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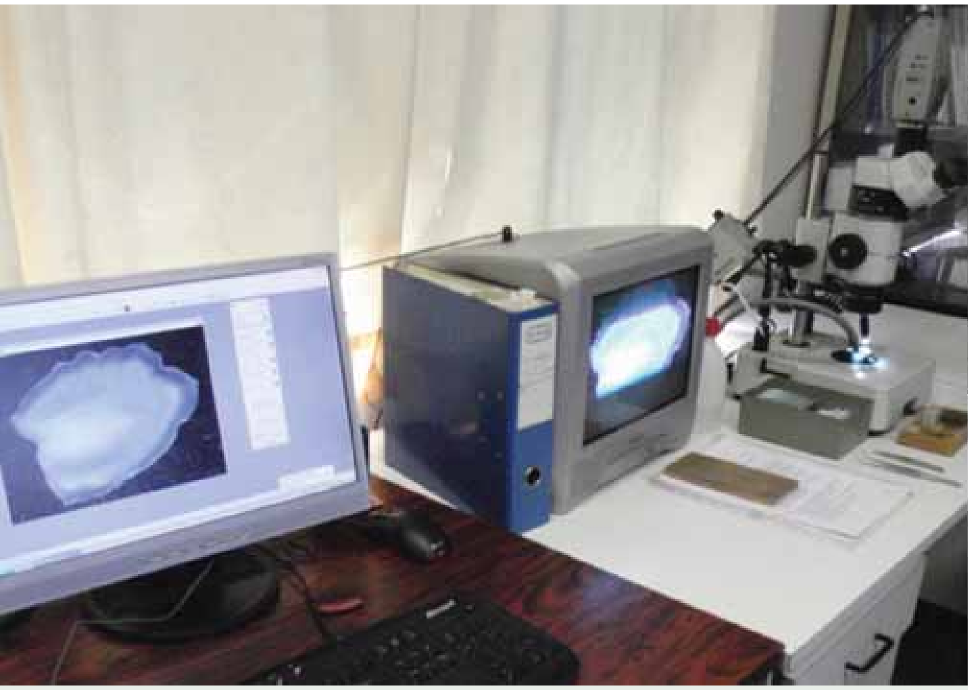

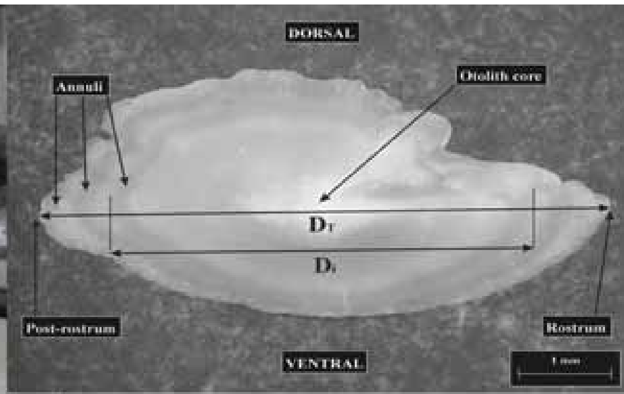

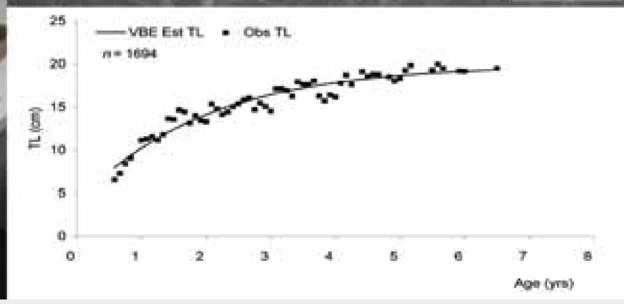

REPORT OF THE AD-HOC ON THE FIELD TRAINING COURSE ON OTOLITH/SPINES SECTIONING AND AGE READING

PIRAEUS, GREECE 19 - 23 JULY 2010

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

eastMed

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PIRAEUS, GREECE

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Hellenic Ministry of Foreign Affairs

Hellenic Ministry of Rural Development and Food

ITALIAN MINISTRY OF AGRICULTURE, FOOD AND FORESTRY POLICIES

GCP/INT/041/EC – GRE – ITA **Piraeus (Greece), 19-23 July 2010**

The conclusions and recommendations given in this and in other documents in the *Scientific and Institutional Cooperation to Support Responsible Fisheries in the Eastern Mediterranean* series are those considered appropriate at the time of preparation. They may be modified in the light of further knowledge gained in subsequent stages of the Project. The designations employed and the presentation of material in this publication do not imply the expression of any opinion on the part of FAO or donors concerning the legal status of any country, territory, city or area, or concerning the determination of its frontiers or boundaries.

Preface

The Project "Scientific and Institutional Cooperation to Support Responsible Fisheries in the Eastern Mediterranean- EastMed is executed by the Food and Agriculture Organization of the United Nations (FAO) and funded by Greece, Italy and EC.

The Eastern Mediterranean countries have for long lacked a cooperation framework as created for other areas of the Mediterranean, namely the FAO sub-regional projects AdriaMed, MedSudMed, CopeMed II and ArtFiMed. This made it more difficult for some countries in the region to participate fully in international and regional initiatives for cooperation on fishery research and management is concerned. Following the very encouraging experience of technical and institutional assistance provided to countries by the other FAO sub-regional Projects,

EastMed

was born to support the development of regional cooperation and the further development of multidisciplinary expertise necessary to formulate appropriate management measures under the FAO Code of Conduct for Responsible Fisheries and the principles of the Ecosystem Approach to Fisheries (EAF) to ensure rational, responsible and participative fisheries management

The project's **longer-term objective** aims at contributing to the sustainable management of marine fisheries in the Eastern Mediterranean, and thereby at supporting national economies and protecting the livelihoods of those involved in the fisheries sector.

The project's **immediate objective** is to support and improve the capacity of national fishery departments in the sub-region to increase their scientific and technical information base for fisheries management and to develop coordinated and participative fisheries management plans in the Eastern Mediterranean sub-region.

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Preparation of this document

This document is the final version of the Report of the *ad-hoc* field training course on otolith/spines sectioning and age reading, organized by the FAO-EastMed Project (Scientific and Institutional Cooperation to Support Responsible Fisheries in the Eastern Mediterranean) in Piraeus (Greece), 19-23 July 2010.

Acknowledgements

The Fisheries Laboratory of the Ministry of Rural Development and Food of Greece, that provided hospitality and technical assistance in the organization of the meeting is gratefully acknowledged.

EastMed, 2010. Report of the ad-hoc on the field training course on otolith/spines sectioning and age reading. GCP/INT/041/EC – GRE – ITA/TD-02. Athens 2010: 32 pp.

ABSTRACT

The ad-hoc on the field training course on otolith/spines sectioning and age reading was held in Athens, Greece from the 19th to 23rd of July 2010. It was organized with the specific purpose of introducing the basic knowledge on the most common techniques and equipment necessary to estimate fish age and growth through the analysis (or reading) of otoliths and other bony structures. This was considered as a first step towards the harmonisation of national expertise in the field of age and growth studies. The training course was divided into several sessions, during which it was possible to focus on the main aspects regarding the age estimation of fish species, from the setting up of the laboratory equipment to the age estimation of selected specimens and the estimation of growth parameters.

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REPORT OF THE AD-HOC ON THE FIELD TRAINING COURSE ON OTOLITH/SPINES SECTIONING AND AGE READING

PIRAEUS, GREECE
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1. Introduction

The estimation of growth parameters is one of the essential information required for describing and understanding the life-history and the state of the stocks of marine living resources. Several methods, both direct and indirect, can be used to estimate age and growth of fish and other marine resources. Among them, the analysis of hard and calcified structures such as vertebrae and otoliths is probably the most common tool in the definition of life history traits in fish and marine vertebrates. In particular, some life history aspects like the age at first sexual maturity, mortality, recruitment, absolute age and other biologically related parameters are all stock descriptors that can be determined from the examination of the bony structures in fish. Therefore, studies on the estimation of age and growth in marine fish are mainly conducted, from the examination of the so-called "calcified structures" (Wright *et al.*, 2002).

One of the objectives of the FAO Project "Scientific and Institutional Cooperation to Support Responsible Fisheries in the Eastern Mediterranean-EastMed", is to support national fishery departments improve their capacity to increase their scientific and technical information for fisheries management and to develop coordinated and participative fisheries management plans in the Eastern Mediterranean sub-region. During the 1st Coordination Committee meeting of EastMed Project (Athens, 19-20 April 2010), the Cypriot focal point requested that Cypriot experts be trained in otolith age reading for some target species exploited by Cyprus. Thus an ad-hoc training course on otolith age reading was agreed upon.

The training course was held at the Fisheries Laboratory (Ministry of Rural Development and Food) Piraeus, Greece from the 19th to 23rd of July 2010. It was organized with the specific purpose to introduce the basic knowledge on the most common techniques and equipment necessary to estimate fish age and growth through the analysis (or reading) of otoliths and other bony structures. This was considered a first step towards the harmonisation of national expertise in the field of age and growth studies.

The training course was divided into several sessions, during which it was possible to focus on the main aspects regarding the age estimation of fish species, from the setting up of the laboratory equipment, up to the age estimation of selected specimens. In particular, the training was organized to deal with the following issues:

- Preparation of laboratory equipment(otolith/spines sectioning, Image Analysis System)
- Principles of age validation
- Techniques for processing of otoliths , case studies : age estimation in *Spicara smaris* and *Mullus barbatus*
- Estimation of growth parameters (VonBit and LFSA)

The training allowed national experts to progress in the investigation of traditional fisheries resources. The course also provided to the trainees the methodology to initiate research activities on species that were never studied before in the region.

