Preliminary study of age, growth and birthdates of juvenile sardine <Sardina pilchardus> (Walbaum, 1792) and European anchovy Engraulis encrasicolus (Linnaeus, 1758) in the east coast of Tunisia

PRELIMINARY STUDY OF AGE, GROWTH AND BIRTHDATES OF JUVENILE SARDINE *SARDINA PILCHARDUS* (WALBAUM, 1792) AND EUROPEAN ANCHOVY *ENGRAULIS ENCRASICOLUS* (LINNAEUS, 1758) IN THE EAST COAST OF TUNISIA

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ملخص

دراسة أولية لعمر ونمو وفترات تفريخ السردينة والأنشوة بالسواحل الشرقية التونسية : يهتم هذا العمل بدراسة النمو اليومي لصغار أسماك السردينة والأنشوة بالمنطقة الشرقية للبلاد التونسية وذلك بتحليل وتعداد حلقات النمو اليومية لحصية الأذن الداخلية. كما وقع تحديد فترات التفريخ بالاستناد على هذه الحلقات. يتراوح عمر العينات ما بين 105 و297 يوم بالنسبة للسردينة و 79 و300 يوم بالنسبة للأنشوة، مع العلم أن طول عينات السردينة يتراوح ما بين 48 و 103 مم وبالنسبة للأنشوة ما بين 42 و86 مم. بالاعتماد على معطيات العمر والطول استخرجنا معادلة قومبارتز للنمو بالنسبة للنوعين من الأسماك. تعتبر النتائج المتحصل عليها في هذه الدراسة مماثلة لتلك المتحصل عليها في المنطقة الغربية للمتوسط. يمتاز الأنشوة بنسبة نمو آني أكبر بالمقارنة مع السردينة. بالاستناد على حلقة النمو عند التفريخ يمكن تحديد فترة التفريخ ما بين أفريل وأكتوبر، الذروة خلال جوان، بالنسبة للأنشوة وما بين أكتوبر وفيفري، الذروة خلال ديسمبر-جانفي، بالنسبة للسردينة.

كلمات مفاتيح : السردينة، الأنشوة ، حصية الأذن الداخلية، حلقات النمو اليومي، النمو اليومي، أيام التفريخ، المياه الشرقية التونسية.

ABSTRACT

The microstructures of the otoliths of juvenile sardines (*Sardina pilchardus*) and anchovies (*Engraulis encrasicolus*), collected in the East coast of Tunisia, were analyzed in order to estimate age, daily growth and hatch date. The fork length of sardines varied from 48 to 103 mm and estimated age ranged from 105 to 297 days. For the anchovies, the estimated age ranged from 79 to 300 days and the fork length from 42 to 86 mm. The length-age data of both species were fitted to the laird-Gompertz model. The corresponding growth equations were $[l_t=10.8\ exp(2.74(1\text{-}exp(-0.006t)))]$ for sardines and $[l_t=3.1\ exp(3.2(1\text{-}exp(-0.02t)))]$ for anchovies.

The instantaneous growth rates of the juvenile of both species in the East of Tunisia were in agreement with the data reported for other areas of the Western Mediterranean Sea. However, the juvenile of sardines had an instantaneous growth rate lower than the juvenile anchovies.

The distribution of the back calculated hatch dates indicated that hatching dates of anchovies occurred from April to October with a peak in June while the hatching dates of sardines occurred from October to February with a peak in December-January.

Keywords: *Sardina pilchardus*, *Engraulis encrasicolus*, otolithometry, microstructure, age, daily growth, hatch dates, east of Tunisia.

RESUME

Etude préliminaire de l'age, de la croissance et des dates de naissance des juvéniles de la sardine *Sardina pilchardus* (Walbaum, 1792) et de l'anchois *Engraulis encrasicolus* (Linnaeus, 1758) des côtes Est tunisiennes : L'estimation de l'âge et de la croissance journalière ainsi que l'analyse de la distribution des dates d'éclosions des juvéniles de la sardine *(Sardina pilchardus)* et de l'anchois *(Engraulis encrasicolus)* ont été effectuées par l'étude des microstries d'otolithe chez ces deux espèces collectées le long des côtes Est de la Tunisie. Les sardines collectées ont des longueurs à la fourche allant de 48 à 103 mm et des âges compris entre 105 et 297 jours. Les âges des juvéniles d'anchois sont échelonnés entre 79 et 300 jours pour des longueurs à la fourche allant de 42 à 86 mm.

