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# Preliminary growth studies of yellowfin and bigeye tuna (*Thunnus albacares* and *T. obesus*) in the Indian Ocean by otolith analysis

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## 1. Introduction

Because of the very high level of commercial catch of yellowfin tuna (*Thunnus albacares*) and bigeye tuna (*Thunnus obesus*) in the three tropical oceans, respectively 1 300 000 tons and 450 000 tons per year on average for the period 2001-2006 (source IOTC, ICCAT, SPC, IATTC), a lot of studies have been conducted on the age and growth of these species. Different methods to determine growth curves have been used: length frequency analysis (Moore, 1951 ; Le Guen and Sakagawa, 1973 ; Marcille and Stéquert, 1976 ; Fonteneau, 1980 ; Gascuel *et al.*, 1992), tag-recapture data analysis (Bard, 1984a et b ; Bard *et al*, 1991 ; Bayliff, 1988) and analyses of calcified structures (scales by Yabuta *et al.*,1960 ; dorsal spines by Lessa and Duarte Neto, 2004 and otoliths by Wild, 1986, Stequert *et al.*, 1996 and Stequert and Conand, 2000), as well as combined analyses of tag-recapture and and otoliths data (Hallier *et al.*,2005).

The Scientific Committee of IOTC (SC) identified during its 6$^{th}$ Session in 2006 (IOTC, 2006) the hypothesis regarding yellowfin growth curve of a two-stanza growth curve (“slow growth” hypothesis between 45 and 60 cm), and a Von Bertalanffy growth curve (“fast-growth” hypothesis, assuming a constant growth rate).

There are debates on what is the most robust method to estimate the growth? (length-frequency, tag-recapture, otoliths). Shuford (2007) thinks that the length-frequency analyses and tag-recapture studies are less robust than direct ageing with otoliths analysis, based on the counts of daily increments. However, each method has its advantages and drawbacks but the otolith method does not need to extrapolate age of smaller fish. Nevertheless, the validation of daily increments must include the smallest and largest fish, while it is not always easy to sample these size classes.

The Working Party on Tropical Tunas of IOTC (WPTT) in 2007 (IOTC, 2007) recommended that a new growth curve using all available tag-recapture data should be derived, including chemical tagging for direct ageing.

Today with data gathered during the “Regional Tuna Tagging Project - Indian Ocean (RTTP-OI)” implemented by the Indian Ocean Commission (IOC) supervised by the Indian Ocean Tuna Commission (IOTC) and funded by the 9$^{th}$ European Development Fund from the

European Commission (9th EDF), it is possible to conduct growth studies in the Indian Ocean based on tagging and otolith readings.

The purpose of the present study is to develop a growth curve that incorporates directly observed estimates of age for the juvenile pre-recruits and recruits. This is determinant to confirm either the one or the two stanza growth curve derived from previous studies. In this study, daily increment analysis of sagitta otoliths collected from fish in the Indian Ocean are used, and several growth curves obtained by different authors are compared with those readings. The present study focus on yellowfin and bigeye tuna, as these two species were the priority of the RTTP-IO. Data collected by the RTTP-IO on skipjack tuna (*Katsuwonus pelamis*) will be considered in the future.

## 2. Materials and Methods

### *2.1 Tagging*

Between May 2005 and September 2007, the RTTP-IO chartered two pole-and-line vessels to tag and release a minimum of 80 000 tuna of the three main tropical species. The tagging method were used - conventional tagging, chemical tagging and electronic tagging. At the end of the project 168 163 tuna were tagged and released in the Western Indian Ocean (WIO), of which 2,018yellowfin and 2,441 bigeye, were chemically tagged with oxytetracycline (OTC).

OTC leaves a mark in the calcified part of the fish such as bones, scales and otoliths. The tuna after being hauled on the tagging cradle were injected, using a syringe, with between 1.5 and 3 ml of OTC, in the muscular part of their back according to the size of the fish. The fish were then tagged with a white spaghetti tag to distinguish them from the conventional yellow tags. Publicity and awareness campaigns have been developed in the coastal fishing countries, but also in the countries where the fish could be process and 224 yellowfin and 169 bigeye, chemically tagged have been recovered. When possible (*ie.* when the fish has been kept intact), at the time of the recovery, both otoliths were sampled, cleaned and store before being sent for analysis. The otoliths are regularly sent for analysis.

### *2.2 Otolith sampling and preparation and reading*

18 yellowfin tunas between 19 and 29cm were sampled from West Sumatra area by the IOTC during the West Sumatra Tuna Tagging Project (WSTTP) funded by the government of Japan and implemented by IOTC. 157 yellowfin tuna withsize at recapture between 47.9 and 115 cm FL and 111 bigeye tunas with size between 46 and 105.3 cm FL, all marked with OTC, were collected from the Western Indian Ocean by the RTTP-IO between 2006 and 2007 and sent to Laboratoire de Sclérochronologie des Animaux Aquatiques (LASAA) in Brest, France, for reading and analysis.