2. Preparation of Laboratory Equipment

The first part of the training was devoted to the illustration of all the procedures necessary to set up a laboratory for otoliths analysis. The following equipment from the Fisheries Laboratory, was used for the needs of the training on “Fish age determination through otoliths reading”.

- One stereomicroscope, a “Leica” model WILD M8, which was connected with a digital camera, a “Sony” model EXWAVE HAD (Fig. 1).
- One PC where images were stored and processed throughout the Image Analysis Software (Image Pro-Plus 5.0) (Fig. 1).
- A grinder/polisher machine, a “Struers” model Dap 6 (Fig. 2)
- A precision cut-off machine with low and high speed operation (up to 3000 rpm), a “Struers” model Accutom-2 (Fig. 3)

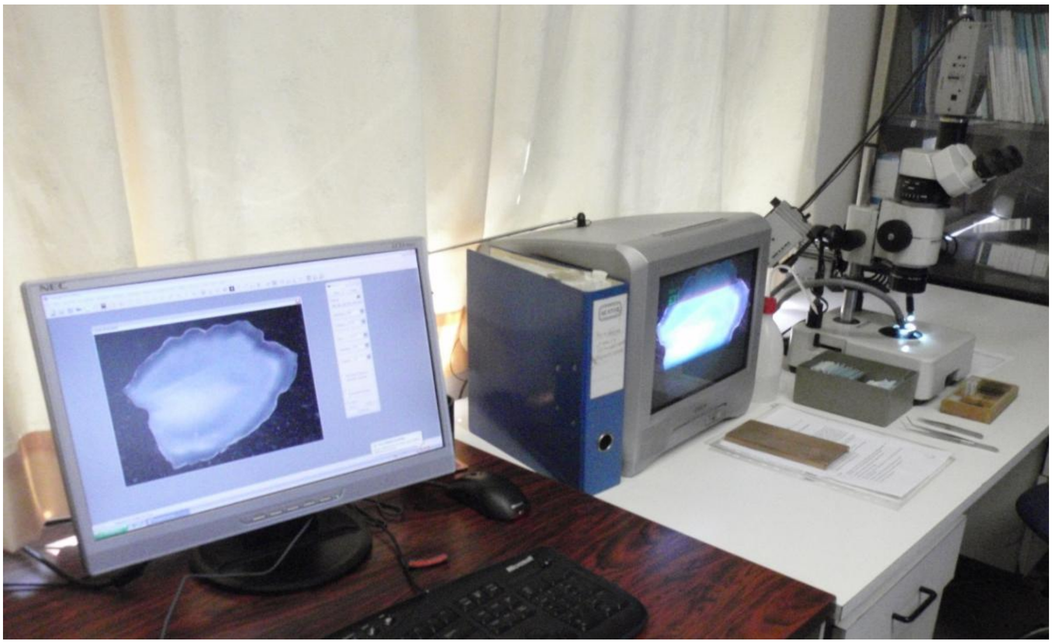

Fig. 1 Stereomicroscope with digital camera and Image Analysis System

Fig. 2 Grinder/polisher machine

Fig. 3 Cut-off machine used during the training course

3. Introduction to the estimation of fish age, growth and basic knowledge of otoliths

At the end of the preparatory phase attention was given to some theoretical aspects of the aging process, however this was done before using the laboratory instruments, describing the best way to handle samples and how to estimate individual fish age through otolith reading. During this part of the training the main properties that make up the otoliths and the essential tools required in the estimation of age and growth were described.

To describe better these aspects, a presentation was given providing the participants with basic information on the several technical and practical processes required to "read" fish otolith for the determination of age. Following this stage, the various theoretical aspects regarding the estimation of growth parameters were presented. Finally, the schemes were used to produce an Age-Length Key (ALK) with a description of their possible applications.

4. Estimation of Age

It is known that hard body parts often reveal the presence of "rings", which are laid down incrementally (daily, monthly or annually). These structures provide a means of aging fish. Thus, the first step in the process for estimating age was to identify in the selected structures (e.g. otolith, spines etc.) the incremental growth pattern and to count the growth rings.

After counting the rings, the consistency of the process for estimating age was determined. This was done through the observation of the same structure by several "readers". The repeatability of the procedures for estimating age was evaluated and the estimated ages were calibrated by more than one operator.

The second step in the estimation of age was **age validation** (Beamish and McFarlane, 1983). Validation is a process that provides information on the accuracy of the method used to estimate the age of fish. In other words the validation is the demonstration of the periodicity of the pattern which is used to infer age. Direct methods to validate the interpretation of age involve the use of fish in which the age is known. Examples of these methods include tagging and recapture experiments (e.g. chemical marking, coded wire tags), radiometric assessment and radiocarbon dating of specimens. Indirect methods to validate the interpretation of age include the use of the length frequency distribution of the samples and looking for a correspondence with modes and/or marginal increment analysis, which is the study of the seasonal pattern of the width outside the last annulus independent of the type of the edge (opaque or hyaline).

5. Practical Exercise

5.1 Techniques for processing Otoliths

The otoliths after their extraction from the cranium, must be cleaned before storage in order to remove any adhering tissue after the dissection. They can be stored completely dry in small glass, plastic vials or paper envelopes.

Otoliths from large fish which tend to be thick and more oval in shape usually require sectioning in order to view the growth zones. For doing this, otoliths must be embedded separately in a support medium. The principal embedding media usually are polyester epoxy resins (Bedford B.C. 1983).

Each otolith has to be embedded in one single mould, which in our case was made of aluminium (Fig. 4). The mould measured 25 cm by 15 cm by 1 cm (L x W x H), had five separate columns. In each column a number of otoliths were embedded.

Fig. 4 Aluminium mould used for embedding otoliths

At the beginning it was very important to cover the mould with a thin layer of a release agent. This made the separation of the hard resin from the mould very easy. The second step was the preparation of the first layer. This layer was made of clear casting resin (100 g), hardener (2 g) and polyester black paste (10 g). The given quantities were estimated after many trials for the specific mould. The above mixture was put into the mould covering half of its height. This layer was allowed to settle for 24 hours at room temperature, or for 5 min at 40-50^{o}C, which then becomes hard enough thus avoiding the otoliths to sink.

After the otoliths were placed in the mould, the second layer of the resin was added. During this step it is very important to mark the spot of the otolith nucleus, since during the sectioning it is essential to cut off the otoliths very close to the nucleus. For that a piece of polyamide monofilament was placed vertically to the otolith above the nucleus. The second layer of resin which is exactly the same as the first one was added afterwards.

When the resin was hard enough the columns were removed from the mould. The “polyamide monofilament finger print” on the columns surface indicated where the exact position of the otolith nucleus was. Using the sectioning machine (Fig. 5) the columns between the “fishing line finger prints” were cut in small cubes in which a single otolith was present. These cubes were then cut into very thin sections (approximately 0.7 mm).

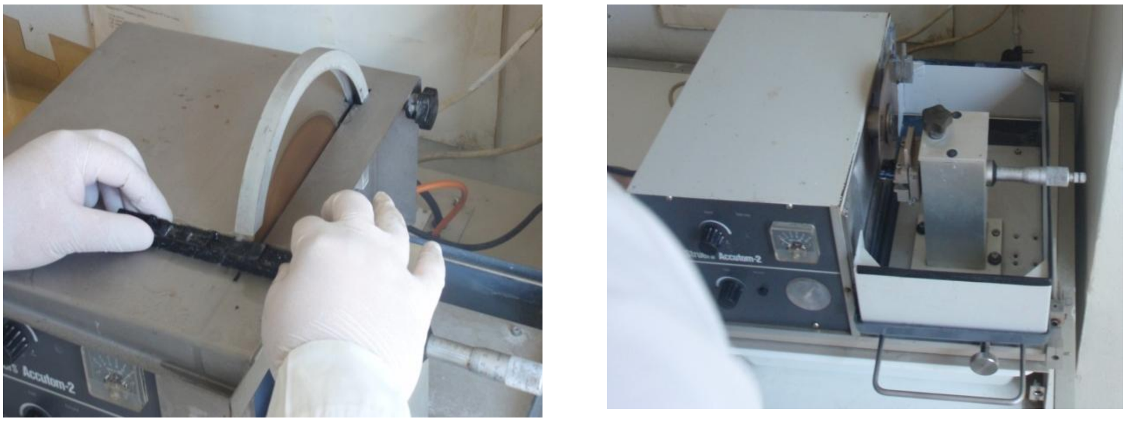

Fig. 5 Otolith sectioning

Figure 6 shows the cubes which were ready to be smoothed at the grinder/polisher machine (see Fig. 2). When grinding, polishing and sectioning were completed, the otolith thin sections were observed under the stereomicroscope with reflected light.

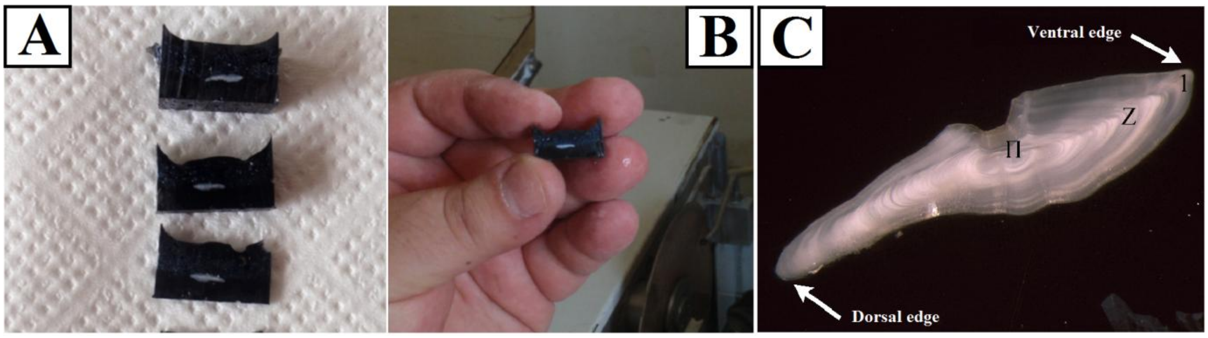

Fig. 6 Otolith in cubes (A and B) and otolith thin sections (C). For the demonstration at the Fisheries Laboratory otoliths from hake (*Merlucius merlucius*) were used. The thin otolith section belongs to a hake of total length 31 cm and has the 2nd annulus in progress

6. Observation of Otoliths

Otolith observations and annuli measurements were made for *Mullus barbatus* and *Spicara smaris*, by the use of Image Analysis System.

