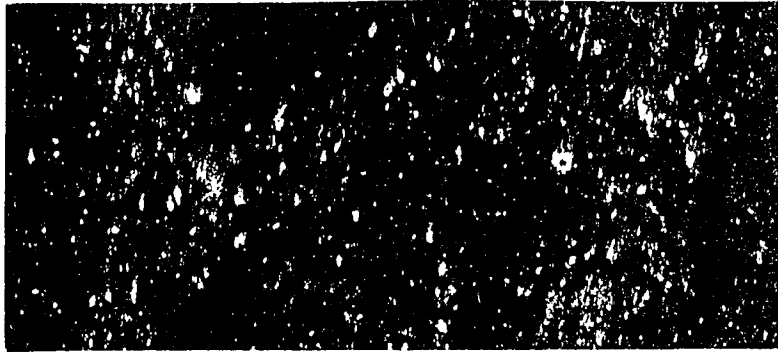


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TITLE XII

Collaborative Research Support Program



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In cooperation with the United States Agency for International Development (Grant No. DAN-4146-G-SS-5071-00) the Fisheries Stock Assessment CRSP involves the following participating institutions:

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1

WORKING PAPER SERIES

Working Paper No. 67

**"AGE DETERMINATION AND GROWTH
FOR TWO CORVINAS,
CYNOSCION STOLZMANNI AND CYNOSCION
SQUAMIPINNIS IN THE GULF OF NICOYA"**

by
Han-Lin Lai and Jorge Campos

The University of Washington

September, 1989

Fisheries Stock Assessment
Title XII
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The Fisheries Stock Assessment CRSP (sponsored in part by USAID Grant No. DAN-4146-G-SS-5071-00) is intended to support collaborative research between U.S. and developing countries' universities and institutions on fisheries stock assessment and management strategies.

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Age Determination and Growth for Two Corvinas,
Cynoscion stoltzmanni and Cynoscion squamipinnis,
in the Gulf of Nicoya

by

Han-Lin Lai and Jorge Campos

Abstract

Age determination and growth for two corvinas, coliamarilla (Cynoscion stoltzmanni) and aguada (Cynoscion squamipinnis), was studied. Validation of age determination on otoliths was carried out by the analysis of length-frequency analysis using the method of Macdonald and Pitcher (1979) and the program MIX. Both species grow 49% of their estimated L_{∞} 's in year one. The estimated L_{∞} for coliamarilla is 96.67 cm and for aguada is 55.65 cm, both over a period of about 7 years. Within reader comparison shows that repeatability of age readings decreases as age increases but the precision of the age readings is high in both cases.

Introduction

Corvinas (SCIAENIDAE) are the dominant fish species in the Gulf of Nicoya, a tropical embayment on the Pacific coast of Costa Rica. The catch of these fishes comprised at least 30% from total landing of artisanal fisheries (Madrigal 1985). Madrigal (1985) summarized the relatively few studies that have been done so far on various fishery resources in the Gulf and none of these deal with age determination.

There has been mixed success in the age determination of tropical fish. The principle difficulties have centered around the identification of the mechanism that would lead to well-defined periodic growth marks. In temperate and high latitude species, growth marks in otoliths are generally believed to be formed by the change in calcium metabolic rate due to seasonal temperature fluctuation (Williams and Bedford 1974), spawning (Rollefson 1934 and 1935, Fitch 1951), food supply (Hatanaka et al. 1952), etc. In tropical climates, however, it is difficult to identify these process as clearly taking place and the formation of periodic growth marks has been called into question.

The two corvinas, coliamarilla (Cynoscion stoltzmanni) and aguada (Cynoscion squamipinnis), were selected as the targets for the development of the ageing techniques. These tropical fish are a major part of the fishermen's corvina catch in the Gulf of Nicoya, 9° to 10° N latitude. Despite the central position that age data plays in stock assessment and population dynamics, the difficulties and uncertainties in estimating ages of tropical fish usually prevent their use. Stevenson (1981a, b) and Madrigal (1985) estimated von Bertalanffy growth parameters based on length-frequency distributions of aguada using the indirect methods of NORMSEP (Hasselblad 1966) and ELEFAN (Pauly and David 1981) but these

results have never been confirmed by a direct ageing method or by growth related studies.