Les données âge - longueur sont ajustées au modèle de Gompertz. Les équations de croissance obtenues sont les suivantes : pour la sardine $[l_t=10.8\ exp(2.74(1\text{-}exp(-0.006t)))]$ et pour l'anchois $[l_t=3.1\ exp(3.2(1\text{-}exp(-0.02t)))]$. Pour les deux espèces, les taux de croissance instantanée obtenus sont comparables à ceux rapportés ailleurs en Méditerranée occidentale. En comparant les deux espèces entre elles, la sardine montre un taux de croissance instantanée inférieur à celui de l'anchois.

La distribution des fréquences des dates d'éclosion rétrocalculées montre que, pour l'anchois, la période d'éclosion dure d'avril à octobre avec un pic au mois de juin alors que, pour la sardine, elle s'étend d'octobre à février avec un maximum en décembre-janvier.
Mots clés: *Sardina pilchardus, Engraulis encrasicolus*, otolithométrie, microstructure, âge, croissance journalière, dates d'éclosion, Côtes Est tunisiennes.

INTRODUCTION

Ageing using daily growth increments is of great importance to study the population dynamics of clupeids, especially, when recruitment models were used, to determine hatching dates and to define the environmental conditions of the spawning period (Methot, 1983; Alvarez and Morales-Nin, 1992; Rasoanarivo, 1997; Pepin *et al*., 2001). Studies of the growth of the 0-age classes are of particular importance for species with high growth potential such as clupeids, which reach 60% of their total growth during their first year of life.
Daily growth increments were observed for the first time by Panella (1971). The daily periodicity of the otolith micro-increments was largely assumed for the larvae and the juvenile phase (Pannella, 1974; Brothers *et al*., 1976; Geffen, 1982; Jones, 1986), however Volk *et al*. (1995) have shown that the increment deposition was not always daily
Previous studies have demonstrated the daily nature of increments in the otoliths of larvae and juveniles *Sardina pilchardus* and *Engraulis encrasicolus* and reported that the age may be estimated with certain accuracy by using daily growth microstructure (Re, 1983 and 1984; Palomera *et al*., 1988; Alvarez and Morales-Nin, 1992; Alemany and Alvarez, 1994; Dulcic, 1997; Rasoanario, 1997; Romanelli *et al*., 2002; Cermeno *et al*., 2003).
The aim of this study is to estimate, for the first time, the age and daily growth rates for class 0 of sardine and anchovy populations in Tunisian waters and to compare our results with those obtained in other areas in relation to environmental factors. Moreover, we analyze the hatching date's distribution of the juveniles *Sardina pilchardus* and *Engraulis encrasicolus* in order to carry out recruitment process studies in a future.

MATERIALS AND METHODS

A total of 75 juveniles of sardines (37 specimens) and anchovies (38 specimens) were collected during hydro acoustics surveys from June to September 2002 in the East of Tunisia (37°04'N 11°02'E-35°N 11°E) with midwater trawl. For each specimen, total and fork length (mm) and weight (g) were measured but only the fork length was kept as the reference (L_F) and was varied from 48 to 103 mm for the sardines and from 42 to 86 mm for the anchovies.
The sagittal otoliths were removed, cleaned and mounted in tack 10 instant glue on a microscope slide with the sulcus facing up. The otoliths were dry ground until the focus was reached using successively fine 30 and 3µm grit paper. The otoliths were then observed with a microscope under transmitted light at magnification of 400x and with immersion oil at magnification of 1000x. Daily and sub-daily increments were differentiated on the basis of their width and appearance when focus was readjusted. Two examinations were made by two readers. Since no significant differences were found between readings made by the readers (student's test t= 1.25) the average of the readings were retained for age calculation.
As in other species the first daily increment was laid down at the end of the yolk absorption period when the jaws became functional (Brothers *et al*., 1976). For the European pilchard, the end of the yolk-sac absorption occurred five days after hatching (Alemany and Alvarez, 1994; Dulcic, 1995) whereas for the anchovy occurred at two days after hatching (Regner, 1985; Palomera *et al*., 1988). According to the species, 5 or 2 days were added to the age of each fish that was subtracted from the dates of capture to calculate the hatching dates (Woodbury *et al*., 1995).
The model used to establish the early growth of sardines and anchovies was the laird- Gompertz Model (Zweifel and Lasker; 1976). The growth equations were calculated using Statistica, a statsoft based on quasi Newton method.
The Formula of the laird- Gompertz Model is:

$$l_t = L_0 \exp\left(G^*(1-\exp(-\alpha^* t))\right)$$

With t: age in days, l_t: estimated length at age t, L_0: length at t=0, $G=A_0/\alpha$, G: growth rate, A_0: instantaneous growth rate at t=0, α: rate of decrease in G.

RESULTS

The sagittae of the juvenile sardines and anchovies are of oval shape, when observed under natural transmitted light, they showed an alternance of dark and light concentric rings from the nucleus to the edge where they became very narrow (Fig. 1). Dark rings called D zones are matrix rich layers whereas light rings (L zones) are mineral rich layers (Kalish et *al*., 1995). One light and one dark ring were considered to form a daily growth increment (Figs. 1 b, d).

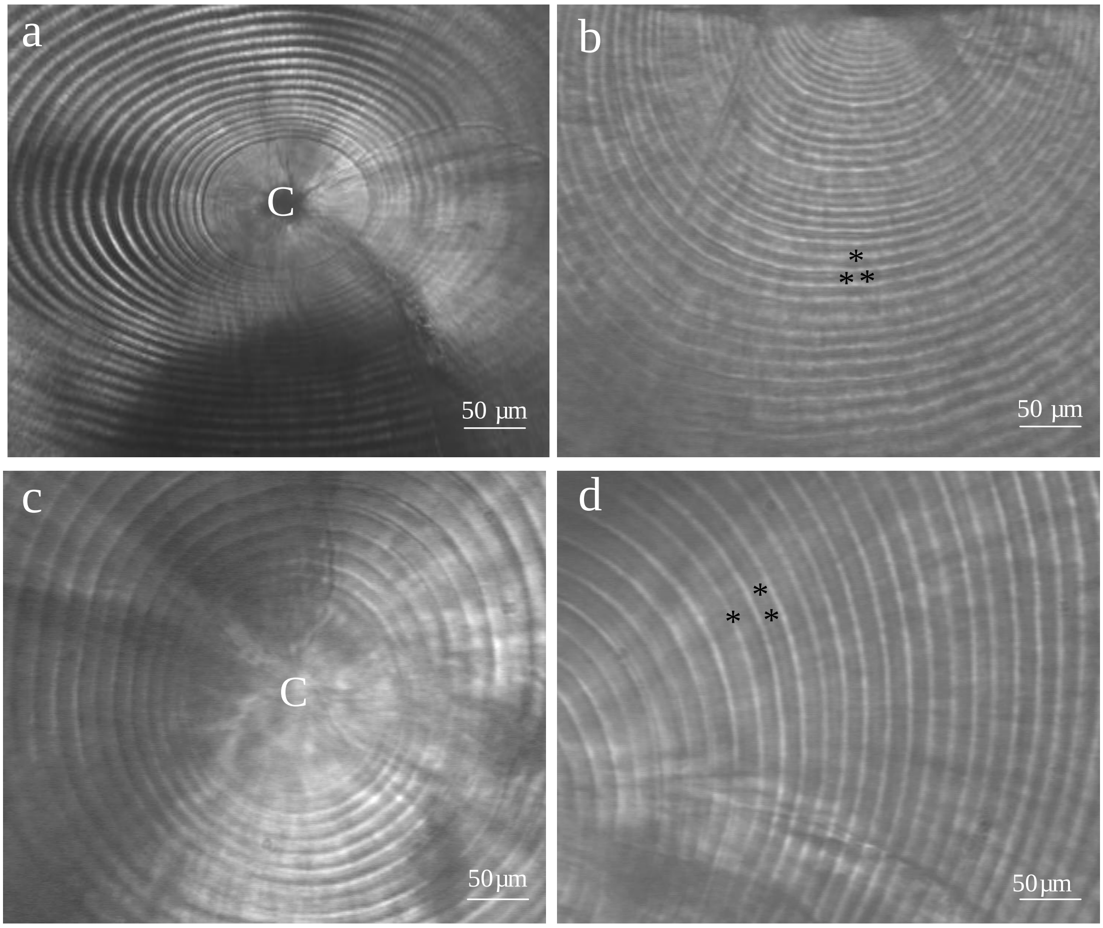

Fig. 1- Sagittae of the juveniles anchovy (a and b) and sardine (c and d) viewed under transmission light microscopy. a. A detail of the core of an anchovy sagittae. C: core. b. A detail of the lateral field of an anchovy sagittae showing the alternation of the D- (**) and L-zones (*). c. A detail of the core of a sardine sagittae. C: core. d. A detail of the lateral field of a sardine sagittae showing the alternation of the D- (**) and L-zones (*).

The rings were arranged regularly over a time scale. In most cases, the pattern of the growth marks was clear and legible.