Sagittae of *Mullus barbatus* (red mullet) and *Spicara smaris* (picarel) specimens are routinely observed whole without sectioning. The otoliths were immersed in a clear liquid (usually water) against a black background and read with a stereomicroscope using the proper magnification, under a source of reflected light. Dark and white rings called hyaline and opaque zones respectively could have been distinguished. These zones follow the central opaque area where a hyaline spot on the centre corresponds to the otolith nucleus. Readings of annual rings were usually made on the concave side and the posterior part (post-rostrum) of the otolith (Fig. 7). However, ring continuity was checked on the anterior (rostrum) and wherever possible on the dorso-lateral edge (Kerstan, 1985; ICSEAF, 1986).

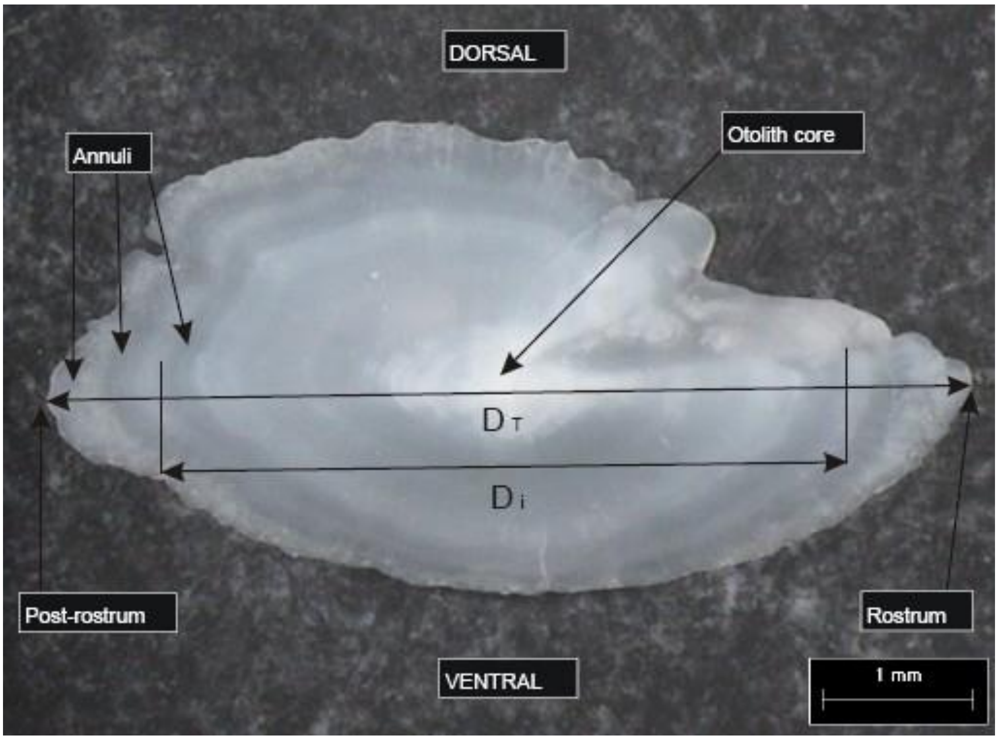

Fig. 7 Concave side of *Spicara smaris* right otolith with tree annuli. D_T= Total otolith diameter, D_i= Diameter of i^{th} annulus

In each otolith (preferably the right one), total otolith radius and annual (hyaline) radii were measured along the post-rostrum axis with Image Analysis. According to Macer (1977), Kerstan (1985) and ICSEAF (1986) the annual radii are measured along the post-rostrum axis because a) they are more readable on this part of the otolith, b) a single hyaline zone on the post-rostrum sometimes separates into two or more rings on the rostrum and c) the rostrum often breaks during otolith removal (Karlou-Riga et al, 1997).

The age was determined by counting the number of hyaline zones; these zones were assumed to be laid down once per year.

As a first approach for demonstration purposes otoliths of the species under study stored at the Fisheries Laboratory were used. After the trainees were shown how the annuli are detected and measured, there was a measurement of annuli for the otoliths brought from Cyprus. Both *M. barbatus* and *S. smaris* otoliths from the Fisheries Laboratory presented a distinct pre-annual ring which was identifiable where the anti-rostrum and rostrum were not yet formed. This pre-annual ring which usually is separated from the first annual hyaline ring, may join with it forming a broad hyaline zone.

7. Annuli measurements and observation of *Spicara smaris* otoliths

The development of the first annulus can be detected by following the progression of the smaller modal fish length group, through successive monthly samples (Karlou-Riga et al., 1997). Monthly length frequencies for picarel (Fig. 8) were followed so as to see the progression of the smaller fish modal length during the year and to study the appearance of the otoliths.

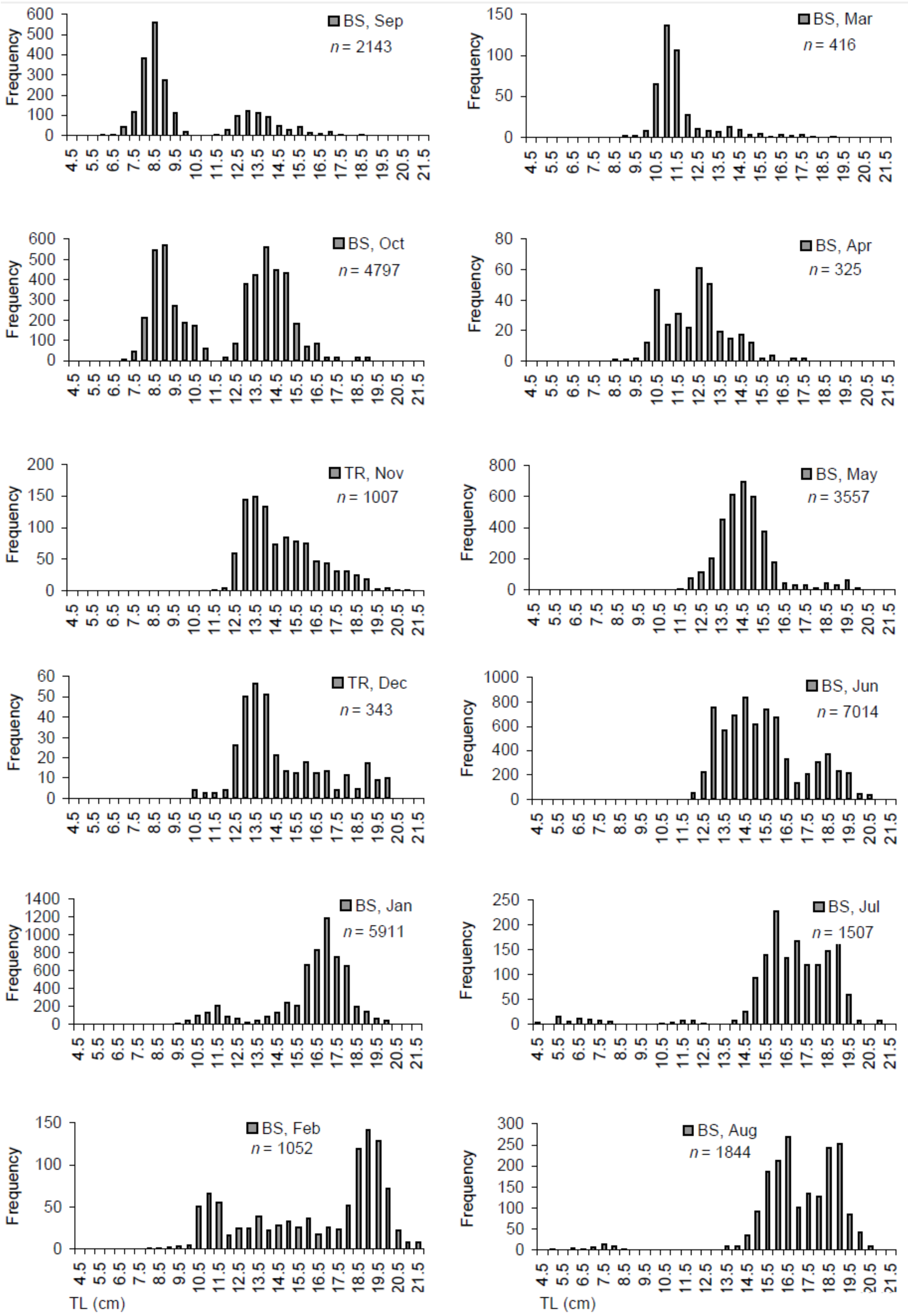

Fig. 8 Monthly length frequency distributions for picarel. BS = Beach seiner, TR= trawler

The smaller specimens, which apparently belonged to the cohort of that year, were caught in July (Fisheries Laboratory, 2001). The otoliths of these fish were very delicate and had a broad hyaline zone as described before (Fig. 9A). Yet, this zone was less distinguishable than the true annuli as it was proved later in the year, while the otoliths from fish of the next modal length showed a distinct separate hyaline zone on the edge (Fig. 9B).

