Age determination of *Encrasicholina purpurea* Fowler (Pisces: Engraulidae) based on otolith analysis.

Xavier Chiappa-Carrara[1],
Manuel Gallardo-Cabello[2]
y Margarita Caso-Chávez[2]

[1]Unidad de Investigación en Ecología Marina, FES-Z, UNAM. Apdo. Postal 9-020, 09230 México, D.F.
[2]Instituto de Ciencias del Mar y Limnología, UNAM. Apdo. Postal 70-305, 04510 México, D.F.

Chiappa-Carrara, X., M. Gallardo-Cabello y M. Caso-Chávez, 1996. Age determination of *Encrasicholina purpurea* Fowler (Pisces: Engraulidae) based on otolith analysis. *Hidrobiológica 6* (1-2): 1-8.

ABSTRACT

In this study, an analysis on the development of *sagittae* of the Hawaiian anchovy, *Encrasicholina purpurea*, from Kaneohe Bay, Oahu, Hawaiian Islands, is presented. Eight age-groups are established by the analysis of daily growth increments. Morphology and a four-step growth of the *sagitta* in relation to body length, are described. The width of the concentric daily growth rings decreases as fish grow, fast-growth rings are wider than slow-growth ones. The time of the formation of the first daily growth ring is determined. Development of daily increments is related to metamorphosis of larvae and the gonadic maturation of adults.

Key words: Ageing, *Encrasicholina purpurea, sagitta.*

RESUMEN

En el presente trabajo, se presenta un estudio sobre el desarrollo de las *sagittae* de la anchoveta hawaiana *Encrasicholina purpurea*, en la bahía de Kaneohe, Oahu, Hawai. Por medio del análisis de los incrementos diarios de crecimiento pudieron reconocerse ocho grupos de edad. Asimismo, se describe la morfología de la *sagitta* y se estudia la relación entre la longitud y la anchura, que viene expresada por cuatro periodos de crecimiento. La amplitud de las bandas concéntricas de crecimiento diario disminuye conforme el pez incrementa su longitud, con la particularidad que las bandas son más anchas durante el periodo de crecimiento rápido que en el de crecimiento lento. Asimismo, se determina el tiempo de formación del primer anillo de crecimiento diario y se relaciona el desarollo de los incrementos de crecimiento diario con la metamorfosis de las larvas y la madurez gonádica de los adultos de esta especie.

Palabras clave: Edad, *Encrasicholina purpurea, sagitta.*

INTRODUCTION

The study of otoliths is important because they are a part of the system which regulates the muscle tone and stimuli reception due to angular acceleration, gravity, and sound. Otoliths are also a permanent register of the physiological and metabolic changes occurring during the life history of fish (Gallardo-Cabello, 1979).

Following Pannella's works (1971; 1974; 1980), several authors have reported daily increments on these calcified structures but few works consider species living in semi-enclosed tropical environments (Struhsaker and Uchiyama, 1976). Sub-daily increments have also been found (Taubert and Coble, 1977; Brothers, 1978; Pannella, 1980; Wilson and Larkin, 1980; Campana and Nelson, 1982), complicating the interpretation of increment's periodicity.

There are few studies on the age and growth of the Hawaiian anchovy (*Encrasicholina purpurea* Fowler), which is the dominant planktivorous fish in semi-enclosed eurihaline waters of the Hawaiian Islands (Clarke, 1989a). *E. purpurea* is used as baitfish in the tuna fishery and its availability is one of the limiting factors of this industry (Comitini, 1977).

Struhsaker and Uchiyama (1976) found that growth is very fast in the 20 days after hatch. Chiappa-Carrara (1993) reported a non-linear growth for this species. Similarly, it is known that the Hawaiian anchovy is a short-lived organism, maximum reported age being around 200 days (Nakamura, 1970; Struhsaker *et al.*, 1975; Clarke, 1989a).

MATERIAL AND METHODS

Biological samples were obtained from Kaneohe Bay, Oahu, Hawaiian Islands, during collections carried out from November 1990 to February 1992. Bimensual sampling of larval stages were accomplished every six hours during the day, using a plankton net, 5 m length, 1 m diameter, and 335 μm mesh. Adults were caught shortly after sunset with a 60 X 12 m purse-seine, with 5 mm mesh. In both cases, samples were preserved immediately in a 70% solution of ethilic alcohol and analyzed within 10 days of capture to prevent shortening due to dehydration.