For yellowfin and bigeye, length at recapture is known for respectively 134 and 73 fishes. So far 99 YFT otoliths were read for growth study and 94 for validation, 60 of them being used for both validation and ageing and 70 BET otoliths were read for growth study and 59 for validation and ageing.

Otoliths (*sagittae*) were prepared for age analysis following the methods as described by Secor *et al.* (1991), Stequert *et al.* (1996) and Panfili *et al.* (2002). They were cleaned in sodium hypochlorite and rinsed with distilled water before been embedded in Sody resin and cut transversally on both sides of the nucleus, which had been previously located in the resin block. The part containing the section was then glued to a glass slide using thermoplastic glue at 150°C, then sanded to the level of the nucleus using different alumina grains from 3 to 0.3 microns. The section was then turned and treated on the other side using the same powders to produce a thin slice of about 100 microns thickness. The surface of the slide was then decalcified with EDTA (tri-sodium-ethylene-diaminetetraacetic acid) to increase contrast between increments. These thin slides were examined under 1000x magnification using an Olympus BX40 microscope for counting increments along the external part of the ventral side of sagitta (Fig. 1). For the OTC tagged fish, the length between the fluorescent mark and edge ($L_{me}$) and between core and extremity of the counted side ($L_{oto}$) were also measured (Fig. 1).

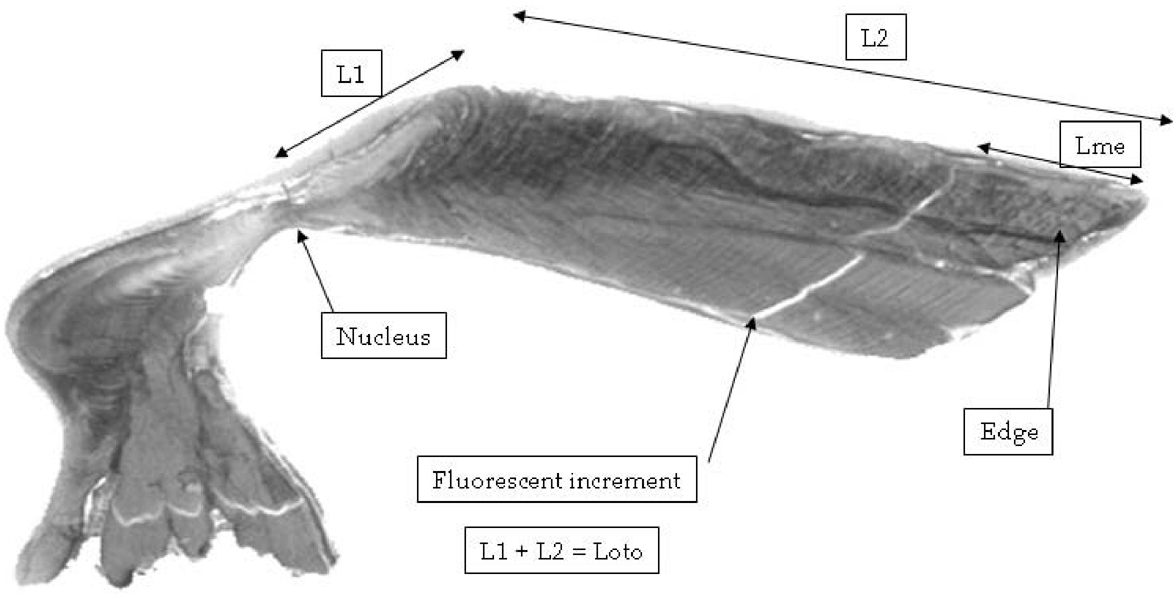


Figure 1: Otolith of bigeye tuna and its different measures.

### *2.3 Daily increments validation*

Although in yellowfin tuna, the frequency of increment deposition had been confirmed to be daily (Wild and Foreman, 1980; Wexler *et al.*, 2001) some problems still persist with the interpretation for the fish smaller than 45 cm and larger than 100 cm. With this sample of RTTP-IO OTC tagged fish that were received at the Laboratory (157 yellowfin and 111 bigeye until now), this study was started by a validation of the daily increment hypothesis.

The relationship between the number of days elapsed after tagging the fish with OTC and the number of increments between the fluorescent increment (OTC marked ring) and the edge of the otolith is calculated from a linear regression:

Numbers of increments = a*numbers of days +b.

If a = 1, the number of increments is related to the number of days.

94 yellowfin have been used for validation, fork length between 45.5 cm and 81 cm at tagging, between 47.9 and 115 cm at recapture and time of liberty between 6 and 502 days. 59

bigeye have been used for validation, fork length between 44 cm and 74 cm at tagging, between 46 and 105.3 cm at recapture and time at liberty between 14 and 555 days.