As can be seen from the length frequency distributions (see Fig. 8) the smaller specimens form a distinct group. The size progression of this group of fish can be easily followed in August, September and October. The respective otoliths showed a progressive increase of the hyaline zone on the edge. In the samples from November and December only a few small fish were collected and did not form a distinct group. In January, the specimens of the 1st mode, which was still discrete, seemed to constitute the progression of the previous year's cohort. Some otoliths of these fish showed a separate distinct hyaline zone on the edge. This hyaline margin increased progressively in the successive months; while in some otoliths from July the hyaline zone appeared completed and was interpreted as the 1st annulus (Fig. 9C). The successive hyaline zones (one per year) were interpreted as annuli based mainly on the gradual decrease of their width and their formation at progressively greater distances from the nucleus (Fig. 9D). In Figure 9D, the annuli next to the 3rd one were shown to be very close, while the contrast between opaque and hyaline area was not clear.

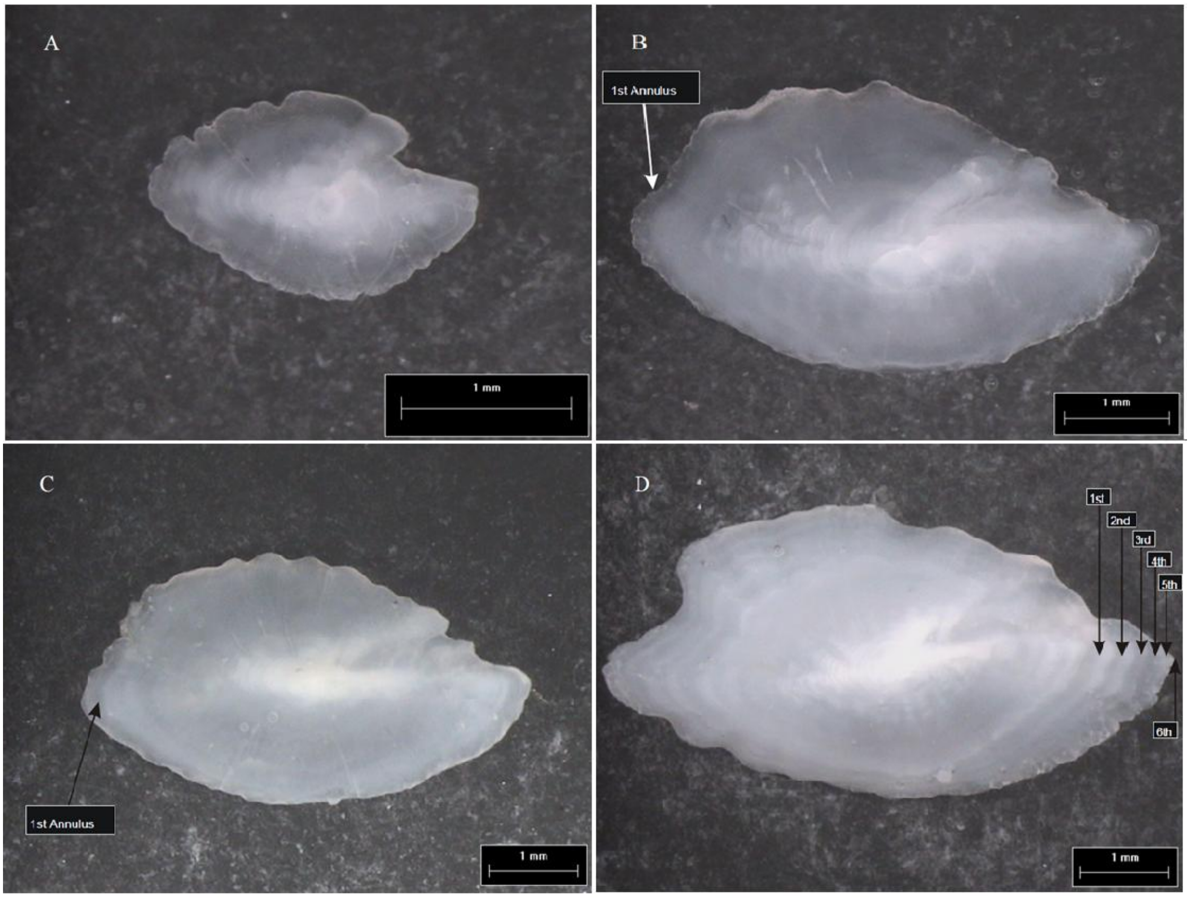

Fig. 9 *Spicara smaris* otoliths. TL= fish total length, DC= date of capture. (**A**) 1st annulus has not been started but only preannual zone can be seen. TL= 51 mm, DC= July 1999. (**B**) 1st annulus is on the otolith edge. TL= 102 mm, DC= July 1999. (**C**) 1st annulus completed and opaque on the edge. TL= 149 mm, DC= July 1999. (**D**) Six annuli with the 6th on the edge, TL= 193 mm, DC= June 1999.

The time of hyaline formation was studied following monthly the Marginal Increments (MIs). The marginal increment was defined as the width outside the last annual hyaline ring independent of the type of the edge (opaque or hyaline). This procedure was followed because it was difficult to detect the beginning of the subsequent annual hyaline ring and thus to measure the opaque increment only. In particular, the time of the 1st hyaline ring formation was estimated by comparing monthly the percentage of the radius R_0 (otolith radius, where 1st annulus is not completed) to R_1 (1st annulus radius completed). Median values, in general, were preferred rather than mean values (Kerstan, 1985, Karlou-Riga et al, 1997). The time of hyaline formation was used to assign the fish to the correct age group (Karlou-Riga et al., 1997). It can be also noted that in general the percentage of hyaline edges increased progressively from January attaining their highest value in June and their lowest in October (Fig. 10).

For picarel, since the otoliths have a symmetrical shape the diameters were used for otoliths measurements. The marginal increments (MIs) widths next to the 1st (MI1), the 2nd (MI2), the 3rd (MI3) and the 4th (MI4) annuli showed a seasonal progression. The lowest values of MIs which were found in October and the highest values in May-June, indicate that the annuli were completed between June and October (Fig. 11). Particularly in the case of the 1st annulus, it was found that this zone is completed next to June (Fig. 12; Karlou-Riga, unpublished data).