This paper develops methods for direct age determination by reading whole otoliths and presents a method of indirect validation of results, for the two selected species. The resulting von Bertalanffy growth parameters are estimated and are compared to the previous studies.

Materials and Methods

At the beginning of the study, otoliths, scales, dorsal fin-rays, cleithrum, gill-covers, and vertebrae were collected from 30 specimens per species. After careful examination of all possible ageing methods related to these age structures, we found that the growth marks are not shown or are too ambiguous in scale, dorsal fin-rays, cleithrum, gillcovers, and vertebrae. Only otoliths are suitable for age determination of these two species. Thereafter, the otoliths of these two species (169 for C. stoltzmanni and 121 for C. squamipinnis) were collected from fishing boats operating in the Gulf of Nicoya from the periods of August 1986 to December 1987.

After measuring the lengths of fish, otoliths are removed from the skull by cutting with a sharp knife across the base of the bone that surrounds the otoliths. This method was used since it would not affect the quality or shape of the fish in market. Otoliths are preserved in 50% alcohol and transported to the laboratory where they are cleansed and preserved in anhydrous glycerine. In our experience, glycerine is a better preservative medium since it produces clear annual marks on the concave surface of otoliths. Dry storage for otoliths is not recommended at any stage of handling. However, greasy glycerine is hard to handle on a field trip, so

50% alcohol is recommended initially to preserve otoliths and to avoid bacterial and fungal infection.

Otoliths are put into a dark-background petri dish (using rubber glue to attach a black color velvet on the bottom of the petri dish) filled with glycerine and are viewed with a dissecting binocular microscope with reflected light. Fiberoptic light is the preferred light source because it is easier to control light intensity and light angle. However, since the growth of these fish is so fast the naked eye or a magnifying glass is adequate for reading otoliths of fish younger than 3 yrs. In general, annual growth rings or annual growth marks can be identified by a series of concentric translucent (transparent) zones which appear dark under reflected light and dark-background. In many studies, these zones are assumed to be formed in winter and are called winter zones or annuli. In these studies, however, it is not recommended that the same terms be used.

The otoliths were read twice, independently, by the senior author. A log-linear model (Fienberg 1981, Lai et al. 1987) was used to test whether there is an association between repeatability (R_i) of the two readings and age of fish (A_j). Table 1 a, b shows a two-way contingency table reading from the two species. The repeatability index measures the deviation between two age readings of the same otolith, i.e., $R_i = (\text{age from the second reading}) - (\text{age from the first reading})$, where $R_1 = +1$, $R_2 = 0$, and $R_3 = -1$. The computer program P4F in BMDP (Dixon 1983) was used for computation and analysis.

To examine if there is a systematic difference between the two readings a linear regression analysis was used to estimate the relationship between the first and second readings and to test the null hypothesis, $H_0: Y = X$, against $H_a: Y = a + bX$; where Y is the second reading and X is the first reading. If H_0 is not rejected, the two readings produce the same age except for systematic differences.

For validation purposes, the length frequency distributions of the two species collected in July-December 1983 were analyzed using the method of Macdonald and Pitcher (1979) and the MIX program by Macdonald and Green (1985). The mean lengths-at-age estimated from age determination and that from MIX were compared. Finally, von Bertalanffy growth parameters were estimated by a non-linear regression method using the SYSTAT software package on a PC.

Results

For the otoliths of Cynoscion stoltzmanni, the first annual mark is identified by the dark zone which is associated with a distinguishing notch on the posterior lobe of the concave surface (Fig. 1). However, this notch may be obscured as the fish grow older. The second and third annual marks are more complicated since there are checks associated with them. This problem becomes more serious when the annual mark is close to the otolith margin which is frequently transparent since it is very thin in this area (Fig. 1a). To identify the associated notch (Fig. 1b), it is helpful to trace the annual growth mark around the otolith and to judge the space between and within the zones. In most cases the annual marks and checks can be more easily distinguished if the otoliths are soaked in glycerine for a period of time. Figures 1c and 1d show the otoliths with 2 and 3 annual growth marks.