### *2.4 Age reading*

99 and 70 otoliths of respectively yellowfin and bigeye tuna have been prepared for this study. Yellowfin tuna were ranged between 19 and 115 cm and bigeye tuna between 46 and 105.3 cm**.** To increase the data set (FL_Number of increments) in the less sampled lengths, for 27 otoliths of yellowfin ranged between 47 and 81 cm at tagging, increments between the core and the marked ring were counted.

Each otolith was read twice without prior knowledge of the species and size. If the coefficient of variation (CV) between the number of increments counted for this two readings was greater than 10 %, a third reading was done. The average of the two or three readings was then calculated.

For some otoliths some regions were illegible and a method of interpolation to estimate the number of increments was used. Campana (1992) finds that this interpolation is reasonable if the number of increments interpolated is small relative to the total count.

### *2.5 Growth*

The Von Bertanlanffy growth curve used in previous studies (Stequert *et al.*, 1996) does not seem to be in agreement with the tagging data of the RTTP-IO. In fact, for both yellowfin and bigeye, it seems that a two stanza growth is applicable (Hillary *et al. 2008* ; Fonteneau and Gascuel, 2008 ; Eveson and Million, 2008). This confirms previous studies based on length frequency analysis in the Indian Ocean (Marsac and Lablache, 1985 ; Marsac, 1991, Lumineau, 2002) or in the Atlantic and Pacific Oceans (Lehodey and Leroy, 1999 ; Gascuel *et al.*, 1992).

The sample size of this study was not large enough to be fitted with a model. So the results obtained for age readings have just been plotted (Number of increment vs FL) and compared with the curves of Stequert *et al.* (1996), Stequert and Conand (2000) and Eveson and Million (2008) for the two species.

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**For yellowfin,** Stequert Von Bertalanffy model and Eveson model are respectively:

:

$FL = 272.7*(1-e^{(-0.176*(t+0.266))})$

and

$FL = 146*(1-e^{(-0,905*(t+1,42))}*(((1+e^{(-10,9654*((t+1,42)-4,1228))}) / (1+e^{(4,1228*10,9654)}))^{((0,1334-0,905)/10,9654)})$

**For bigeye,** Stequert Von Bertalanffy model and Eveson model are respectively:

$FL = 303.9*(1-e^{(-0.145*(t+0.0.48))})$

and

$$FL = 160*(1-e^{(-0,4207*(t+3,09))}*(((1+e^{(-2,999*((t+3,09)-5,6033))})/(1+e^{(5,6033*2,999)}))^{((0,071-0,4207)/2))}$$

