IRREGULAR MICROINCREMENT DEPOSITON ON THE OTOLITHS OF SKIPJACK TUNA (*KATSUWONUS PELAMIS*) FROM THE MALDIVES

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ABSTRACT

The rate of microincrement deposition on otoliths of skipjack tuna (Katsuwonus pelamis) from Maldives was studied using injections of a fluorescent marker (tetracycline) in tagged fish. The number of increments was counted on transverse sections of otoliths from recaptured skipjack, between the fluorescent mark and the outer edge of the otolith. By comparing the number of increments with the number of days at liberty, it was concluded that on average one microincrement was formed every 2.3 days. The frequency of microincrement deposition varied between individual fish, so the number of increments on otoliths cannot be used for age determination of skipjack tuna.

INTRODUCTION

The Maldives has a large traditional pole-and-line tuna fishery. Skipjack tuna (*Katsuwonus pelamis*) is the main target, accounting for some 70% of the total national catch. Skipjack tuna is the major source of protein for the Maldivian people. In addition, the skipjack fishery provides a major source of employment and a major source of export earnings.

It is believed that skipjack caught in the Maldives are part of an Indian Ocean stock. There is increasing concern that Maldivian tuna catches may be adversely affected by the growing catches of skipjack tuna being made elsewhere in the Indian Ocean. There is particular concern about the disastrous consequences for the Maldives that would result if the Indian Ocean skipjack tuna stock collapsed as a result of overfishing. To date there has been no comprehensive stock assessment of Indian Ocean skipjack. A prerequisite for such a stock assessment would be a sound understanding of Indian Ocean skipjack growth rates. The study of 'daily rings' in otoliths (Panella, 1971 and 1974) offers perhaps the best method for elucidating fish growth rates, provided that the periodic nature of the rings is properly validated.

The aim of this study was to test the periodic nature of microstructures in Maldivian skipjack tuna otoliths, as a first step towards determining growth rates. Skipjack tunas were injected with tetracycline during the course of a tuna-tagging programme (Waheed and Anderson, 1994; Anderson, Adam and Waheed, 1995). Otoliths from recaptured fish were examined to determine the number of microincrements between their outer edges and the fluorescent marks caused by the tetracycline. It was planned to inject and release an initial 500 skipjack, so that methods could be tested prior to undertaking a larger study if required.

METHODS

Marking of fish and collection of otoliths

A tuna-tagging programme was carried out in the Maldives during 1993-95 by the Marine Research Section (MRS) of the Ministry of Fisheries and Agriculture (Waheed and Anderson, 1994; Anderson, Adam and Waheed, 1995). During the course of that programme a total of 494 skipjack (out of a planned total of 500) were injected with tetracycline prior to tagging and release. All these skipjack were tagged in the south of the Maldives in the vicinity of the One-and-a-Half-Degree Channel. Thirty-four of these skipjack were tagged and released during tetracycline injection trials in February and April 1994. The great majority (460) were injected and tagged in August 1994.

The length-frequency distribution of the tetracycline-injected fish is illustrated in Figure 1. One fish was not measured. The remaining 493 skipjack were within the size range 35-65 cm FL, with a modal length of about 46 cm. The weight of a modal-length fish is estimated at about 2.0 kg; the mean weight of all the skipjack injected is estimated at 2.1 kg.

The dose injected was about 1ml of 100mg/ml oxytetracycline for an average-sized skipjack (*i.e.* nominally about 50 mg/kg). Minor seepage of oxytetracycline was often observed from the injection site, so the effective dose injected would often have been less than this. It was not practical to adjust dosage for individual fish, although the largest skipjack were injected with 2ml of 100 mg/ml oxytetracycline. Injections were made intramuscularly, just

below the first dorsal fin origin, using a continuous pippetting syringe dispenser.