For the otoliths from Cynoscion squamipinnis, the appearance of annual growth rings is determined from dark bands under reflected light against a dark-background (Fig. 2a). Figure 2a shows an otolith with 4 annual growth marks. The first two marks are frequently associated with a deep notch. The transparent appearance at the margin of the otoliths presents a problem for the identification of annual growth marks. This problem is more significant for one and two year old

fish since the growth is fast in these stages and the annual growth marks are wider than for older fish. Figures 2b and 2c are a comparison of 2 year old otoliths. Figure 2b shows that the second annual growth mark is exactly at the margin and Fig. 2c shows that there is an extra growth mark after the formation of the second annual growth mark at the margin. Also shown in Fig. 2c is a false check laid between the first and second annual growth marks. This check is identified as false because it is not associated with a notch and is relatively thinner, when compared with the other regular growth marks. Figure 2d shows ambiguous checks close to the marginal area. Although the space between checks and the continuity of the checks on the otolith are helpful, these criteria cannot be used consistently.

Tables 1a and b show the frequency of agreement between two readings by age groups. In general, percent agreement decreases as age increases for the two species. A log-linear model was fitted to the data in Tables 1a and b. The test results show that the full model,

$$\theta_{ij} = \mu + \lambda_i^R + \lambda_j^A + \lambda_{ij}^{RA},$$

is the best model for the two species ($G^2 = 36.16^{**}$ and 22.36^* for coliamarilla and aguada, respectively). This indicates that the agreement between readings is related to the age group.

Table 2 shows the results of linear regression analysis for the two species, and indicates that there are no systematic differences between the two age readings. These results are confirmed by the paired t-test on $H_0: \bar{Y} = \bar{X}$, where \bar{X} and \bar{Y} , respectively, are the mean ages of the first and second age readings. The null hypothesis is not rejected for either species at the 1% significance level. However, the index of variation (Lai 1985) of aguada is 2.30% which is smaller than that of

coliamarilla (3.82%). For a fixed sample size, this indicated that a higher precision can be attained for aguada.

Validation of age readings is based on analysis of the length-frequency distributions of the two species (Figures 3a and b) using the MIX program. Assuming that the length-frequency distribution of fish is a mixture of several normal distributions and each distribution has a mean length-at-age and a variance, the estimated proportions, mean lengths, and their variances are computed by the MIX program. Table 3 shows the results obtained from the program and from direct age determination. Examination of the estimated standard deviation and the estimated mean lengths-at-age for each age class clearly shows that no statistical difference exists between the two methods. This is a strong suggestion that the direct ageing method used for the two species is accurate.

The von Bertalanffy growth parameters and their standard deviations are estimated for both species from the mean lengths-at-age (Table 4). Both species grow about 49% of L_{∞} in their first year of life, i.e., 46.91 cm vs. 96.68cm for Coliamarilla and 27.13cm vs. 55.65cm for aguada.

Discussion

Since the determination of the age of tropical fish is difficult it has sometimes led to the interpretation that it is impossible. Although age determination is a science, the method has some elements of being an art. This is because the methodology relies mainly on subjective observations which can be affected by human preference, age-reader training, and mechanical factors such as quality of microscope and light source. It has also been said (Pannella 1974) that analyses fine enough to detect daily increments of growth are needed to age tropical fish but there are examples, e.g., Lutjanus sanguineus (Lai and Liu 1977, 1983), Nemipterus

japonica (Kuo and Liu 1974), and this study on sciaenidae where otolith daily increments were not necessary.

The use of length-frequency data for validation of direct age reading is not the preferred method. An independent experiment such as an oxytetracycline-tagging experiment or the use of known age specimens would be preferable. We used the analysis of length-frequency distributions as an alternative to a tagging study which would be expensive and extremely difficult to carry out in a remote tropical environment.

The growth rates in the first year are very high for the two species. If the growth curves are fitted and constrained to pass through $l_0=0\text{cm}$ at $t=0$, we found that $K=0.506$ for coliamarilla and 0.647 for aguada while the values of L_∞ remain nearly the same as prior to the translation on the age axis. We also found that the length distributions at age cover a wide range suggesting continuous or semi-continuous recruitment and spawning during a year. Continuous spawning and recruitment is not uncommon in tropical fish (Lam 1983, Bye 1984, Munro et al. 1973). If this is the case for these species, the growth rate in the first year becomes an important factor related to "year-class" strength and yield potential because the growth rate largely decides how soon the fish recruit to the fishery and certainly is central to the time of sexual maturity.

