been marked in this way. Validation of otolithic ageing was to be achieved by two methods: (1) Examination of oxytetracycline marks on the otoliths of recaptured tagged fish. (2) Measurement of the marginal increment on the otoliths of fish sampled throughout the year.

# Oxytetracycline marking.

When tetracycline is injected into a fish, it quickly becomes incorporated in bones, otoliths etc. and, when these structures are subsequently examined under UV light, a fluorescing band will appear corresponding to growth immediately after tagging. The

position of these marks on hard parts corresponds to the time of tagging. The position of the time mark on the otoliths of recaptured fish can be used to validate ageing based on otolith sub-structure. The time mark can be used to determine when checks are laid down and whether these occur anually or more frequently. Tetracycline marking is thus an accurate technique for validating methods of age determination and was an integral part of the current study.

Otoliths from the 31 recaptured fish that were marked with oxytetracycline failed to show any flourescence. These were examined initially using an Oliphant longwave magnifying flourescent lamp and subsequently using a Zeiss fluorescence microscope. The light source for the latter was a 50 watt mercury burner which was used in conjunction with a G436 exciter filter and an LP520 barrier filter. These techniques should have revealed flourescence from the tetracycline if it had been present. All precautions, such as storing otoliths in darkness, were taken with the biological material when it was obtained. We have sent some of these otoliths to the National Marine Fisheries Service in California (where some success has been achieved with other species of tuna) to see whether they can detect flourescence in the otoliths, but we expect they will only confirm our findings.

We suspect that southern bluefin tuna were not given sufficient dosages of oxytetracycline to mark otoliths. Fish marked in Esperance and Albany in 1983 were injected with Oxytetracycline 100 which was only 10% oxytetracycline chloride, and it appears that dilutions and dosages used resulted in injecting about 1/10th of the amount required to produce a mark. Fish marked in South Australia were apparently given the correct dose as pure oxytetracycline was used and diluted appropriately. However, it was noted that there was considerable loss of the drug after injection, apparently due to muscular contractions forcing the liquid out of the tissue. This wastage may have been sufficient to render the dosage ineffective. With our prime method for validating otolithic ageing no longer possible, we then had to resort to a seasonal study of otolith marginal increments to validate ageing.

2

83

### Marginal increments.

The marginal increment is a measure of growth since the formation of the last check on a structure used for ageing. By measuring this increment on a sufficiently large number of fish taken at differnt times of the year and plotting these against the time of year should indicate if check formation occurs seasonally. One would expect small marginal increments immediately after checks have formed and large increments just prior to the period of check formation. A seasonal examination of marginal increments becomes less clear if one or more of the following situations occurs: (1) Check formation occurs over a protracted period. (2) There is considerable individual variation in the timing of its formation. (3) More than one check is formed each year. (4) The position of the check cannot be measured accurately.

It was found that the burning technique developed previously by CSIRO did not always produce bands. The checks, especially the first two, often appeared diffuse and faint. This meant that measurement of the marginal increment was subject to much error and seasonal patterns in marginal increments could not be clearly demonstrated. A number of techniques including sectioning, polishing, etching and producing acetate peels did not provide a simple and accurate method for determining the exact position of the last check and hence an accurate measurement of the marginal increment. The problem was not just limited to being unable to determine the position and size of the checks with acceptable accuracy. There has been considerable disagreement between the results of ageing of burned otoliths done prior to the present program and recent ageing carried out on the same otoliths (i.e. the number of checks present in an otolith did not agree between readings). Without validation, the unsubstantiated ageing of the present collection of otoliths would be inappropriate, and possibly misleading.

We feel at this point that accurate positioning of checks and measurement of marginal increments requires examination of otolith microstructure (daily banding). This work was initiated but not perfected, as it requires special equipment and the technique is

3

Sen

extremely time consuming. A study of otolith microstructure requires more development work than can be achieved with the available resources. We find it difficult to justify increased involvement in this project until more elaborate techniques are developed to accurately identify the position of checks and measure the marginal increment. We chose to terminate the programme at this stage and possibly restart it once these techniques have been developed satisfactorily.

4

I	0	1	Q			8	4	2			•	7	1	-
	U	Ŧ	v	٠	٠	÷	•	•	•	٠	٠	٠	٠	٠

- D NEW PROPOSAL
- CONTINUING PROJECT
- FINAL REPORT
- PROGRESS REPORT

# FISHING INDUSTRY RESEARCH TRUST ACCOUNT

TITLE OF PROPOSAL/PROJECT: Direct otolithic ageing of southern bluefin tuna, Thunnus maccoyii (Castlenau), exploited by the Australian fishery

ORGANISATION: CSIRO, Division of Fisheries Research

PERSON(S) RESPONSIBLE: Dr FR Harden Jones

FUNDS SOUGHT 'GRANTED

YEAR	SOUGHT	GRANTED \$35,544
1984/85		\$46,984
1985/86		

ATED APPLICATIONS:	
	· · · · · · · · · · · · · · · · · · ·
	2. The second s second second se second second sec second second sec
	and the second
	.)0
CEIVED 3.0 4 19.86	DISTRIBUTED

Secteda

Listing industry research Committee

F139 12-8

FINAL REPORT : PROJECT 84/71 APRIL 1986

#### 1. Title of project

Direct otolithic ageing of southern bluefin tuna, Thunnus maccoyii (Castlenau), exploited by the Australian fishery.