Orange tags were used to mark tetracycline-injected fish, to distinguish them from the yellow-tagged normal fish (Anderson, 1995). Arrangements were made with the Government-owned Maldives Industrial Fisheries Company (MIFCO) to collect recaptured orange-tagged skipjack from fishermen, and return them frozen to MRS in Malé Fishermen were informed of the programme and of arrangements for the return of orange-tagged skipjack through a nation-wide publicity campaign, which included radio and TV broadcasts, and posters distributed to every fishing island and every MIFCO collector/freezer vessel. A premium price of MRf 200 (about US$17) was paid for each skipjack returned with orange tag in place and with full recapture information. Recapture information was recorded on printed forms distributed in advance to every island and collector/freezer vessel.

Not all Maldivian fishermen have access to collector/freezer vessels. Information was therefore broadcast recommending that such fishermen gut any orange-tagged skipjack that they might catch and preserve them in salt prior to forwarding to MRS in Malé.

In Malé all fish were measured to the nearest millimetre, and beheaded with a hacksaw. The top of the head was then cut off, again with a hacksaw, to expose the top of the brain. It was found easiest to cut the fish while it was frozen, and then allow the head to thaw before removing the otoliths. Removing the brain with coarse forceps exposed the cavities of the membranous labyrinths and semicircular canals containing the otoliths. Sagittae were extracted with fine forceps and stored in a small numbered plastic tube. A few vertebrae and the first dorsal spine were removed from each fish at the same time for separate study.

Otolith preparation and procedures

In the ORSTOM laboratory, sagittae were cleaned in sodium hypochlorite (household bleach), and distilled water, then dried in alcohol. Each otolith was embedded in polyester resin (Sody 33) and a transverse section made with a low-speed saw (Isomet Buchler) to obtain a slice containing the primordium. This slice was attached to a glass microscope slide with thermoplastic glue (Crystalbond 109) and then ground with wet sandpaper (800 and 1200 grit sizes) sprinkled with aluminium powder (0.5❍µm). It was then polished on a polishing plate with water and aluminium powder (0.3µm and 0.1µm) until the primordium was very close to the surface. The microscope slide was then placed on a hot plate for a few seconds to soften the glue, making it possible to turn the section. The turned section was polished again until a preparation of 50-100µm thickness was obtained.

The characteristic yellow tetracycline mark was identified under an optical microscope by means of ultraviolet light emitted from a 100-watt mercury burner. Excitation wavelength was limited by a filter to 355-420 nm, and autofluorescence was minimized by a 390 nm barrier filter. The position of the fluorescent mark was noted on a photographic print. The surface of the section was then partially decalcified with 5-7% EDTA (Ethylenediaminetetraacetic acid) to emphasize the increments. Under a separate microscope, using a Metallographic lens and a total magnification of 1000x, the number of increments between the position of the fluorescent mark and the outer edge of the otolith was counted. A minimum of six counts at different times were made on each otolith by two different readers, without prior knowledge of the previous counts.

A few skipjack otoliths were observed under a scanning electron microscope (SEM) to confirm the status of microincrements observed under the optical microscope.

RESULTS

To the end of August 1995 a total of 58 returns were made, as follows:

Orange-tagged skipjack, frozen	32
Orange-tagged skipjack, salted	2
Orange tag without skipjack	24
Total	58

Thus, the recapture rate for all tetracycline-injected skipjack was 11.7% (58/494). The return rate for all skipjack tagged but not injected during the period of September 1993 to August 1994 was 8.0% (481/5980).

Two skipjack preserved in salt were returned by fishermen to MRS. One had been gilled as well as gutted, and no trace of the otoliths could be found. The other had been gutted but not gilled, and one otolith was recovered. However, the outer layers of this otolith were badly deformed, with numerous microscopic cracks, making it unreadable.