For the sardine juveniles, the age obtained by counting the rings, varied from 105 to 297 days and the L_F varied from 48 to 103 mm. For the anchovy juveniles the age varied from 79 to 300 days and the L_F ranged from 42 to 80 mm.

The age-length data of the juveniles of sardines and anchovies were fitted to the laird-Gompertz Model which provided a good description (r=0.80 for the sardine and 0.81 for the anchovy). The corresponding growth curves were represented in figures 2 and 3. The growth equations of the sardines and of the anchovies were respectively:

Sardine: l_t =10.8exp(2.74(1-exp(-0.006t))).

Anchovy: l_t =3.1exp(3.2(1-exp(-0.02t))).

Growth in length of sardine juveniles was fast in the first 166 days, thereafter the growth rate decreased to 0.28 mm per day in the 305th day. The decline was most marked and continuous for the anchovy

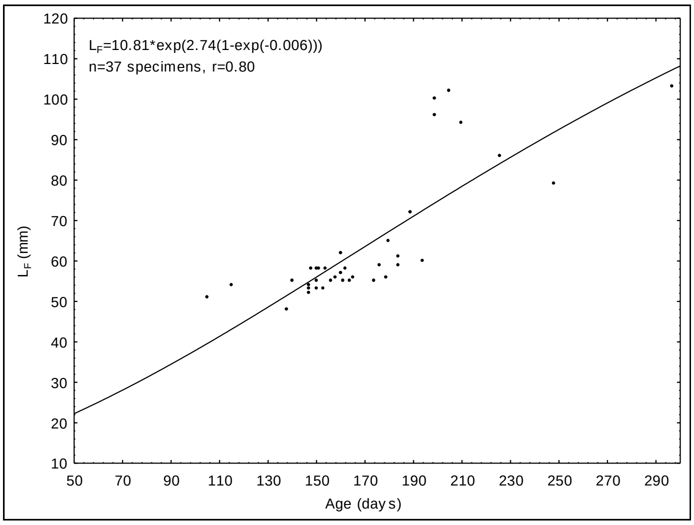

Fig. 2- Growth curve of *Sardina pilchardus* from the East of Tunisia fitted to the Laird-Gompertz Model.

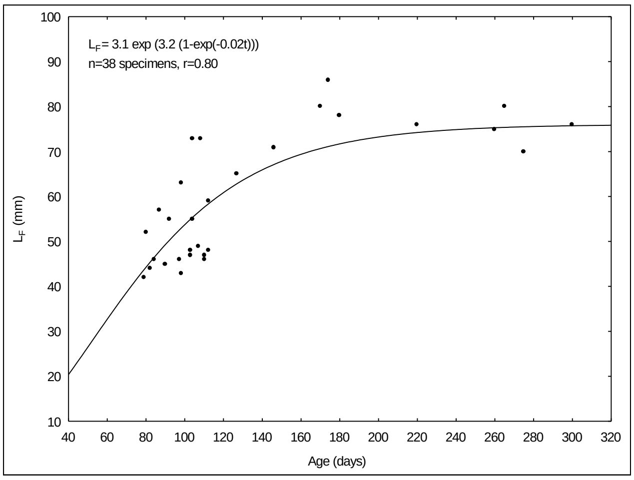

Fig. 3- Growth curve of *Engraulis encrasicolus* from the East of Tunisia fitted to the Laird-Gompertz Model.

juveniles where growth rate fell from 0.56mm/day in the 75th to 0.01mm/day in the 315th day.

