

Preliminary results of the empirical validation of daily increments in otoliths of jack mackerel *Trachurus symmetricus* (Ayres, 1855) marked with oxytetracycline*

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SUMMARY: The frequency of microincrement formation in sagittae otoliths of jack mackerel *Trachurus symmetricus* was validated using experiments on captive fish. Adult jack mackerel were injected with a dose of 100 mg of oxytetracycline/kg of fish. A second injection was performed 30 days later. The fish were then sacrificed and their sagittae otoliths were extracted. Thin sections of the otoliths were prepared and observed through an epifluorescent microscope using ultraviolet light. Two fluorescent marks corresponding to the two injections were clearly visible. The average number of microincrements between the two fluorescent marks was 29 (n=10; S.D.=1.63) and the median was 29.3. The Wilcoxon signed-rank test indicated that this value was not significantly different from 30. This result indicates that microincrements in otoliths of adult jack mackerel of between 28.4 and 37.7 cm fork length are formed with a daily frequency.

Key words: otolith, validation, microincrements, *Trachurus*, jack mackerel.

RESUMEN: RESULTADOS PRELIMINARES DE LA VALIDACIÓN EMPÍRICA DE LOS INCREMENTOS DIARIOS EN OTOLITOS DE JUREL *TRACHURUS SYMMETRICUS* (AYRES, 1855) MARCADO CON OXYTETRACICLINA. – La frecuencia de formación de los microincrementos en los otolitos sagittae del jurel chileno *Trachurus symmetricus* fue validado mediante un experimento en cautiverio. Ejemplares adultos fueron inyectados con una dosis de 100 mg de oxytetraciclina/kg de pez. Una segunda inyección fue aplicada 30 días después. Los peces fueron sacrificados y sus otolitos sagittae fueron extraídos. Se preparó secciones delgadas y se las observó bajo un microscopio de epifluorescencia. Dos marcas fluorescente fueron visibles las que corresponden a las dos inyecciones. EL número promedio de microincrementos entre las dos marcas fluorescentes fue de 29 (n=10; S.D.=1.63) y la mediana de 29.3. El test de Wilcoxon de rangos con signos indica que este valor no es significativamente diferente de 30. Este resultado indica que los microincrementos presentes en los otolitos de jurel, entre 28,4 y 37,7 cm de longitud horquilla son formados con una frecuencia diaria.

Palabras claves: otolito, validación, microincrementos, *Trachurus*, jurel.

INTRODUCTION

The discovery of daily growth microincrements in fish otoliths (Pannella, 1971) opened new possibilities

for studies on fish population dynamics through age and growth estimation (Methot and Kramer, 1979), duration of larval stages and hatching dates (Moksness and Fossum 1992), individual growth analysis (Moksness and Weststad 1989), and recruitment patterns based on age structure of juveniles (Methot, 1983).

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It has been suggested that the formation of daily microincrements is a universal character of fish (Campana and Neilson, 1985). Daily microincrement formation has been demonstrated for most fish species in which experiments in captivity have been performed (Campana and Neilson, 1985). Exceptions have occurred when fish were exposed to extreme light, temperature or feeding conditions (Lough *et al.*, 1982). Other reports of non-daily microincrement formation may be due to poor preparation of the otoliths, or the relatively low resolution of light microscopes (Neilson and Geen, 1982).

Various techniques have been used to test the frequency of microincrement formation. These include the culture of known-age eggs and larvae in the laboratory (Tanaka *et al.*, 1981; Jones and Brothers, 1987), changes in the average number of microincrements over a known time period during both field and laboratory experiments (Struhsaker and Uchiyama, 1976), and use of marks produced in the otolith by external factors (Campana, 1983) or chemical agents as reference points during otolith growth (Campana and Neilson, 1982). Chemicals used to mark otoliths may be administered by feeding, immersion or injection. Oxytetracycline (OTC) is one of the most widely used chemical markers due to its effectiveness in producing a mark that is easily visible with ultraviolet light (Brothers, 1990).

The jack mackerel, *Trachurus symmetricus*, is the most important fishery resource for the purse-seine fleet from northern and central-southern Chile (Arancibia *et al.*, 1995). Age estimates of the species have been based on both annual marks (Castillo and Arrizaga, 1987) and microincrements (Araya *et al.*, 1993, Araya *et al.*, 2001). Significant discrepancies between the results of these studies may be due to incorrect interpretation of the first annulus compounded by difficulties in correctly interpreting subsequent growth marks, particularly in larger individuals (Kochkin, 1994).