From the 32 frozen skipjack returned to MRS, otoliths were obtained from 30. Not all of these 30 recovered otolith sets were usable for this study. On examination under UV light, only 8 showed a visible fluorescent mark on their otolith section. The pertinent information for increment counts in these 8 skipjack tuna otoliths is presented in Table 1. This sample included fish with fork lengths at recapture in the range 48.0 to 56.6cm, and which had been at liberty for between 32 and 225 days.

Table 1. **Skipjack measurement data and otolith increment counts Key: FL = Fork length; Dt = number of days at liberty; Ci = increment count number i**

Fish no.	*FL (cm)*	*Sex*	*Δt (days)*	*Different increment counts*						*C (mean)*	*Std. error*	*C/Δt*
				C1	*C2*	*C3*	*C4*	*C5*	*C6*			
31	47	F	32	42	38	37	41	42	41	40.2	0.87	1.26
32	49	M	35	27	26	27	27	28	27	27.0	0.26	0.77
34	46	F	69	33	33	31	31	34	28	31.7	0.88	0.46
49	46	F	153	96	103	96	92	97	99	97.2	1.49	0.64
50	49	F	193	91	87	92	89	86	92	89.5	1.06	0.46
51	52	M	187	54	54	49	52	48	50	51.2	1.05	0.27
52	45	F	225	71	68	67	70	72	68	69.3	0.80	0.31
53	45	F	179	65	61	66	62	63	65	63.7	0.80	0.36

The mean number of increments counted between the tetracycline mark and the edge of otolith was in 7 out of 8 cases less than the number of days at liberty (Table 1 and Figure1). In fact, the estimated number of increments deposited per day varied greatly between individuals, from 1.26 to 0.27. The weighted average was 0.44 increments per day, *i.e.* an average of 1 increment every 2.3 days.

Because the number of increments deposited per day varies greatly between individuals, there is not a precise relationship between the number of increments and the number of days at liberty (Figure 2). Nevertheless, the best linear relationship between the number of increments (N_i, dependent variable) and the number of days at liberty (N_d, independent variable) is:

$$N_i = 0.245\ N_d + 25.9\ (r = 0.73)$$

The confidence limit (1.96 SE) for the estimate of the slope (0.245) is 0.18. The slope of the relationship is significantly different from 1 ($p>0.95$). Since the slope might be expected to pass through the origin, the relationship (assuming it is linear) may also be represented as follows:

$$N_i = 0.395\ N_d \qquad (r = 0.52)$$

The confidence limit (1.96 SE) for the estimate of this slope (0.395) is 0.10, and is again significantly different from 1.

DISCUSSION

From these results it is concluded that the formation and deposition of otolith increments in Maldivian skipjack is not daily. Furthermore, since the number of microstriae deposited per day varies between individuals, otoliths cannot be used for age determination in Maldivian skipjack tuna. These results differ greatly from the results of Panella (1971, 1974) and most subsequent studies, which show that for most fish species increment deposition is daily.

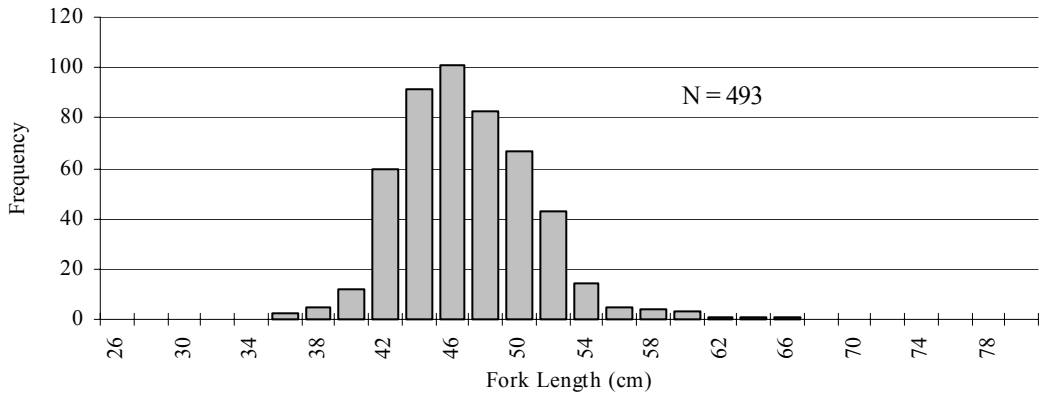
Findings of non-daily increment deposition are known but are not common. Brothers *et al*. (1976) showed that the use of increment counts underestimated the age of 7- to 13-year-old hake (*Merluccius angustimanus*) by 2 to 3 years. Caillart and Morize (1989) found that, on average, one microstria was formed only every 2 days in a tropical grouper (*Epinephelus microdon*) from French Polynesia. Le Guen (1976) demonstrated that in a tropical sciaenid (*Pseudotolitus elongatus*) incremental age agreed with age determined by seasonal marks in immature fish, but underestimated age in mature fish by up to 30%.