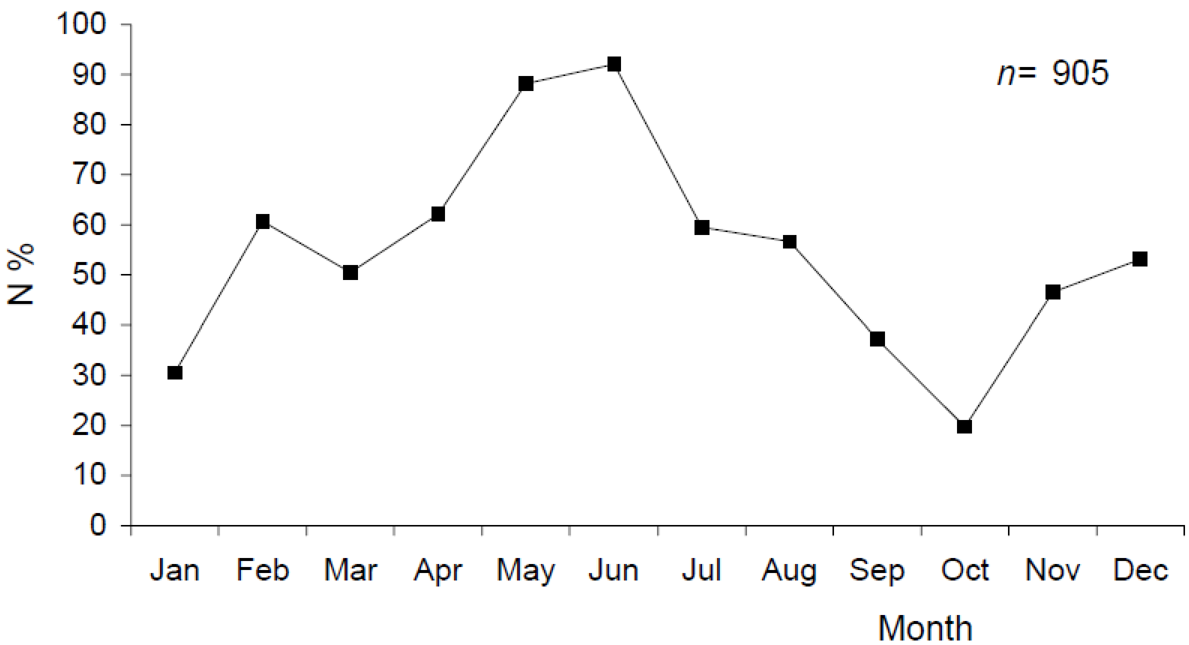

Fig. 10 Monthly percentage of *Spicara smaris* otoliths with hyaline on the edge

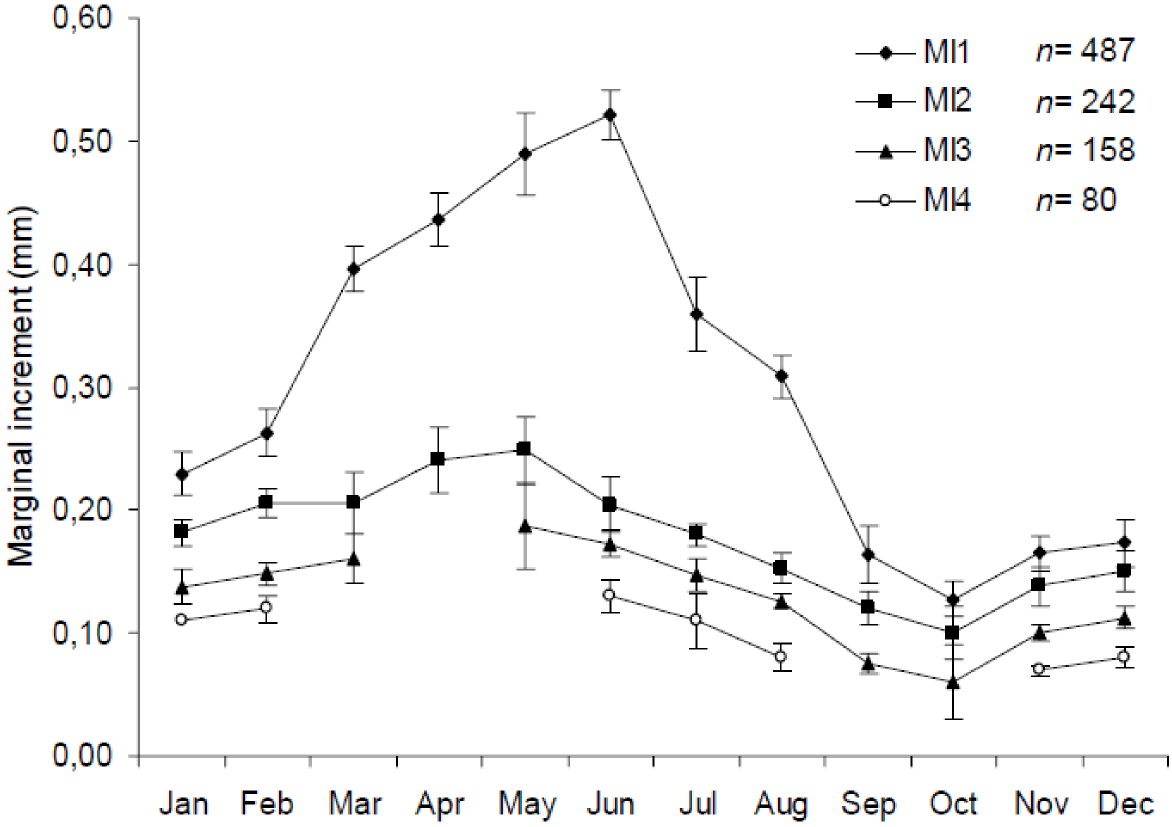

Fig. 11 Monthly mean values of *Spicara smaris* otolith marginal increments next to the 1st (MI1), the 2nd (MI2), the 3rd (MI3) and the 4th (MI4) annulus. Bars are 2 SE of the mean.

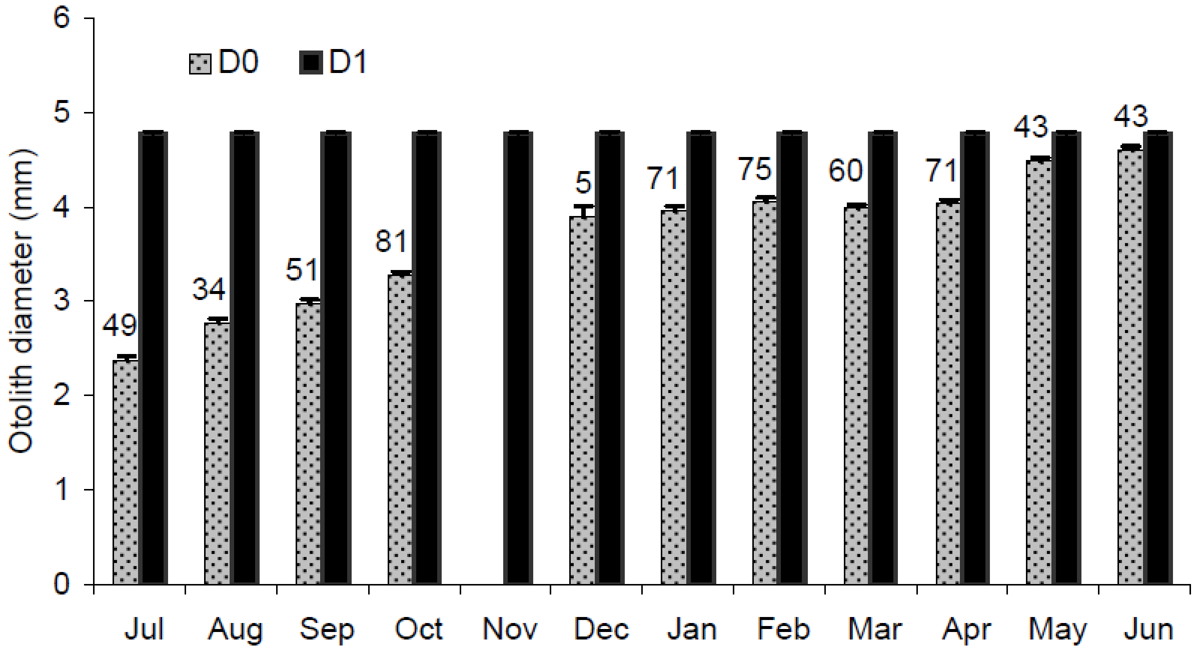

Fig. 12 Monthly relation of *Spicara smaris* otolith diameter (D_0) to the 1st annulus diameter (D_1). D_0= otolith diameter, where the 1st annulus has not been started or finished yet. Vertical lines above the bars are 1 SE of the mean. Numbers shown are the numbers of otoliths with D_0. D_1 is measured from 487 individuals.

Criteria for Age interpretation

The time of annulus formation (2nd semester) including the date of capture, the otolith edge and the spawning period were used as criteria for age interpretation. Since picarel spawning period lasts from March to June (Karlou-Riga and Makrakos, 2001), the fish, which were spawned on this period, constituted one cohort. Consequently, if **n** was the number of annual hyaline rings, the age interpretation was as follows:

a. Fish caught from 1st of January to 30th of June
Otolith edge hyaline: Age group equal to n (including that on the edge).
Otolith edge opaque: Age group equal to n+1. In this case the n+1 annulus will be laid down during the current year.

b. Fish caught from 1st of July to 31st of December
Otolith edge hyaline: Age group equal to n (including that on the edge). The annulus on the edge is expected to be completed before the end of the year.
Otolith edge opaque: Age group equal to n. (Karlou-Riga, unpublished data)

8. Annuli measurements and observation of *Mullus barbatus* otoliths

The same procedure was followed for the specimens of red mullet. As it can be seen from the length frequencies distributions (Fig. 13) the smaller specimens appear in August forming a clear distinct mode (Fisheries Laboratory, 2001).

The otoliths of smaller specimens of the first modal length group are homogeneous and opaque, except of the nucleus, which is hyaline (Fig. 14). In the bigger specimens of the same mode, alternate thin opaque and hyaline rings appear forming a broad semi-opaque zone (Fig. 15), which is better distinguished during the next months of autumn and particularly in January when a distinct hyaline edge is formed (Fig. 16). This hyaline edge increases during the next months, forming at last the first annulus (Fig. 17). The first annulus in red mullet has the formation of a double ring and this is the formation also shown for the other annuli (Fig. 18; Kalagia et al., 2000).