Morphometric, ponderal, and biological data obtained for each individual are as follow:

Length: In individuals $\leq$25 mm, whose flexion process had not finished yet, length from the tip of the mouth to the ending point of notochord was recorded ($\pm$ 0.01 mm). From that length on, standard length (SL) was measured with approximation of 0.1 mm.

Weight: Total weight of individuals was measured with an approximation of 0.01 mg.

Sex: *In visu* observations were carried out following Clarke's (1987) criteria.

A total of 550 pairs of *sagittae* were obtained from the otical cavities. Since differences between morphometric data among right and left *sagittae* were not significant ("t" = 3.123; $p < 0.01$), in this study only right-side otoliths were analyzed. *Sagittae* were immersed for ten days into Euparal® milieu, to improve contrast between fast and slow growth increments, and then observed with an optical microscope with a graduate ocular lens. Readings of number of rings were carried out twice. During the first count, it was possible to assign each otolith to some age. However, 2% of *sagittae* were discarded because on the second reading differences in the number of rings were larger than 5%.

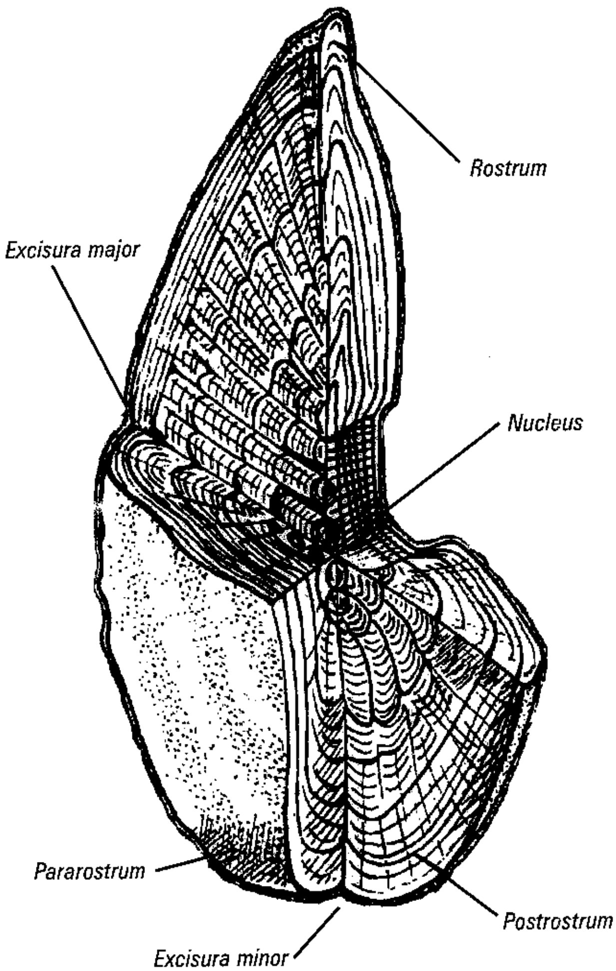

Figure 1. Diagram of the *sagitta* showing its main parts.

Total length, width, and length from the *postrostrum* to the center of the otolith (postrostral length, PL) were registered for every otolith. Age was established by counting the number of rings surrounding the *nucleus* of the *sagitta*. Even though the number of rings between otoliths of the right and left cavity was not significantly different ("t" = 1.76, $p < 0.05$), growth rings were always counted in the right-side otolith.

RESULTS AND DISCUSSION

Description of the *sagitta*

E. purpurea's sagittae are elongated, maximum length comprised 53.75 times in standard length of fish. Figure 1 exhibits a diagram of the otolith, showing its main parts.