The hatching dates distribution of survivors juvenile sardine and anchovy compared with the percentage of ripe females from January to December, showed that spawning of sardines took place from October to March whereas survival peaks of larvae were concentrated mostly between December and January (Fig. 4) while the spawning period of the anchovies occurred from April to October but survivors displayed an important peak in June (Fig. 5)

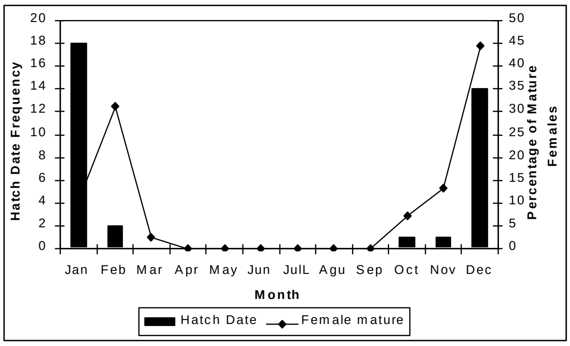

Fig. 4- Hatch date frequency distribution and percentage of monthly ripe female of *Sardina pilchardus* in the East of Tunisia.

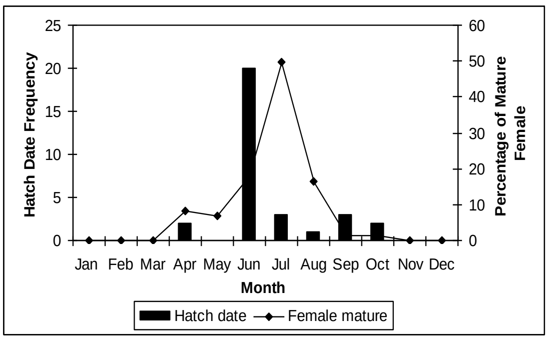

Fig. 5- Hatch date frequency distribution and percentage of monthly ripe female of *Engraulis encrasicolus* in the East of Tunisia.

DISCUSSION

Growth in length

The deposition of daily growth increments was directly ascribable to an endogenous circadian rhythm (Mugiya *et al*., 1981; Campana and Neilson, 1985), synchronized by environmental factors. Numerous synchronizing factors have been proposed including photoperiod (Campana and Neilson, 1985), feeding activity (Pannella, 1980; Campana, 1983) and temperature (Brothers, 1981).

In the present study, we considered that each otolith increment represented one day. Since methods to validate the daily nature of micorincrements deposition were not available, we referred to previous works performed on otoliths of anchovy and sardine. Effectively, the daily periodicity has been widely demonstrated for both species (Re, 1983, 1984; Cermeno *et al*., 2003). However a recent study (Cermeno *et al*., 2003) showed, for the anchovy, a loss of daily rhythm over long periods but these results were attributed to the aquarium conditions.

The age-length data showed a relatively high dispersion. It seemed possible to relate this to the protracted spawning periods for both species in the studied area, which last for the sardine from October to March and for the anchovy from April to October. Both growth curves fitted to the data sets did not display the lower part of the S-shaped Gompertz curve that usually occurred in the early growth phases (Moreau, 1987). This requires samples that cover the larvae phase of sardine and anchovy. Furthermore these samples will allow to improve the estimated parameters. In our case, such samplings were not possible with midwater trawl. Nevertheless, the predicted parameters from fitting the growth curves were in range with those obtained in the other Mediterranean regions. The estimated value of the instantaneous growth rate A_0= 0.016 (with L_0=10.8mm) of the sardine obtained in this study was in agreement with the value recorded by Alvarez and Morales-Nin (1992) who found a value of A_0= 0.02 (with L_0=12.28mm) in the Western Mediterranean Sea. Thus it could be hypotheses that the juvenile sardines of the East of Tunisia grew approximately with the same rate as those of the Western Mediterranean Sea. However compared to the growth rates reported for the sardine larvae (Dulcic, 1995; Rasoanarivo, 1997), the value obtained in the present study was much lower which is expected. For the anchovy, the growth rates were in good accordance with previous reports Palomera *et al*. (1988) and Dulcic (1997), in spite of the considered length range (3.9 to 23 mm; 2.8 to 35 mm). Also, the estimated value of the initial length when t=0 (L_0=3.1mm) obtained for the anchovy was in range with those found by Palomera *et al*. (1988) L_0=3.7mm in the