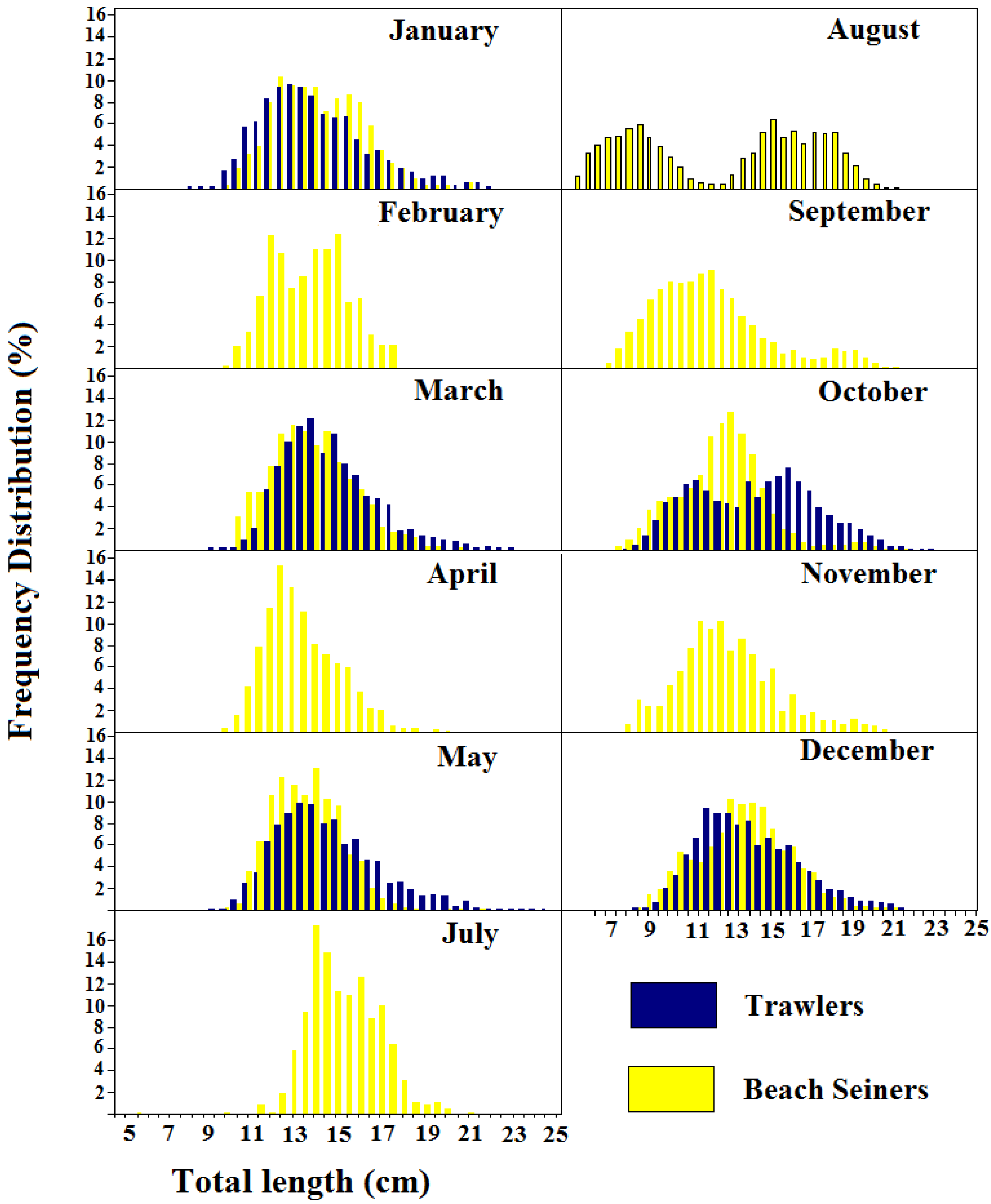

Fig. 13 Monthly length frequency distribution of *Mullus barbatus*

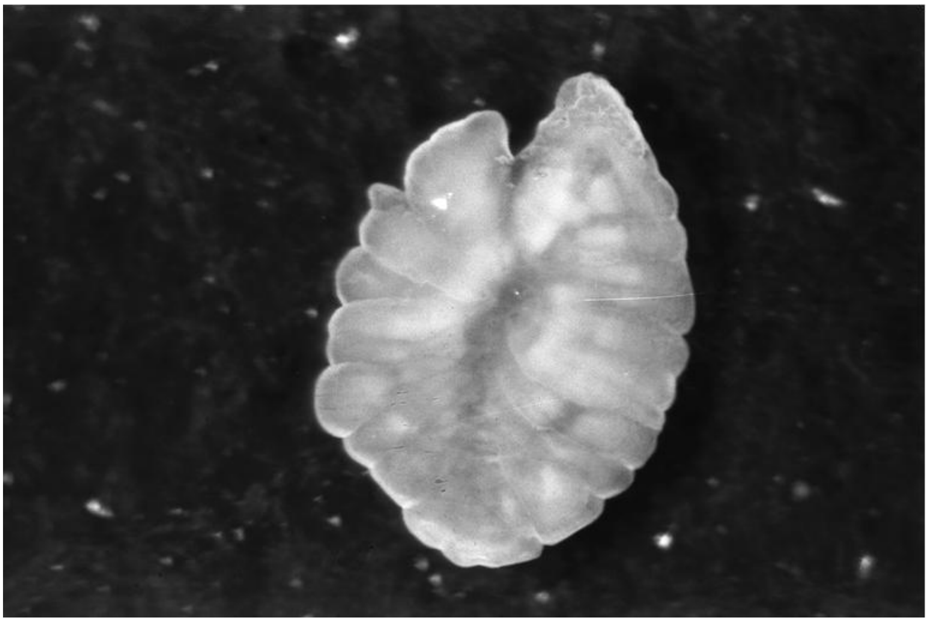

Fig. 14 Otolith of red mullet caught in August - fish total length 4.6 cm (age group 0**)**

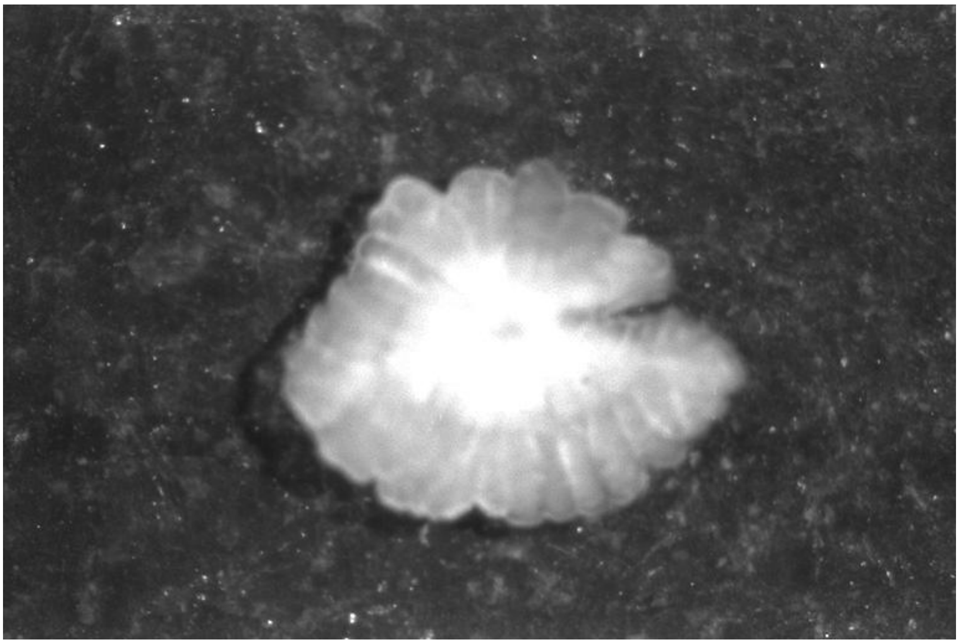

Fig. 15. Otolith of red mullet caught in August - fish total length 9.6 cm (age group 0).

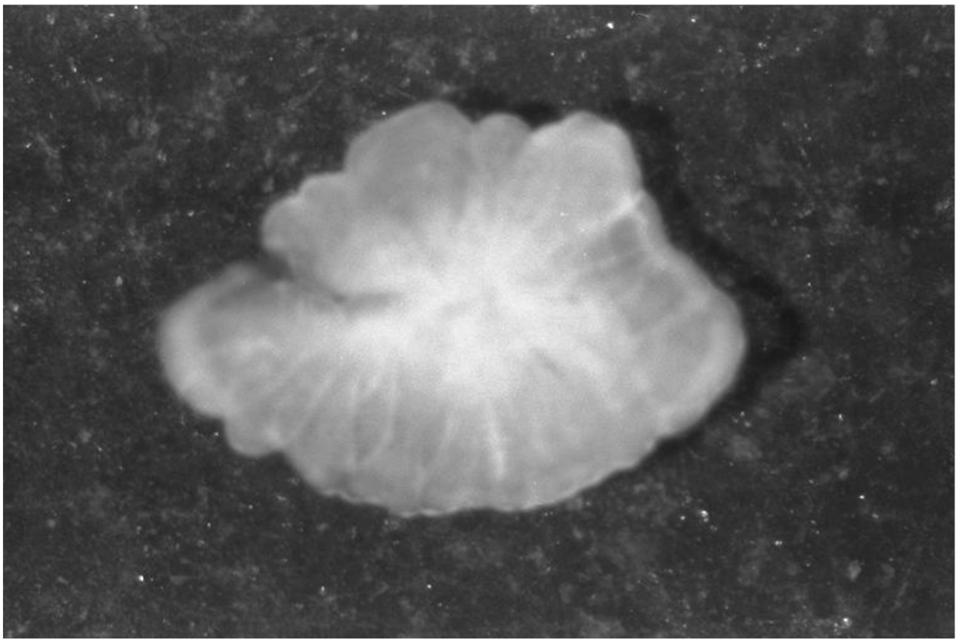

Fig. 16 Otolith of red mullet caught in January, hyaline on the edge - fish total length 12.3 cm,

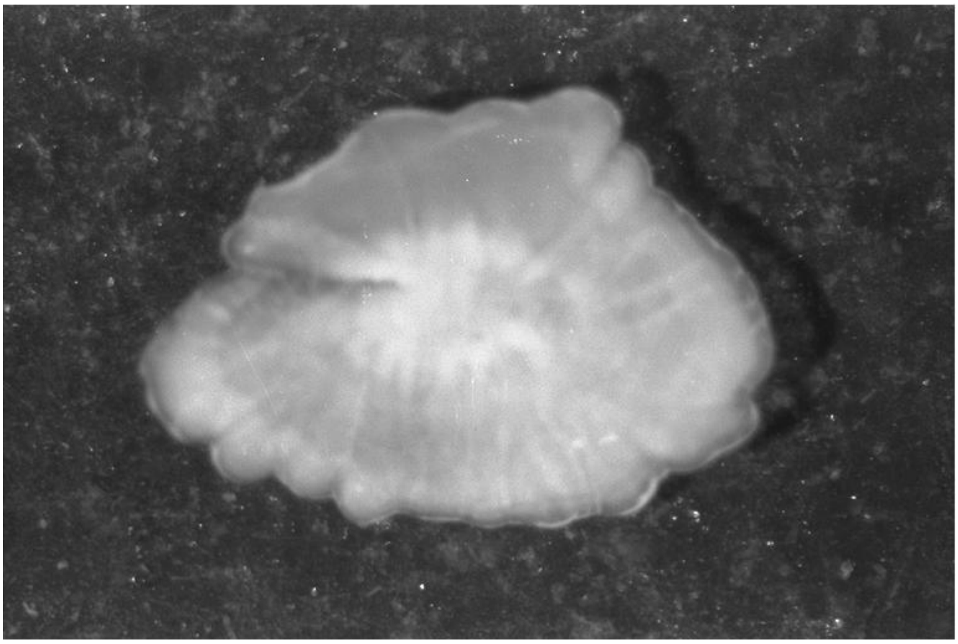

Fig. 17 Otolith of red mullet caught in April, the first annulus on the edge - fish total length 14.9 cm