## 3. Results

### *3.1 Validation*

**For yellowfin tuna** the equation of the linear regression "Number of increment Vs days of liberty" is (Fig. 2):

$$Y = 3.34 + 0.93*X \text{ with } R^2 = 0.98$$

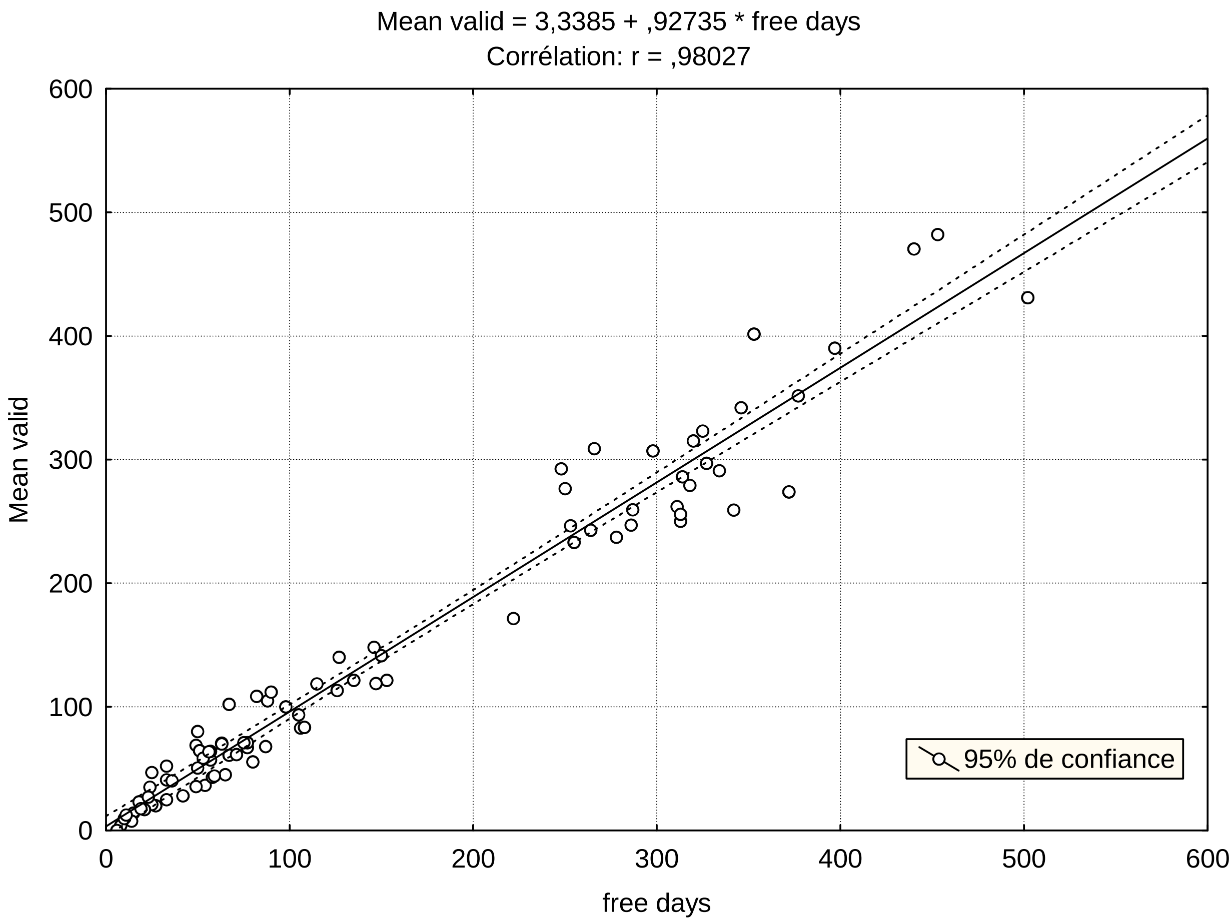


Figure 2: Yellowfin, relashionship between Number of increments (Mean valid) and Number of days at liberty (free days)

Using a t test, 0.93 is not statistically different from 1 and 3.33 is not neither statistically different from 0, so we can assume that one increment is laid by day and that the fluorescent mark is laid immediately after tagging.

**And for bigeye tuna** the same linear regression is (Fig. 3):

$$Y = 24.0 + 0.85*X \text{ with } R^2 = 0.97$$

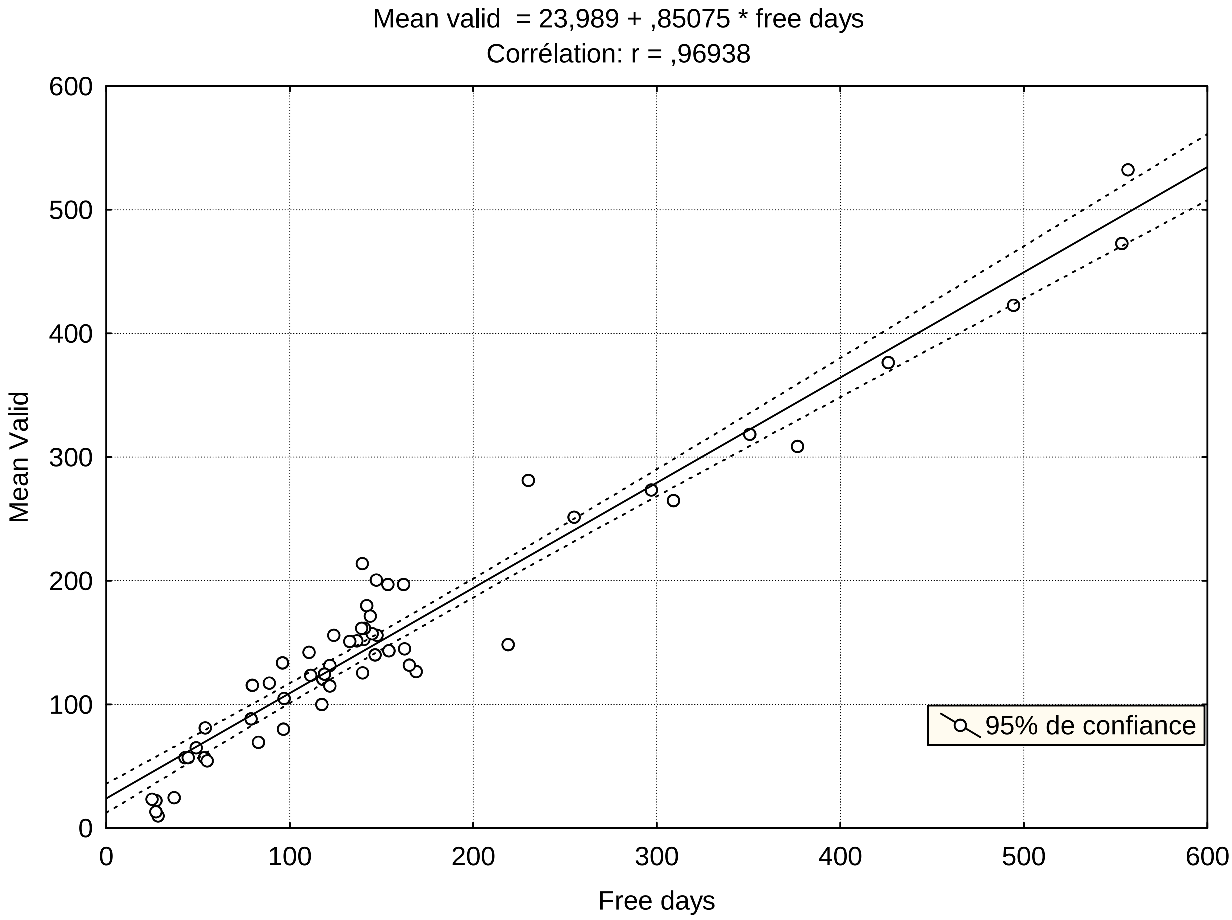


Figure 3: Bigeye, relashionship between Number of increment (Mean valid) and Number of days at liberty (free days)

Then, at p=0.05, we cannot estimate that on average the number of increments is related to the number of days for bigeye tuna between 46 and 105.3. The numbers of days is underestimated by the number of counted increments.