Based on the age and length-frequency distributions of the catches, the two corvinas show different harvesting patterns. The age of first and full recruitment for coliamarilla is 1 year old. Aguada is fully recruited to the fishery at 3 years old, but can enter into the fishery at 2 years old. Evaluation of the consequences of these harvesting patterns requires a knowledge of the reproductive biology of the two species. Therefore, studies on fecundity, age of sexual maturity, growth and survivorship of pre-recruitments are essential to a better understanding of the dynamics and management for the two corvina stocks.

The von Bertalanffy growth parameters of aguada have been estimated by Stevenson (1981a, b) using the method of NORMSEP (Hasselblad 1966; Young and Skillman 1975) on survey length-frequency distributions (LFD) in 1976-77; by Madrigal (1985) using ELEFAN (Pauly and David 1981) on catch LFD in 1979 and 1982. We also used ELEFAN (Sims 1985) to estimate L_{∞} and K from a time series of monthly catch LFD from 1983. Table 5 clearly shows that L_{∞} and K estimated by ELEFAN and NORMSEP are different from that estimated by the direct age method using otoliths. The problem evidently results from the interpretation of modes in the LFDs. All length-frequency distributions show a dominant mode around 45cm, which appears to include at least 3 age classes, based on otolith readings, which cannot be easily separated by the methods of length-frequency analysis. Madrigal's L_{∞} estimates are especially large because large-sized fish which are relatively rare in the LFD are given too much weight in the analysis. To overcome this problem we eliminated 11 fish greater than 60 cm from the 12 LFD from 1983, but ELEFAN still over-estimates L_{∞} by 11% (61 cm vs. 55.65 cm) and under-estimates K by 40% (0.22/yr vs. 0.37/yr). There are other possibilities to explain differences between the estimates based on 1979/1982 data and 1983 data. These include changes in population structure due to recruitment fluctuation, gear selectivity, growth variation, and harvesting pressure. While not very much is known about these possibilities, none of the evidence we have at hand supports any dramatic shifts in the fishery over the years involved.

The results of age determination are the optimal data upon which to estimate growth rate, mortality rate, age of sexual maturity, and age of recruitment. Furthermore, these estimates are central for the evaluation of biological critical points, such as $F_{0.1}$, F_{MSY} , and F_{MEY} , and thus for the definition of alternative harvest strategies. It is therefore clear that accurate age determination is essential for accurate stock assessment for the fishery resources in the Gulf of Nicoya. To

achieve this goal, further studies such as validation by a tagging experiment, estimation of juvenile growth rate by daily increment analysis, and the estimation of reproductive potential by fecundity studies are recommended.

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Table 1. Frequency of agreement and disagreement between the two age readings for Cynoscion stoltzmanni and Cynoscion squamipinnis. Percent agreement is shown in parenthesis.

A. Cynoscion stoltzmanni

Age	Deviation of two age readings			Total
	+1	0	-1	
0	0	5 (100%)	0	5
1	0	111 (99%)	1	112
2	1	26 (93%)	1	28
3	1	9 (82%)	1	11
4	0	4 (100%)	0	4
5	1	2 (50%)	1	4
6	0	0 (0%)	1	1
7	0	1 (50%)	1	2
9	1	0 (0%)	1	2
Total	4	158 (93%)	7	169

B. Cynoscion squamipinnis.

Age	Deviation of two age readings			Total
	+1	0	-1	
0	0	1(100%)	0	1
1	0	14(100%)	0	14
2	0	31(100%)	0	31
3	0	25 (95%)	1	26
4	1	26 (94%)	1	33
5	3	9 (64%)	2	14
6	1	1 (50%)	0	2
Total	5	107 (88%)	4	121

Table 2. Test of systematic difference between two age readings using linear regression analysis. (** : significant at 1% level)

A. Cynoscion stoltzmanni

	df	SS	MS	F	R ²
Regression	1	261.35	261.35	4355.83	96.4%
Error	166	9.64	0.06		
Total	167	270.98			

$$a = 0.0561 \quad s_a = 0.0293 \quad t = 1.91$$

$$b = 0.9644 \quad s_b = 0.0144 \quad t = 67.07^{**}$$

B. Cynoscion squamipinnis

	df	SS	MS	F	R ²
Regression	1	193.00	193.00	2757.14 ^{**}	95.6%
Error	119	8.87	0.07		
Total	120	201.87			

$$a = 0.0661 \quad s_a = 0.0634 \quad t = 1.04$$

$$b = 0.9756 \quad s_b = 0.0192 \quad t = 50.88^{**}$$

Table 3. Mean lengths-at-age and standard deviation (S.D.) of Cynoscion stoltzmanni and Cynoscion squamipinnis estimated by age determination and MIX.