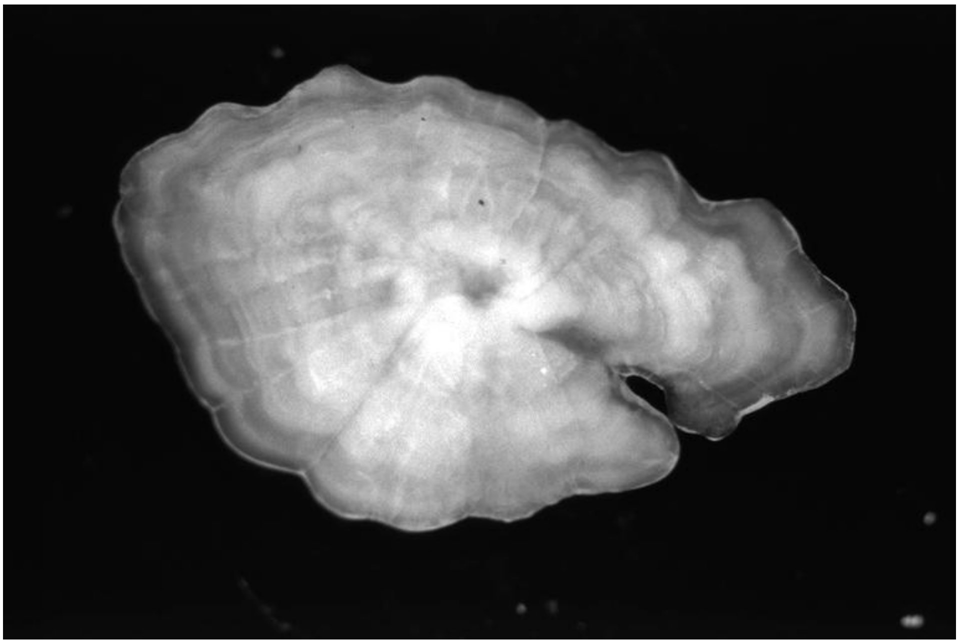

Fig. 18 Otolith of red mullet caught in May with three annuli - fish total length 18.6 cm

For red mullet, the marginal increments present their lowest value in August and their highest in January. This means that the annual ring is formed after January and before August (Fig. 19; Fisheries Laboratory, 1998).

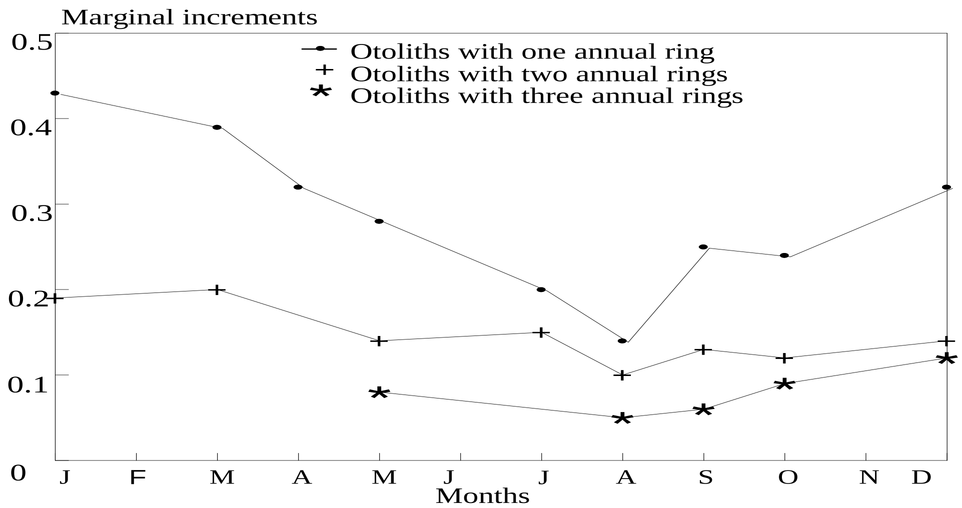

Fig. 19 Red mullet mean marginal increment (1989 - 1993)

Criteria for Age interpretation

The time of annuls formation (1st semester) including the date of capture, the otolith edge and the spawning period were used as criteria for age interpretation. Since red mullet spawning occurs in April-May (Fisheries Laboratory, 1998), the fish, which spawned on this period, constituted one cohort. Consequently, if **n** was the number of annual hyaline rings, the age interpretation was as follows:

a. Fish caught from 1st of January to 30th of June
Otolith edge hyaline: Age group equal to n. In this case the n annulus will be laid down during the current year.
Otolith edge opaque: Age group equal to n

b. Fish caught from 1st of July to 31st of December
Otolith edge hyaline: Age group equal to n-1
Otolith edge opaque: Age group equal to n. (Fisheries Laboratory, 1998)

9. Estimation of growth parameters

To estimate growth parameters, the von Bertalanffy growth equation was fitted to the mean observed lengths at age. During the early life of eastern Mediterranean species there is fast growth, when these young specimens are fully recruited to fishing (Caddy, 1989a, 1989b). Therefore, the mean lengths at age must be expressed over time intervals of less than 1 year. This forces the growth curve close to the origin and provides a better fit of data. Consequently, for each month of sampling of each year an age-length key was constructed (based on the total number of fish collected). Assuming January 1st, as a conventional birthday, the age was expressed in months (Karlou-Riga et al., 1997).

The data used were those of the Fisheries Laboratory. This is because neither the Cypriot length frequencies showed a clear modal length progression nor the annuli growth pattern was similar to the Greek one. Thus mean lengths at age as constructed for each month of sampling were used for the estimation of growth parameters.

For the von Bertalanffy growth parameters $\mathbf{L_\infty}$, **K,** $\mathbf{t_0}$**,** the programs VONBER from the LFSA software package (Sparre, P., 1987) and the VONBIT (Stamatopoulos, C., 2005) were used.

LFSA is a computer software, designed to run under DOS. The program **VONBER** estimates the growth parameters $\mathbf{L_\infty}$**, K,** $\mathbf{t_0}$**,** in the ordinary von Bertalanffy (1934) model:

$$L_i = L_\infty \cdot \left(1 - e^{-K \cdot (t_i - t_0)}\right)$$

from observed age and length **($t_{(i)}$, $L_{(i)}$), i=1,2,…n**

The program uses a nonlinear least squares procedure, where the estimates of $\mathbf{L_\infty}$**, K** and $\mathbf{t_0}$ are those that minimize the sum of squared deviations (SSD) between the observations and the estimated growth curve. The procedure is iterative and requires an initial guess of the parameters (which may be determined by the program or given by the user).

VONBIT stands for von Bertalanffy Iterative Approach and it is a computer software designed to run under Windows. The presented linear regression method for fitting the von Bertalanffy growth function to data on size at age is also directly applicable to tag and recapture data, thus offering a more integrated approach than other similar methods.

For this method the driving parameter is **K**, trial values of which are used in simple linear regressions, with age as the independent variable and observed size as the dependent variable. The advantage over general-purpose non-linear procedures is that the optimization process concentrates on values of **K** only and deals with stationary points on a curve, rather than on a surface. This makes it possible to select a single population value for **K** as a global optimum. The method involves very few computational steps, and although it remains an iterative approach (in that trial values of **K** are examined), the number of iteration steps as well as the accuracy of parameter estimation are under the control of the user.
Given data on size at age:

$(t_1, L_1), (t_2, L_2), \ldots (t_n, L_n)$

it can be shown (see derivation of formulae) that for a given value of **K**, size can be expressed linearly in the general form:

$$L_t = a_0 + a_1 X_t \qquad (1)$$

$L_t = a_0 + a_1 X_t$ where a_0 and a_1 are parameters of a simple linear regression, and the independent variable X_t is a function of **t**. Expression (1) of length-at-age does not depend on a knowledge of the secondary parameters L_∞, and t_0. The constant a_0 represents size at any age t_a and the coefficient a_1 refers to size increase over the period t_a and any other age t_b . (Stamatopoulos and Caddy, 1999)

The estimated parameters for the Fisheries Laboratory *Spicara smaris* **(a)** and *Mullus barbatus* **(b)** data were :

(a)

$L_\infty = 19.91$, $K = 0.51$, $t_0 = -0.41$	From LFSA program and
$L_\infty = 19.93$, $K = 0.51$, $t_0 = -0.42$	From VONBIT program

The von Bertalanffy growth curve is illustrated in Figure 20

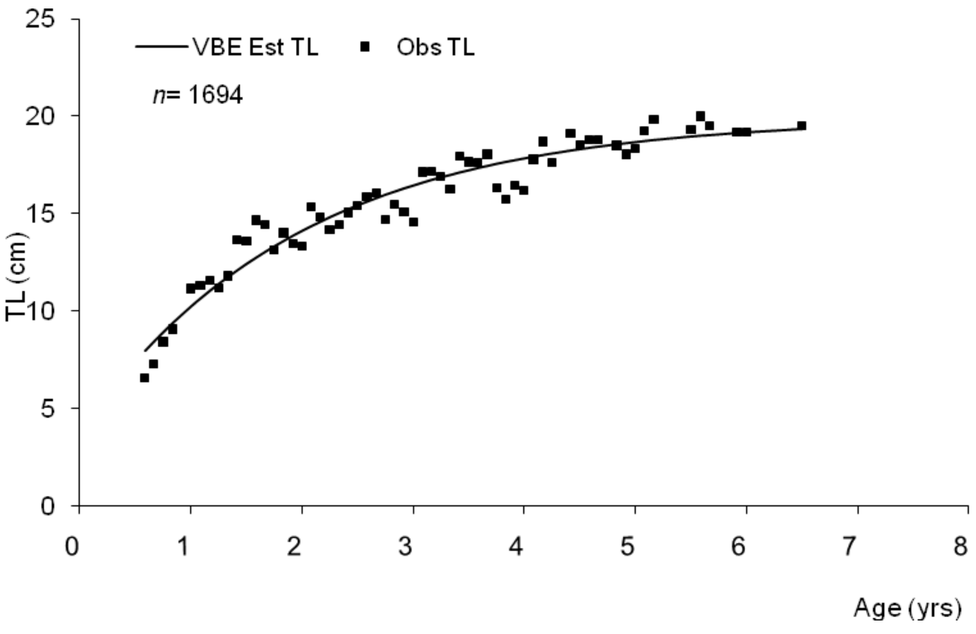

Fig. 20 Von Bertalanffy growth curve of *Spicara smaris* based on otolith age reading.