In order to help in this problem and generate accurate age estimates of adult *T. symmetricus*, this study is a preliminary attempt to validate experimentally the frequency of microincrement formation in the *sagittae* otoliths using oxytetracycline as a marker. Although the microincrement technique has been applied to larvae of *T. symmetricus* from the coast of California (Hewitt *et al.*, 1985) and *T. declivis* from Tasmanian waters (Jordan, 1994), this is the first study applying the technique to adult *T. symmetricus*.

MATERIALS AND METHODS

The experiment was performed in a pool with a surface area of 100 m² and depths ranging from 1.2 to 2.2 m located in Iquique (20°14'S; 70°08'W). Live jack mackerel were collected by the purse-seine vessel "Towerkoop" in October 1992 and maintained on board in 500-litre basins supplied with a constant flow of air and fresh seawater until being transferred to the experimental pool. The fish were acclimatised to conditions in the pool for a period of fifteen days. The mean sea water temperature in the pool was 18.7°C (range 16.5-20°C), and the mean salinity was 34.92 UPS. During both the acclimation and subsequent experimental periods, the fish were fed twice a day with fresh meat of anchovy (*Engraulis ringens*) and Pacific sardine (*Sardinops sagax*). After acclimation, 42 individuals were marked with a dose of 100 mg of OTC per kg of fish, injected directly into the abdominal cavity (Villavicencio de Muck, 1989; Brothers, 1990). The dose was repeated 30 days later to induce a second mark. In the jack mackerel fisheries in northern Chile, only specimens in the size range between 25 to 35 cm fork length appeared during the research period, so this was the range considered in this research.

Twenty days after the second injection, the fish were sacrificed, their length and weight were recorded, and the *sagittae* otoliths were extracted. The otoliths were prepared for microscopic examination using the method described by Brothers *et al.* (1976) and Campana and Neilson (1985). Because there is no difference between the left and right otoliths (Araya *et al.*, 1993), only the right otolith was used for the preparation and observation. Thin sections of the frontal plane were made by embedding otolith blocks of polyester resin, grinding and polishing them to the frontal plane using a Buehler-Ecomet 2®, with a graded series of aluminium oxide compounds (400, 600, and 800 grit) and abrasives (1500 grades), and finishing with Brasso® liquid metal cleaner. The polished surface was attached to a microscope slide with thermoplastic cement. The procedure was repeated from the other end of the block, leaving a thin section of between 200 and 300 µm.

The fluorescent marks in the otolith were observed with an epifluorescent microscope equipped with filters of 450-490 nm. The otolith preparations were photographed at magnifications of 400X and 1000X using both transmitted and UV light. The numbers of microincrements between the

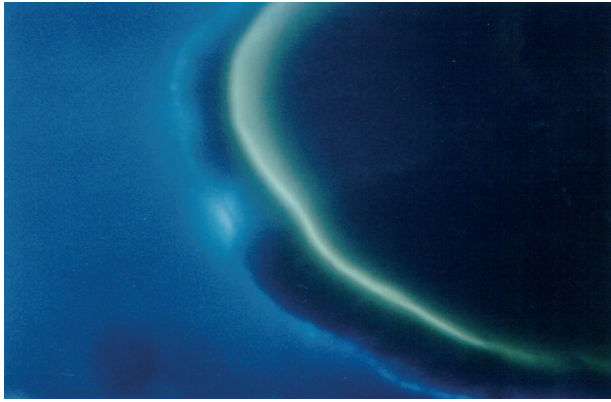


FIG. 1. – Photomicrograph frontal plane of sagittal otolith (postrostrum) of an adult jack mackerel injected once with tetracycline. The fluorescent mark corresponding to the injection is clearly visible, while no autofluorescence at the edge of the otolith is apparent. Scale 1 cm = 56.4 μ m.

two fluorescent marks were counted from photographs. Two non-consecutive readings of each specimen were conducted, and the average was calculated. If the difference between the two counts was greater than three increments, a third and in some cases a fourth reading was conducted. If the difference persisted, the otolith was discarded. To ensure that potential autofluorescence at the otolith edge would not interfere with the examination, thin sections of fish injected only once with OTC were obtained and prepared; these fish died during the experiment before the second injection. These otoliths displayed a single fluorescent mark that did not correspond to the edge of the otolith (Fig. 1).

RESULTS

During the three days after the first injection, 21 individuals survived out of the original 42, with only 14 individuals surviving to the end of the experiment. These mortalities were attributed to stress resulting from capture, transport and the marking procedure. From the 14 otoliths that were prepared for examination, 10 specimens showing two clear fluorescent marks were prepared satisfactorily. The remaining 4 specimens were discarded because it was not possible to observe microincrements in these preparation or the readings differed by more than three microincrements.