In conclusion for the two species, as we read the same otolith for validation and growth we have adjusted the total number of counted increments by the ratio: 1/0.93 = 1.075 for yellowfin and by 1/.85 = 1.176 for bigeye tuna for estimating the age of the fish.

### *3.2 Otolith relationship*

Analyses show that linear relationships exist between number of increments between the fluorescent mark and the edge (Nbr_valid) and the growth during time-at-liberty (GrF) and between LF and $L_{oto}$ for the two species.

**Yellowfin tuna**:

- Nbr_valid = 5,06+0,722*GrF with $R^2$ = 0.97
- $L_{oto}$ = 695,1 + 16,32*LF ($R^2$=0.90)

This last linear relationship is different for fish <60 cm and > 60 cm (Fig. 4).
For fish smaller than 60 cm : $L_{oto}$ = 321.32 + 25.00*FL ($R^2$=0.96), and for fish higher than 60 cm : $L_{oto}$ = 1037.7 + 12.36FL ($R^2$=0.78)

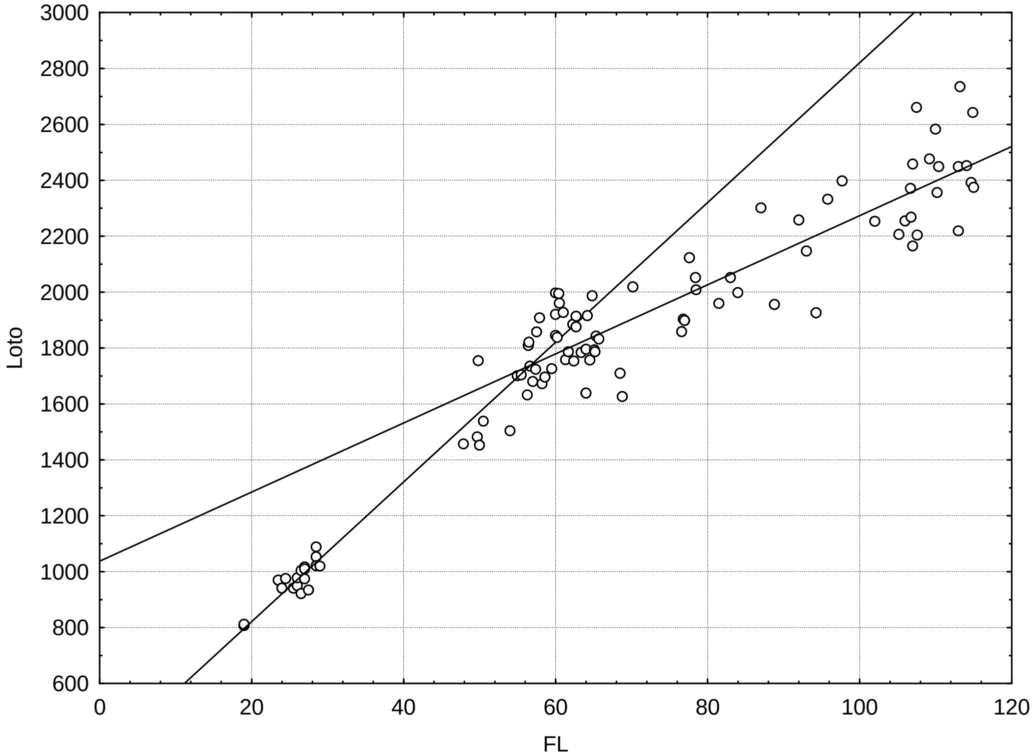


Figure 4: Yellowfin, relationships between $L_{oto}$ and FL for FL < and > 60 cm.

The otolith growth vs the fish growth seems to be slower for the fish of a FL>60cm than for the fish of a FL<60cm.

**Bigeye tuna** :

- Nbr_valid = 17.55 + 0.70 * GrF ($R^2$= 0.93)
- $L_{oto}$ = 601 + 18,94 * LF ($R^2$=0.89)

No difference is observed in the case of the bigeye between small and large fish (Fig. 5) but no fish of a FL<46cm could be sampled.