(b)

L_∞ = 24.74, K= 0.38, t_0= -0.80 From LFSA program and
L_∞ = 24.87, K= 0.37, t_0= -0.71 From VONBIT program

The von Bertalanffy growth curve is illustrated in Figure 21

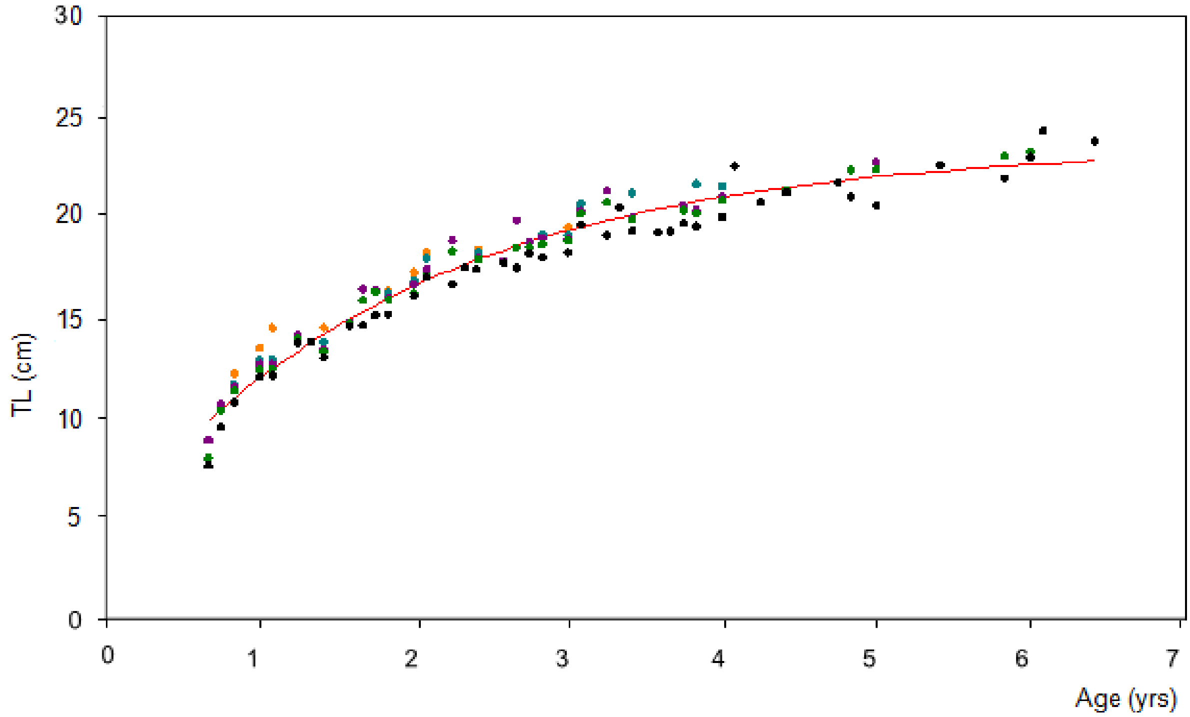

Fig. 21. *Mullus barbatus* von Bertalanffy growth curve based on otolith age reading.

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ANNEXES

Annex I. List of participants

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Annex II. Agenda

FAO-EastMed Working Document.
ToR for the ad-hoc on the field training course on otolith age reading

Ad-hoc on the Field Training Course on Otolith/Spines sectioning and Age reading

Piraeus, Greece, 19-23 July 2010

Tentative agenda and time table

1st day

Monday 19 July, 9:00-17:00 :

1. Introduction to the equipments used for the otolith/spines sectioning
2. Preparation of non transparent (black) resin blocks with embedded otoliths/spines
   The blocks should be remained until dry for two days
3. Introduction to the use of Image Analysis System

1st coffee break: 11:00 am (15 min)
Lunch: 13:30 (half an hour)
2nd coffee break: 15:30 (15 min)

2nd day

Tuesday 20 July, 9:00-17:00

4. Introduction to the otolith use for age reading (otoliths structure, otolith preservation and observation, sectioning, true annuli/pseudo-annuli, annuli measurement)
5. Principles of age validation (length frequency, marginal increment)
6. Information on *Spicara smaris* biology
7. Otolith observation and annuli measurement by the use of Image Analysis System of *Spicara smaris* otoliths
8. Construction of marginal increments charts
9. Age assignment to age groups – age length key
10. Estimation of growth parameters

1st coffee break: 11:00 am (15 min)
Lunch: 13:30 (half an hour)
2nd coffee break: 15:30 (15 min)

3rd day

11. Sectioning the embedded blocks of otoliths/spines (done on the 1st day) for getting thin sections
12. Smoothing sections surfaces
13. Mounting the thin sections with transparent resin
    The mounted sections should be kept until dry for one day

1st coffee break: 11:00 am (15 min)
Lunch: 13:30 (half an hour)
2nd coffee break: 15:30 (15 min)

4th day

14. Information of the *Mullus barbatus* biology
15. Otolith observation and annuli measurement by the use of Image Analysis System of *Mullus barbatus* otoliths
16. Construction of marginal increments charts
17. Age assignment to age groups – age length key
18. Estimation of growth parameters

1st coffee break: 11:00 am (15 min)
Lunch: 13:30 (half an hour)
2nd coffee break: 15:30 (15 min)

5th day

19. Observation of otoliths/spines sections
20. Annuli measurements in otolith/spines sections by the use of Image Analysis System

1st coffee break: 11:00 am (15 min)
Lunch: 13:30 (half an hour)
2nd coffee break: 15:30 (15 min)

Annex III. Terms of Reference

FAO-EastMed Working Document.
ToR for the ad-hoc on the field training course on otolith age reading

Terms of Reference for the Ad-hoc on the Field Training Course on Otolith/Spines sectioning and Age Reading

Piraeus, Greece, 19-23 July 2010

During the 1st Coordination Committee meeting (Athens 19-20 April, 2010), the Cypriot focal point requested for Cypriot experts to be trained on otolith age reading for some target species in Cyprus. The focal point when communicating with the Project, explained the urgency of the request. Cyprus participates to the EC Data Collection and age reading is one of the program duties, which estimates are needed for the current year. Besides the urgency of the request, it shall be noted that age reading is needed for local species having probably few interest in the whole sub-region. In order for strengthening the Cypriot capacity for implementing their international duties, an ad-hoc on the field training course was agreed to be convened in the Fisheries Laboratory of the Ministry of Rural Development and Food, Piraeus, Greece during the week from 19-23 July 2010.

The training course also foresees to spines/otolith sectioning and the use of Image Analysis System for annuli reading. The training course will be scheduled according to the following:

1st day:

- Introduction to the equipments used for the otolith/spines sectioning
- Preparation of non transparent (black) resin blocks with embedded otoliths/spines
  The blocks should be remained until dry for two days
- Introduction to the use of Image Analysis System

2nd day

- Introduction to the otolith use for age reading (otoliths structure, otolith preservation and observation, sectioning, true annuli/pseudo-annuli, annuli measurement)
- Principles of age validation (length frequency, marginal increment)
- Information on *Spicara smaris* biology
- Otolith observation and annuli measurement by the use of Image Analysis System of *Spicara smaris* otoliths
- Construction of marginal increments charts for *Spicara smaris* otoliths
- Age assignment to *Spicara smaris* age groups – age length key

- Estimation of growth parameters

3rd day

- Sectioning the embedded blocks of otoliths/spines (done on the 1st day) for getting thin sections
- Smoothing sections surfaces
- Mounting the thin sections with transparent resin
  The mounted sections should be kept until dry for one day

4th day

- Information of the *Mullus barbatus* biology
- Otolith observation and annuli measurement by the use of Image Analysis System of *Mullus barbatus* otoliths
- Construction of marginal increments charts for *Mullus barbatus* otoliths
- Age assignment to *Mullus barbatus* age groups – age length key
- Estimation of growth parameters

5th day

- Observation of otoliths/spines sections
- Annuli measurements in otolith/spines sections by the use of Image Analysis System

Experts from Fisheries Laboratory

1. will show:
   - the use of equipments needed for otolith/spines sectioning including information on reagents necessary
   - the otoliths/spines sectioning process
   - the modules of the Image Analysis System and its use with examples
   - the annuli on whole otoliths for *Spicara smaris* and *Mullus barbatus*
   - age interpretation – age length key
   - estimation of growth parameters
2. will contribute to the preparation of the report

Training material and specific demonstrative material (e.g. published scientific paper and grey literature references) will be provided

Beneficiary countries

Countries with waters included in the GFCM Geographical Sub-Areas (GSAs) 19-20 and 22-28

Donors

Greece

- Ministry of Foreign Affairs
- Ministry of Rural Development and Food

Italy

- Ministry of Agriculture Food and Forestry Policies

European Community

- Directorate General of Maritime Affairs and Fisheries (DG-MARE)

Hellenic Ministry of Foreign Affairs
Hellenic Ministry of Rural Development and Food

ITALIAN MINISTRY OF AGRICULTURE, FOOD AND FORESTRY POLICIES

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