On the anterior edge, a projecting *rostrum* with small dentitions is present. The *excisura major* is prominent and the *antirostrum* is well developed. Otoliths have an ascending dorsal edge from the *rostrum* to their center, descending as far as the posterior edge. Dorsal edge has 3 to 5 dentitions which tend to be very irregular in adult fish (≥ 35 mm SL). A sharp and well developed *postrostrum* on the posterior edge is visible. The *excisura minor, pararostrum*, and *postrostrum* are well developed. The ventral edge is slightly curved from the *rostrum* until the center of the otolith, being flat from the center to the posterior edge. There are many dentitions irregularly disposed. This unevenness increases as the fish grows.

Otolith internal face is slightly concave and presents a flat surface. This face shows a shallow *sulcus* which runs uniformly through this surface. The external face is slightly convex and covered by numerous and small granules in adult stages. Dorsal edge is thinner in the longitudinal axis.

Gallardo-Cabello (1985) described *Engraulis mordax's sagittae* finding similar characteristics. Nevertheless, these structures are larger in *E. mordax* than those of *E. purpurea*.

Otolith development

Figure 2 shows the relationship between total length and width of *sagittae*. The observed inflections indicate that differential growth occurs in these longitudinal planes. The first inflection point pertains to individuals between 15 and 18 mm SL. In this length range the metamorphosis occurs. To this point, the allometric index is 0.9419 being not significantly different from isometry ($n = 44$; $p > 0.05$). During this period, the shape of the the *sagitta* resembles a disc.

The next inflection point appears when length of organisms is between 27 and 32 mm SL. Value of the exponent is 0.2646, showing a strong negative allometric relation ($n = 26$; $p < 0.05$). Since sexual maturity is reached at this stage

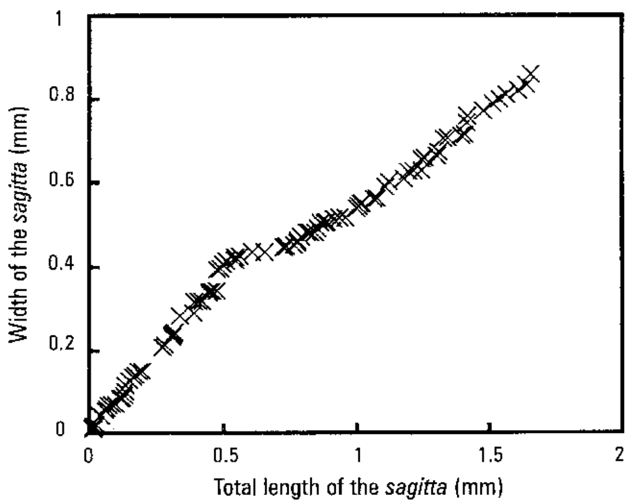

Figure 2. Total length and width dimensions of *E. purpurea's sagittae*.

Table I. Mean number and width (mm) of last deposited daily growth rings, as a function of otolith average postrostral length (mm) and fish mean standard length (mm).

Otholith postrostral length (mm)	Fish SL	Mean number of daily growth rings	Width of daily growth rings (mm)
0.05	4.49	2.47	0.020
0.10	8.75	7.33	0.014
0.15	10.45	10.42	0.014
0.20	13.14	15.24	0.013
0.25	16.13	22.34	0.011
0.30	19.50	31.41	0.010
0.35	20.75	34.26	0.010
0.40	23.50	44.56	0.009
0.45	27.64	62.36	0.007
0.50	30.50	73.34	0.007
0.55	33.25	87.98	0.006
0.60	36.63	105.36	0.006
0.65	39.10	118.88	0.005
0.70	42.00	136.20	0.005
0.75	44.50	154.91	0.005
0.80	47.00	179.79	0.004
0.85	48.50	198.97	0.004

(Clarke, 1987; 1989b), a certain amount of energy is used for gonadic maturation. During this stage the longitudinal growth of otoliths is bigger than width growth. Otoliths acquire their adult, lengthened shape.

From the second inflection point until organisms reach 40 mm SL, allometric index reflects departure from isometry ($b = 0.6961$; $n = 23$; $p < 0.05$). Subsequent values are close to isometric growth ($b = 0.9594$, $n = 20$; $p > 0.05$).