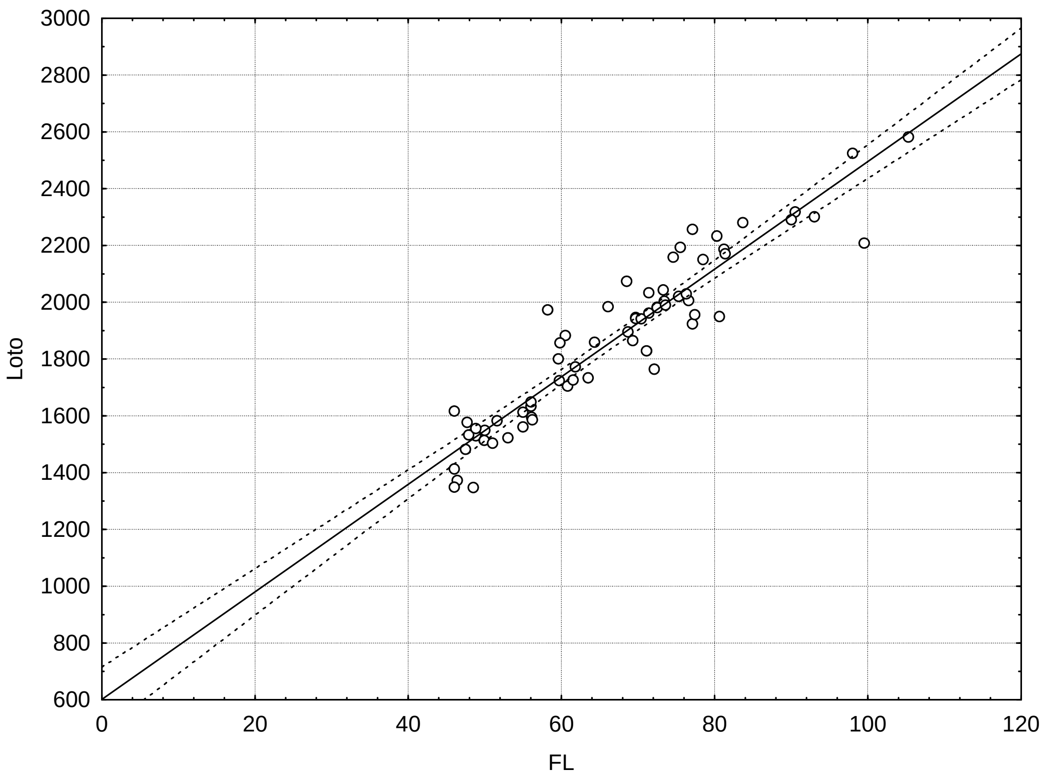


Figure 5: Bigeye, relationships between $L_{oto}$ and FL.

### *3.2 Growth*

99and 70 sets of data of respectively yellowfin and bigeye have been used for growth study. 6 readings for yellowfin and 2 for bigeye were included in this study. The samples for both species are quite different in term of the range of FL analysed. Lengths range from 19 to 115 cm for YFT and from 46 to 98 cm for BET (Fig.6). Unfortunately, at the time of the study, for yellowfin sample are missing between FL 39 and 46 cm and FL 68 and 103 cm.

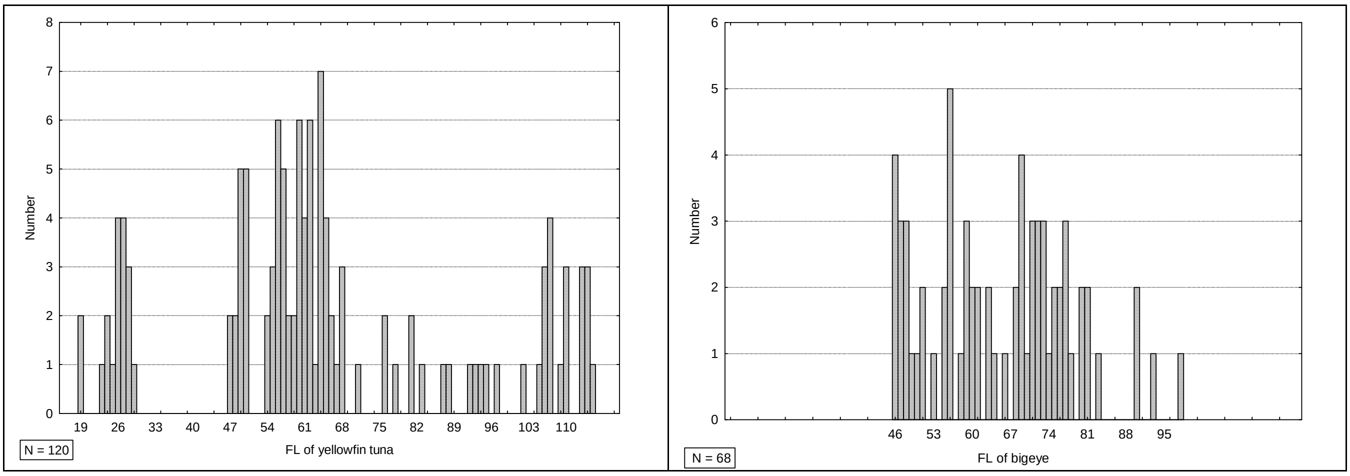


Figure 6: Range of fish length used for studying age and growth of yellowfin and bigeye tunas

For the two species the mean CV of the age counts is 7, which shows that the counts are relatively precise, replicable, and reliable for the same reader.