Western Mediterranean sea and by Regner (1985) L_0=3.54mm estimated from artificially reared larvae.
Comparing both species, the juvenile sardines had an instantaneous growth rate lower than the juvenile anchovies. This was also reported by Alvarez and Morales-Nin (1992) for the Western Mediterranean sea. The faster growth rate observed for juvenile anchovy may in part be related to the temperature. In Tunisian coasts, the spawning period of the anchovy corresponds to the time when the mean sea water temperature was the highest. A positive correlation between instantaneous growth rate and temperature was reported for the anchovy and the sardine larvae (Dulcic, 1995 and 1997). Houde (1989) found a positive correlation between weight-specific growth rate and water temperature for 26 species of fish larvae. Therefore in the investigated area, anchovy larvae were present when environmental conditions were optimal for growth contrary to the sardine that spawned in winter. According to Morales-Nin and Pertierra (1990) the difference in growth between these species remained until the adult stage.
The evolution of the growth rates of the juvenile sardine and anchovy were not similar. In deed for the sardine, the growth rate followed a dome-shaped trajectory; it increased initially then declined, with a peak recorded in the 166^{th} day. Accordingly growth in length of juvenile sardine remained rapid after metamorphosis until 166^{th} day whereas for the anchovy the growth rate fell continually. Such evolution was reported elsewhere for the northern anchovy, the bay anchovy and the Walleye Pollock (Houde, 1997). According to Houde (1997) species that displayed declining growth rate during early life have, apparently, more typical pelagic life histories during this period.

Hatch date analysis
The protracted spawning period is characteristic of small pelagic fishes, it could be interpreted as an adaptation to unpredictable environmental conditions. This spawning strategy is supposed to ensure for the newly hatched larva environmental conditions favoring their survival (Lasker, 1989; Alvarez and Morales-Nin, 1992; Le Page, 1995). The determination of the hatching dates allowed us to identify the time favorable for larva survival and subsequently to identify environmental conditions involved in the recruitment success. Our results showed that higher survival rates were observed for the anchovy in June while for the sardine the highest rate is recorded in December–January.
In the East of Tunisia, the mean sea surface temperature is about 25°C in summer. A strong thermoclin appears in June-July. In this season the homogeneity of the temperature and salinity may favor, in some conditions, an up-welling. In winter, the water column is mixed with temperatures around 14.5°C. An inflow of surface freshwater from the Atlantic is observed in this region. This water is characterized by a high productivity (Brandhorst, 1977; Sammari et Gana, 1995). These data showed that apparently high survival rates of the juvenile sardine and anchovy corresponded to periods of high productivity and/or thermal stratification. These factors are consistent with the three major classes of physical processes defined by Bakun (1996 *in* Agostini, 2000) as the "fundamental triad" favorable for larval success. Our results must be considered as a preliminary attempt to hatching date analysis because the smallest age group of the recruit was not represented due to gear selectivity.

CONCLUSION

The present paper is a preliminary attempt to determine age, growth and hatching date of juvenile sardine and anchovy using daily growth increments of otoliths. For both species, estimated parameters from fitting the Laird-Gompertz curve to the sets of data are roughly comparable to those obtained in the Mediterranean regions. The birthdates analyses indicate that highest survival rates of juvenile sardine and anchovy occur respectively in December-January and June.
It seems essential to include samples that cover the early larvae phase to improve the reliability of growth parameters. Also, a large data set on larvae and juvenile environment, egg production and juvenile survivors are needed in order to determine environment mechanisms that insure juvenile survival and then to obtain a better prediction of recruitment.

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