A. Cynoscion stoltzmanni

Age	I	II	III	IV	V	VI	VII	VIII	IX
I. from MIX									
Proportion	0.711	0.016	0.088	0.032	0.153*				
S.D.	0.055	0.031	0.032	0.021	0.038				
Mean (cm)	49.54	57.35	64.78	71.22	85.41*				
S.D.	4.54	0.61	2.21	1.99	3.92				
II. from age determination									
Mean (cm)	46.91	55.77	68.06	72.95	82.63	98.60	84.90	--	88.90
S.D.	3.94	4.30	3.99	1.02	6.18	--	2.97	--	--
N	114	26	11	4	4	1	2	--	1

B. Cynoscion squamipinnis

Age	I	II	III	IV	V	VI	VII
I. from MIX							
Proportion	--	0.035	0.712	0.170	0.057	0.016	0.009*
S.D.	--	0.008	0.025	0.028	0.015	0.009	0.004
Mean (cm)	--	35.22	41.99	44.95	49.51	52.21	55.78*
S.D.	--	3.29	2.65	1.94	1.48	2.27	2.86
II. from age determination							
Mean (cm)	27.13	35.67	41.88	46.72	48.94	51.10	
S.D.	5.03	2.31	3.78	3.62	2.77	2.97	
N	14	30	26	33	14	2	

* Include the ages equal and greater than the corresponding age category.

Table 4. Estimated parameters of the von Bertalanffy growth curves for Cynoscion stoltzmanni and Cynoscion squamipinnis.

Parameters	<u>Cynoscion stoltzmanni</u>		<u>Cynoscion squamipinnis</u>	
	Estimates	S.D.	Estimates	S.D.
L_{∞}	96.682	7.271	55.651	0.915
K	0.318	0.146	0.370	0.026
t_0	-0.946	0.935	-0.797	0.106

Table 5. Estimated growth parameter of Cynoscion squamipinnis by various studies.

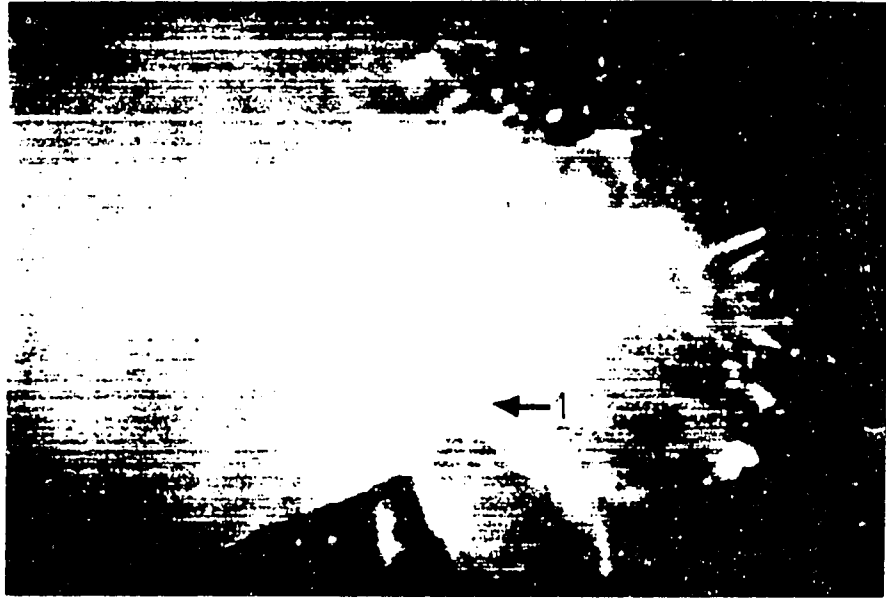
	L_{∞} (cm)	K	t_0	Remarks
Stevenson (1981a,b)	60.00	0.26	?	Head of Gulf (1976-77), NORMSEP
	60.00	0.65	?	Middle of Gulf (1976-77), NORMSEP
Madrigal (1985)	75.5	0.4	-0.1607	1979 survey, ELEFAN
	72.5	0.4	-0.1621	1982 survey, ELEFAN
Present study	55.651 (0.915)	0.37 (0.026)	-0.797 (0.106)	Age determination, Non-linear least squares
	61.0	0.22	-0.514	1983 catch data, ELEFAN

(): standard deviation of the estimates.