**For Yellowfin tuna**, theresults are showing differences from the growth curves obtained by the other authors (Fig. 7). The otolith data seems to show an intermediate growth between the two.The mean growth rate is 2.3 cm/month between 30 and 60 and 5.6 cm/month between 60 and 110 cm.

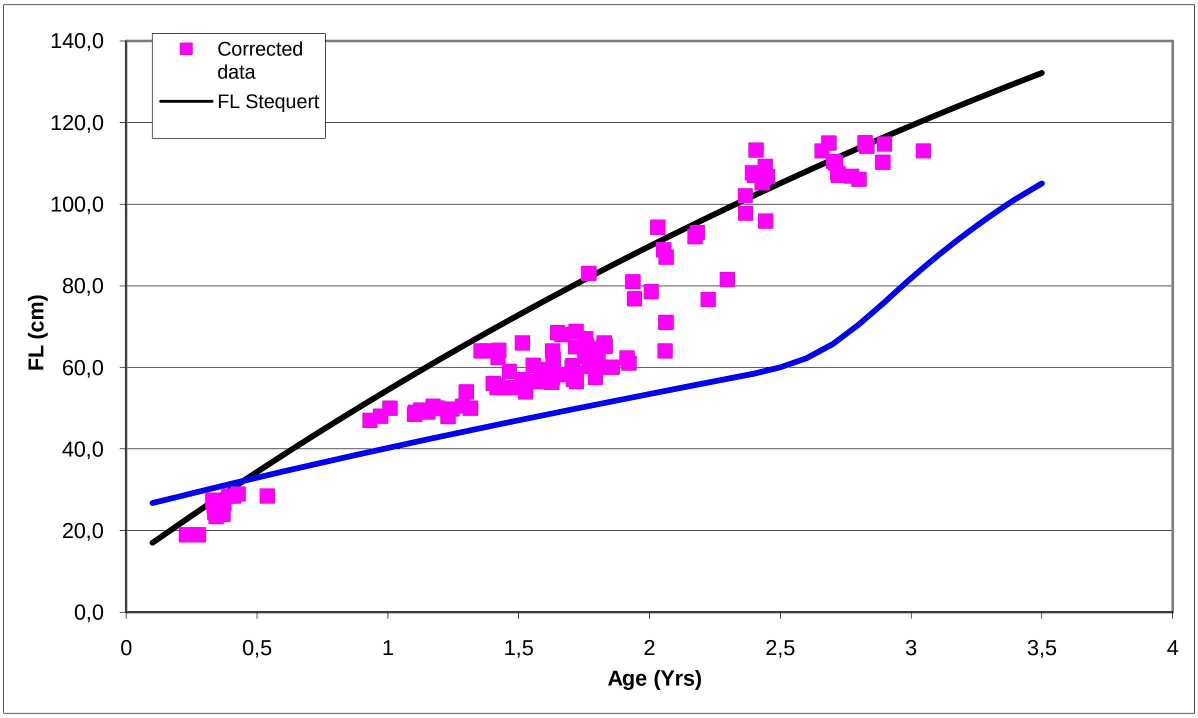


Figure 7 : Yellowfin tuna growth according to our adjusted results, Stequert and Paige curve.

**For Bigeye tuna**, the results are more scattered but also show differences with the previous authors (Fig. 8), and again the otolith data seem to indicate an intermediate growth. The mean growth rate is 2.5 cm/month between 60 and 90 cm. Concerning the two previous growth models (Stequert and Everson), the growth rates seems to be similar but shifted by 6 months with the Stequert curve and by 12 months with the Eveson curve.