# 2. Person and Organization responsible for programme

Dr F.R. Harden Jones, Chief, CSIRO Division of Fisheries Research GPO Box 1538, Hobart, Tasmania 7001

#### 3. Project objectives

a. To validate otolithic ageing techniques developed by CSIRO for southern bluefin tuna.

b. To determine the timing and periodicity of otolithic check formation and hence the growth season of southern bluefin tuna at different ages and in different geographical areas.

#### 4. Staff employed on the Program

T.L.O. Davis, PhD, Senior Research Scientist K.J. McLoughlin, BSc, Experimental Scientist

# 5. Date project commenced and terminated

January 1985 - 31 January 1986

#### 6. Final report

To satisfy the objectives of this project, it was proposed to collect otoliths from fish caught in the three areas of the Australian fishery, i.e. Western Australia, South Australia and New South Wales. In the first year of the project, otoliths had been collected from 689 fish from South Australia and 529 fish from Western Australia. These samples represent a complete time series from the 1984/85 and 1985/86 fishing seasons. No otoliths were collected from New South Wales due to the lack of tuna caught there. A key part of the study was the collection of otoliths from recaptured fish that had been marked with oxytetracycline during a previous tagging programme carried out by CSIRO. A total of 31 fish were recovered that had previously been marked in this way. Validation of otolithic ageing was to be achieved by two methods: 1) Examination of oxytetracycline marks on the otoliths of recaptured tagged fish; 2) Measurement of the marginal increment on the otoliths of fish sampled throughout the year.

#### Oxytetracycline marking

When tetracycline is injected into a fish, it quickly becomes incorporated in bones, otoliths, etc. and, when these structures are subsequently examined under UV light, a fluorescing band will appear corresponding to growth immediately after tagging. The position of these marks on hard parts corresponds to the time of tagging. The position of the time mark on the otoliths of recaptured fish can be used to validate ageing based on otolith sub-structure. The time mark can be used to determine when checks are laid down and whether these occur annually or more frequently. Tetracycline marking is thus an accurate technique for validating methods of age determination and was an integral part of the current study.

Otoliths from the 31 recaptured fish that were marked with oxytetracycline failed to show any fluorescence. These were examined initially using an Oliphant longwave magnifying fluorescent lamp and subsequently using a Zeiss fluorescent The light source for the latter was a 50 watt microscope. mercury burner which was used in conjunction with a G436 exciter filter and an LP520 barrier filter. These techniques should have revealed fluorescence from the tetracycline if it had been present. All precautions, such as storing otoliths in darkness, were taken with the biological material when it was obtained. We have sent some of these otoliths to the National Marine Fisheries Service in California (where some success has been achieved with other species of tuna) to see whether they can detect fluorescence in the otoliths, but we expect they will only confirm our findings.

We suspect that southern bluefin tuna were not given sufficient dosages of oxytetracycline to mark otoliths. Fish marked in Esperance and Albany in 1983 were injected with Oxytetracycline 100 which was only 10% oxytetracycline chloride, and it appears that dilutions and dosages used resulted in injecting about 1/10th of the amount required to produce a mark. Fish marked in South Australia were apparently given the correct dose as pure oxytetracycline was used and diluted appropriately. However, it was noted that there was considerable loss of the drug after injection, apparently due to muscular contractions forcing the liquid out of the tissue. This wastage may have been sufficient to render the dosage ineffective. With our prime method for validating otolithic ageing no longer possible, we then had to resort to a seasonal study of otolith marginal increments to validate ageing.

#### Marginal increments

The marginal increment is a measure of growth since the formation of the last check on a structure used for ageing. By measuring this increment on a sufficiently large number of fish taken at different times of the year and plotting these against the time of year, should indicate if check formation occurs seasonally. One would expect small marginal increments immediately after checks have formed and large increments just prior to the period of check formation. A seasonal examination of marginal increments becomes less clear if one or more of the following situations occurs: 1) Check formation occurs over a protracted period; 2) There is considerable individual variation in the timing of its formation; 3) More than one check is formed each year; 4) The position of the check cannot be measured accurately.

It was found that the burning technique developed previously by CSIRO did not always produce bands. The checks, especially the first two, often appeared diffuse and faint. This meant that measurement of the marginal increment was subject to much error and seasonal patterns in marginal increments could not be clearly demonstrated. A number of techniques including sectioning, polishing, etching and producing acetate peels did not provide a simple and accurate method for determining the exact position of the last check and hence an accurate measurement of the marginal increment. The problem was not just limited to being unable to determine the position and size of the checks with acceptable accuracy. There has been considerable disagreement between the results of ageing of burned otoliths done prior to the present program and recent ageing carried out on the same otoliths (i.e. the number of checks present in an otolith did not agree between readings). Without validation, the unsubstantiated ageing of the present collection of otoliths would be inappropriate, and possibly misleading.

We feel at this point that accurate positioning of checks and measurement of marginal increments requires examination of otolith microstructure (daily banding). This work was initiated but not perfected, as it requires special equipment and the technique is extremely time consuming. A study of otolith microstructure requires more development work than can be achieved with the available resources. We find it difficult to justify increased involvement in this project until more elaborate techniques are developed to accurately identify the position of checks and measure the marginal increment. We chose to terminate the programme at this stage and possibly submit a new application once these techniques have been developed satisfactorily.