Among Scombrid fishes, most of the studies on larvae or juveniles (Brothers *et al*., 1983; Radtke, 1983; De Vries *et al*., 1990; Jenkins and Davis, 1990; Wexler, 1993), and on adults (Wild and Foreman, 1980; Wild, 1986; Stéquert *et al*., 1995) have demonstrated that increment deposition is daily. However, Wild and Foreman (1980) found that skipjack tuna in the eastern Pacific (Revillagigedo Islands - Baja California region) also deposit significantly less than one increment per day. Their results suggested an average deposition rate of one increment every 1.3 days (0.76±0.09 [mean ± 1.96SE] increments per day). Our results indicate an average deposition rate of one increment every 2.3 days (weighted average, equivalent to 0.44 increments per day) or one increment every 1.8 days (unweighted average, 0.57±0.23 increments per day).

Comparing Wild and Foreman's (1980) original data on individual skipjack increment deposition rates (from their Table 8, but excluding their deleted sample K4334) with ours (Table 1), it is assumed that the two samples do not have equal variances ($F=3.27 > F_{0.025(7,24)}$). Given this, it is concluded that mean deposition rates are not significantly different ($t=1.78 < t_{0.05(8)}$).

It should be noted that there was a difference in otolith-reading technique between the study of Wild and Foreman (1980) and this study (cellulose acetate replica of external etched surface versus transverse section). Wild and Foreman (1980) noted that, using the cellulose acetate replica technique, ventral edge counts (*i.e*. the external equivalent of a transverse section) were significantly lower than ros-

Figure. 1. *Size frequency distribution of OTC injected skipjack tuna.*



tral or postrostral counts in yellowfin tuna (*Thunnus albacares*). They therefore counted skipjack increments only along the rostrum. However, Stéquert, Panfili and Dean (1995) have demonstrated that in yellowfin tuna the cellulose acetate technique underestimates ventral edge counts, because increments overlap within the otolith and so cannot be seen on the external face. These increments are separable in transverse sections, under suitable magnification. The use of transverse sections in this study of skipjack otoliths is therefore not thought to be a source of error in estimating increment deposition rates Nevertheless, if the opportunity arises, the second otolith of each of the eight skipjack otolith pairs showing OTC marks will be examined in longitudinal/oblique section.