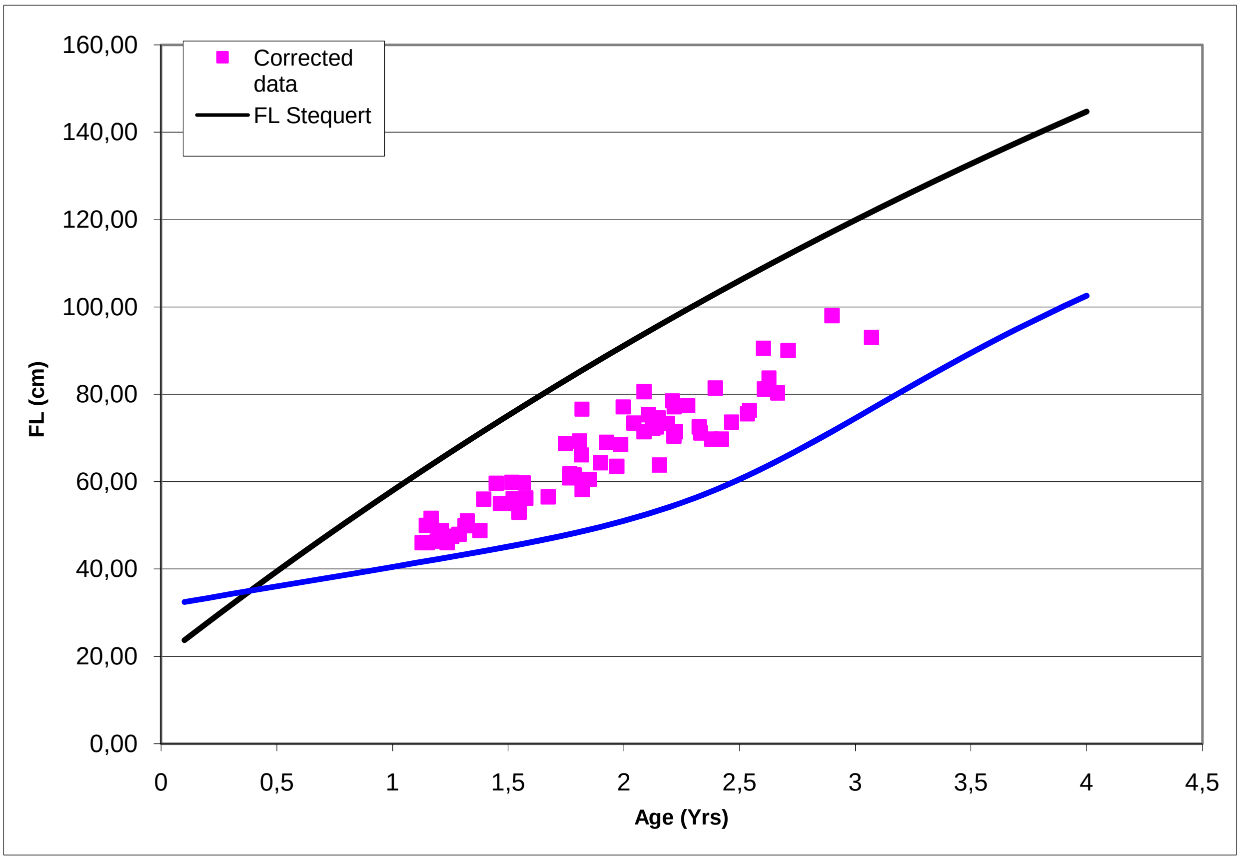


Figure 8 : Bigeye tuna growth according to our adjusted results, Stequert and Eveson curves.

## 4. Discussion

Concerning the validation, for all the analysed otoliths marked with OTC and in the sampled range length, daily increments have statistically be validated for the yellowfin. For bigeye, the validation did not show daily increment but a bit less than one increment per day. Wild and Foreman (1980) have shown that daily increment is formed on the otolith for the yellowfin tuna in the eastern Pacific and Schaefer and Fuller (2006) in the central and eastern Pacific Ocean for bigeye tuna – FL range from 38 to 135 cm.

More analysis of the readings of the otolith of the bigeye tuna shall be undertaken, in particular to see if the daily increment could be validated for the smaller sizes. Hallier *et al.* (2005) showed that after a certain size, rings are too close to the other and counts under optical microscope could be biased. However, utilisation of electronic microscope could permit to correct those readings and proved a daily increment. Problem of overlapping has also been showed by Stequert *et al.* (1996) on transverse section. For these authors, some increments cannot be seen on the external face of the sagitta. Shuford (2007) met the same problem and had to adjust the counts by extrapolation as already mentioned (Campana, 1992).

Due to large variability in the results, 6 otoliths from yellowfin and 2 from bigeye were not used. This could come from the readings, from a default in the calcification of the otolith itself but also from the length or date of recapture that was not correct (dates of recapture are estimated from the logbook from the purse-seiner).

For yellowfin tuna, the slope of the linear regression between the measurement of the otolith and the fish length decreases for fish bigger than 60 cm. The otolith growth is in general well correlated with the fish growth but the otolith grows proportionally less than the fish. For bigeye tuna, this difference between smaller and larger fish has not been shown.. Nevertheless the mean width of increment is larger than 1 micron (1.34 and 1.24 micron respectively for yellowfin and bigeye) and they could be read by optical microscope.

It seems that a Von Bertalanffy curve cannot describe correctly fish the otoliths data of this study which tends to confirm a two-stanza growth. However the sample size is still not large enough to fit a model. The data analysed in this study also seems to be in disagreement with the growth curve developed after the Working Party on Tagging Data Analysis (WPTDA) by Eveson (2008) with a gap of around 12 months. We found that for the same length, yellowfin tuna is one year younger. This otolith data could maybe be included in the tag-recapture analysis for growth, in order to give a direct anchor to the curve. This has also been shown for bigeye tuna, however, more reading and re-reading shall be undertaken.

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