Counts of the microincrement between the fluorescent marks in the otoliths from these ten fish are shown in Table 1. The average number of microincrements between the marks was 29 (s.d.=1.63) and the median was 29.3. The Wilcoxon signed-rank test

TABLE 1. – Length, weight and increments numbers of two counts of *T. symmetricus* otoliths maintained in captivity.

Fork Length (cm)	Weight (gr)	Increments	
		1°	2°
27.4	279.5	-	-
28.4	325	27	28
28.9	320.4	-	-
29.1	331.6	31	30
30	302	26	27
30	355.3	-	-
30.5	382.5	29	29
30.8	393.5	31	30
30.9	375.5	29	30
31	409.7	-	-
32.4	476.5	27	27
32.8	437.6	28	29
32.9	481.5	30	29
37.7	439.9	32	31

demonstrated that this value is not significantly different from 30 ($P>0.1$), corresponding to the time between the two injections (Fig. 2).

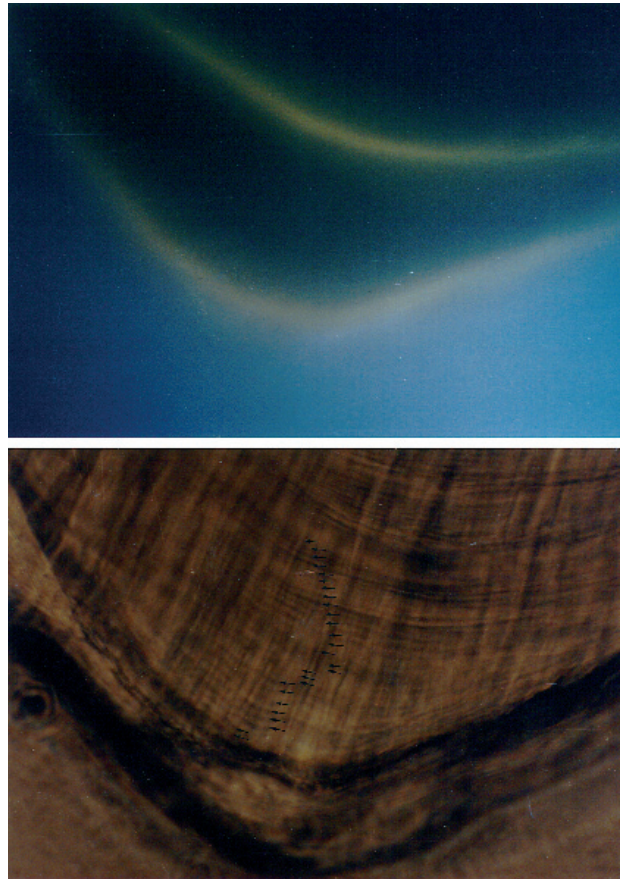


FIG. 2. – Photomicrograph frontal plane of sagittal otolith (postrostrum) of an adult *T. symmetricus* injected twice (30 days between the injections) with oxytetracycline. (A) Illuminated with ultraviolet light; (B) illuminated with normal transmitted light. 30 microincrements are visible between the two fluorescent marks. Scale 1 cm = 30.2 μ m.

DISCUSSION

The relatively high mortality rate we observed during the course of the experiment (only 14 of the original 42 fish surviving to the end of the experiment) limits the scope of our results to a certain extent. Considering that experiments of this nature require clear marks in the otoliths with minimal associated mortalities, the selection of OTC as a chemical agent to mark the otoliths of *T. symmetricus* was based on documented evidence (Villavicencio de Muck, 1989).

However, McFarlane and Beamish (1987) have reported that incorrect dosages of OTC administered by means of injection to mark otoliths of *Anoplopoma fimbria* from the coast of Vancouver Island did result in some mortalities. Selection of a suitable dose is consequently crucial to minimise mortality and maximise the number of OTC-marked fish. In accordance with the results of McFarlane and Beamish (1987), the dose we administered during the experiment should be appropriate for *T. symmetricus*. The mortalities we observed are probably a result of the stress of captivity and handling during the marking procedure, rather than a toxic response to the OTC.

Our results support the hypothesis that microincrements are deposited with a daily frequency in *sagittae* otoliths of adult *T. symmetricus* (28.4-37.7 cm fork length) from northern Chile. This conclusion cannot be extrapolated to fish larger than those used in this study because daily microincrement formation may cease in older fish, or the microincrements may not be detectable using an optical microscope (Pannella, 1971). In addition, more work is necessary to validate the method.

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