Figure 1. Otoliths of Cynoscion stoltzmanni viewed under reflected light and dark background.

- A. Otolith from 26cm fish which is 1yr old. Note the check and transparent area at margin of the otolith.
- B. Side view of otolith in A. Showing the notch associated with the first annual growth mark.
- C. Otolith of 2 yrs old fish (38cm). Note the growth at margin after formation of the second annual growth mark.
- D. Otolith of 3yrs old fish (43cm).

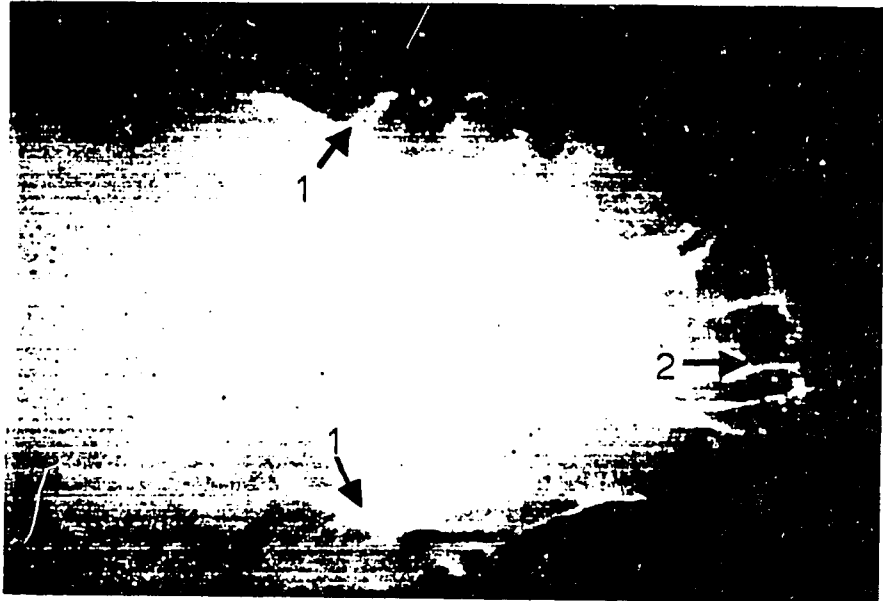
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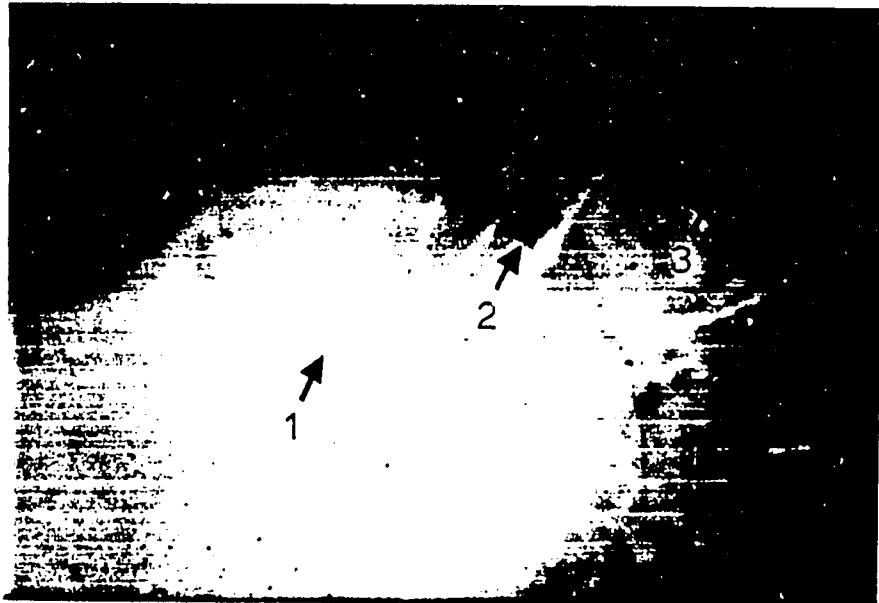
B



C



D



- 21'

Figure 2. Otoliths of Cynoscion squamipinnis viewed under reflected light and dark background.