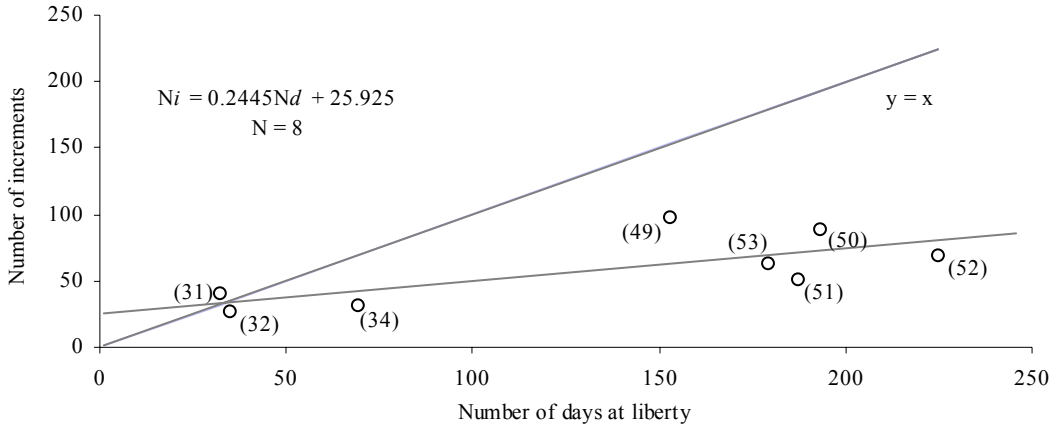
There are several explanations for deviations in increment deposition from the generally observed daily rate. Starvation experiments have been shown to lower deposition rates in larval anchovy (*Engraulis mordax*) (Methot and Kramer, 1979) and in rainbow trout (*Onchorhynchus mykiss*) (Brothers, 1978). Reduction in temperature and photoperiod has been shown to inhibit the formation of increments in sunfish (*Lepomis cyanellus*) (Taubert and Coble, 1977). These results suggest that in the natural environment stresses such as thermal changes, lack of food, and perhaps also reproductive events, might be able to induce some breaks in growth that lead to a reduction in the deposition of increments.

For skipjack tuna, temperature changes may not be a significant factor affecting increment deposition rates, since they live in the upper layers of tropical waters where temperature variations are generally rather small. A more important factor responsible for the observed reduction in increment deposition rate may be reproductive activity, which in this species is carried out all year round (Stéquert and Ramcharrun, 1995). However, the most significant factor affecting increment deposition rates in skipjack otoliths may well be food availability. This species is an opportunistic feeder, and can survive for several days without food when moving through unproductive areas. Such behaviour seems likely to reduce increment deposition rates.

One other possible explanation for the reduced increment deposition rate in skipjack tuna (namely that daily increments exist but are not visible to the observer) can be discounted. Davies *et al* (1988) studied the otoliths of smooth oreo (*Pseudocyttus maculatus*) and black oreo (Allocyttus sp.). Using a scanning electron microscope (SEM) they demonstrated that the crystalline structures in some areas of those otoliths were so complex and confused that they obscured the microincrements. Our observations on skipjack otolith sections under SEM did not reveal any such structures. All increments appeared well formed and clearly distinguishable from each other.

It is not known why only 8 skipjack out of 32 showed tetracycline marks in their otoliths. In the 8 skipjack that did have fluorescent marks the marks were clear, so it seems unlikely that the dosage of oxytetracycline administered was inadequate. Nor is it likely that mistakes were made in the labelling of otoliths, resulting in a mix-up between tetracycline-marked and unmarked otoliths. One explanation might be that some fish were frozen for much longer before being returned to MRS than others, resulting in deterioration of the tetracycline mark. Since those fish that did have useable otoliths (nos. 31, 32 and 34, and numbers 49-53) were grouped according to time, and hence batch, of return to MRS this is certainly a possibility. Unfortunately records of times spent frozen were not kept, but in any case in future experiments they will be kept to a minimum. It should be noted that the brand of tetracycline used in this experiment ("Terramycin" manufactured by Pfizer Inc.) is labelled "Do not freeze". However, Mr. Vince Petersen (Quality Operations Manager, Pfizer Pty. Ltd., Sydney, Australia, pers. comm.) informs us that "we have re-

Figure 2. *Relationship between number of otolith increments (from OTC mark to the otolith edge) and number of days at liberty, for Maldivian skipjack tuna. Individual fish reference numbers in parentheses.*



searched our archives and also undertaken some practical work in our laboratory and this indicates that there appears to be no effect on the fluorescence of the material due to thawing and freezing".

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