- A. Otolith of 4 yrs old fish (70cm). Note the notches associated with the first and second annual growth marks.
- B. Otolith of a 2 yrs old fish (59cm). The second annual growth mark is formed at margin.
- C. Otolith of a 2yrs old fish (57cm). There is growth after formation of the second annual growth mark. Also note the false check (C).
- D. An otolith classified as unreadable because of ambiguous marks (arrows).

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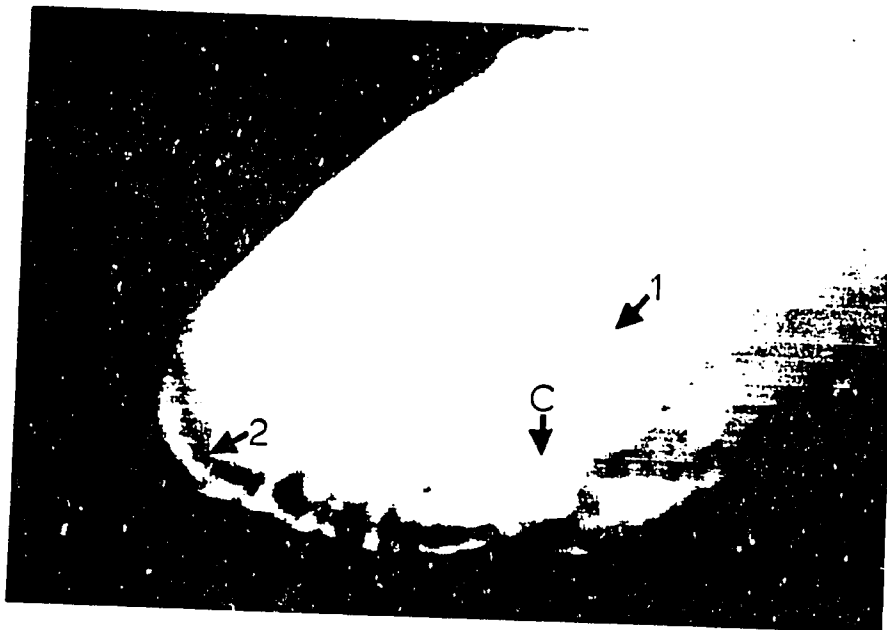
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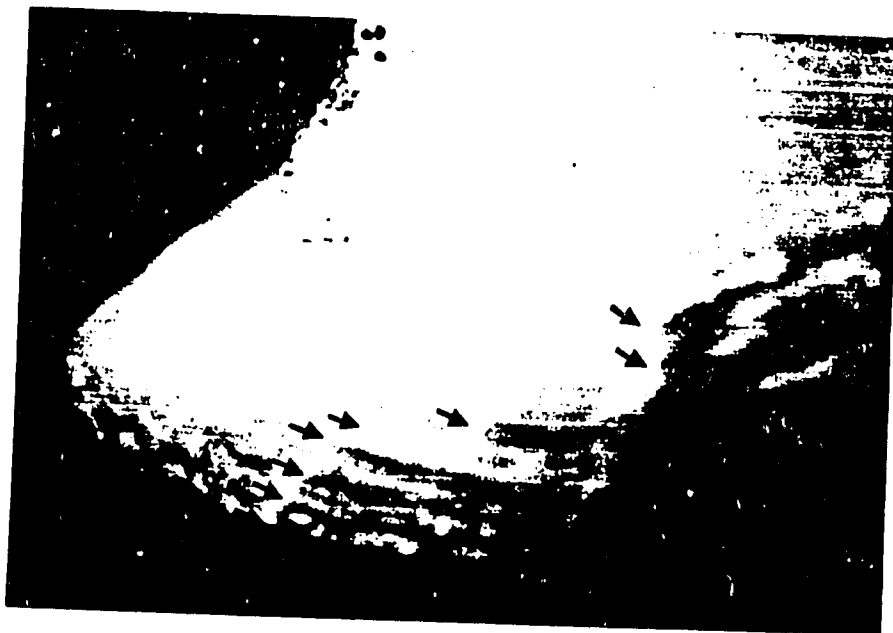
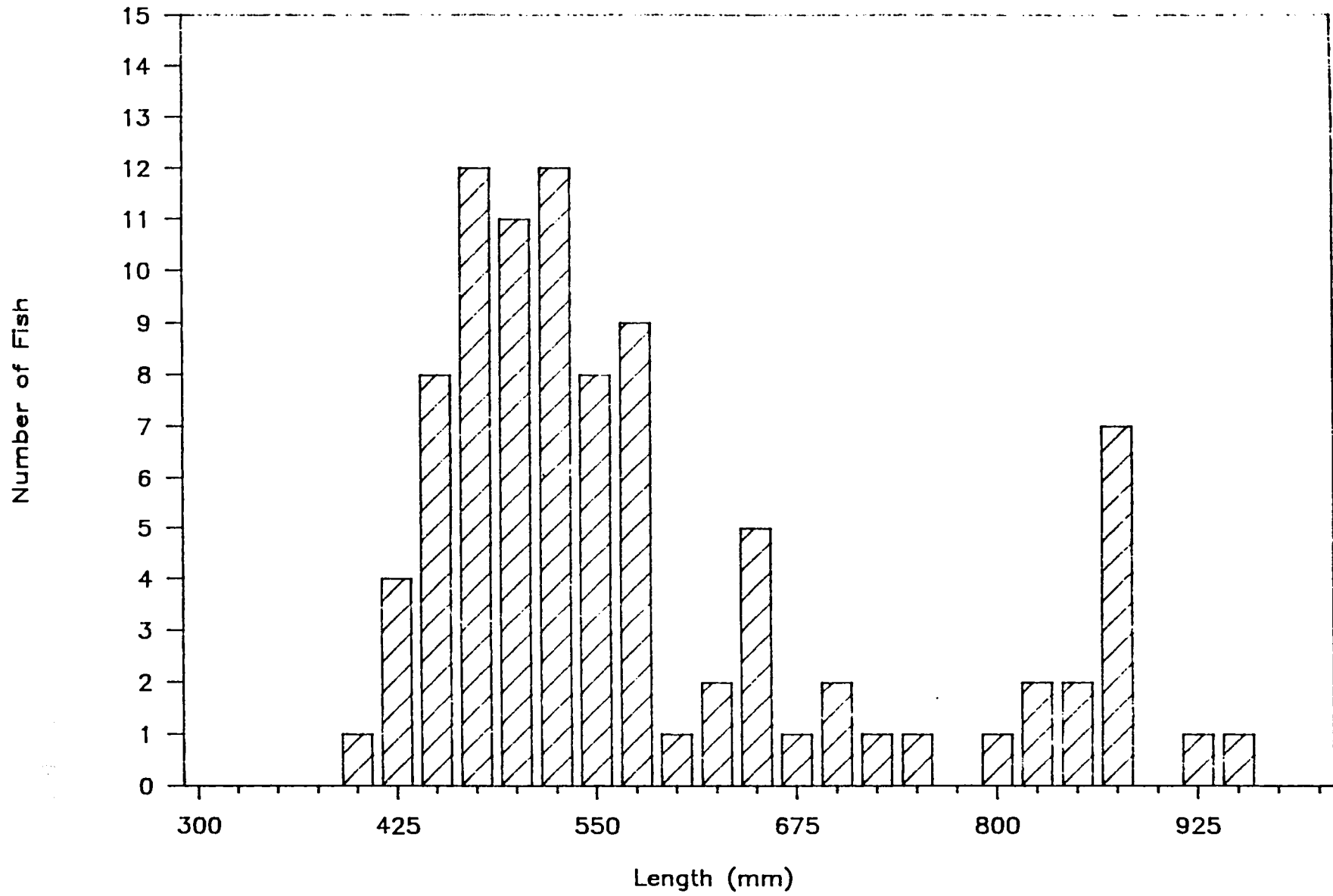


Figure 3. Length-frequency distributions of Cynoscion stoltzmanni (A) and Cynoscion squamipinnis (B) used in the method of Macdonald and Pitcher (1979).

A. Coliamarilla (C. stoltzmanni)

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B. Aguada (C. squamipinnis)