Growth marks

Two daily marks or rings, a dark and a light one, are formed around the nucleus of otoliths. Appearance of rings is determined by differences in deposition rates, and therefore in relative concentrations of calcium carbonate and otolin protein (Pannella, 1971). Once the nucleus is formed, the accretion rate of new material decreases as fish grows. Because the accumulation of new material is not a uniform process, the first growth rings are much wider than the ones formed on later stages (Table I). There is a differential deposition of new material around the nucleus on both longitu-

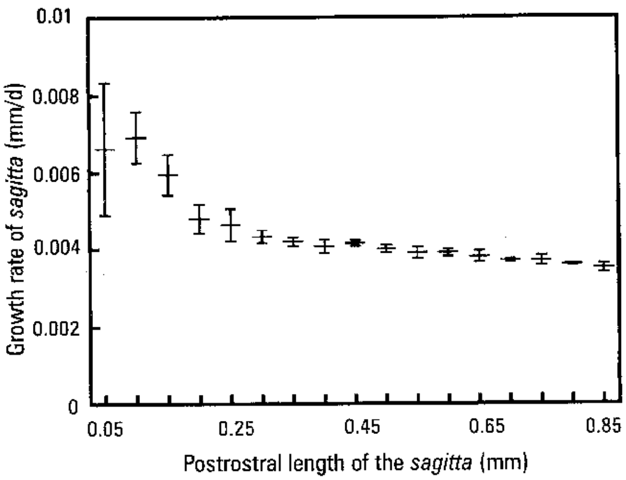

Figure 3. Average growth rates (mm d^{-1}) of the *sagitta* of *E. purpurea*. Vertical lines indicate standard deviations of rate esfimates.

dinal axis. Nuclei of otoliths in *E. purpurea* are eccentric on each surface plane, because the rostral side grows faster than the postrostral one, and the ventral section grows faster than the dorsal one. In figure 3, average growth rates (mm d^{-1}) calculated from postrostral dimensions, are presentes as a function of PL.

Figure 4 shows the diameter of nuclei as a function of PL. Nuclei dimensions range from 0.0190 mm in *E. purpurea's* otoliths with a length of 0.05 mm to 0.0263 mm for otoliths with a length of 0.80 mm. Two inflection points, corresponding to PL = 0.1 and 0.5 mm, are observed. Allometric exponent for the first part of graph (PL $\leq$ 0.1 mm) is 0.0668. After a sudden slope change at PL $\approx$ 0.5 mm, allometric coefficient is 0.3953. It is possible to establish at least two well marked developmental phases. In the first stages, apparent increments in the diameter of the nucleus

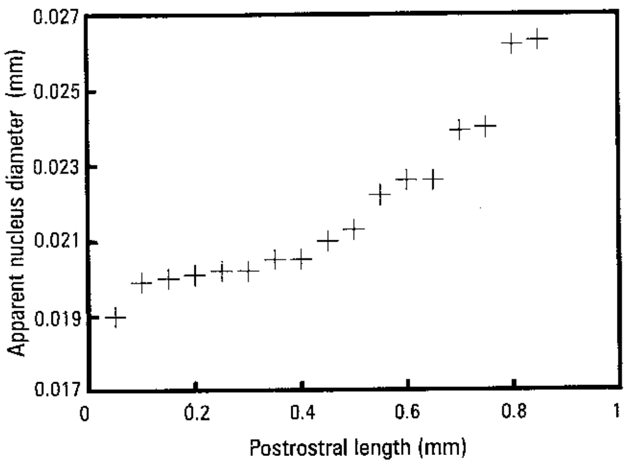

Figure 4. Apparent nucleus diameter as a function of postrostral length of otoliths.

are not very significant and the slope of the relation is not significantly different from zero ($p < 0.01$).

Near the moment of sexual maturity of *E. purpurea*, which occurs at SL $\approx$32 mm (Clarke, 1989b), corresponding in this case to data belonging to the second section of the curve, it is very difficult to differentiate the first growth rings because the nucleus appears as if it increased its dimensions. Ratio between the diameter of the nucleus and the postrostral length of the otolith is not constant because consecutive *annuli* depositions merge with the nucleus. Estimates of age in organisms belonging to stages after sexual maturity might be underestimated if based upon enumeration of rings.

Estimation of the formation time of first daily growth ring

Based on certain morphological features, such as the presence or absence of yolk sac, implantation, development and number of rays of pectoral fins, and progression of eye

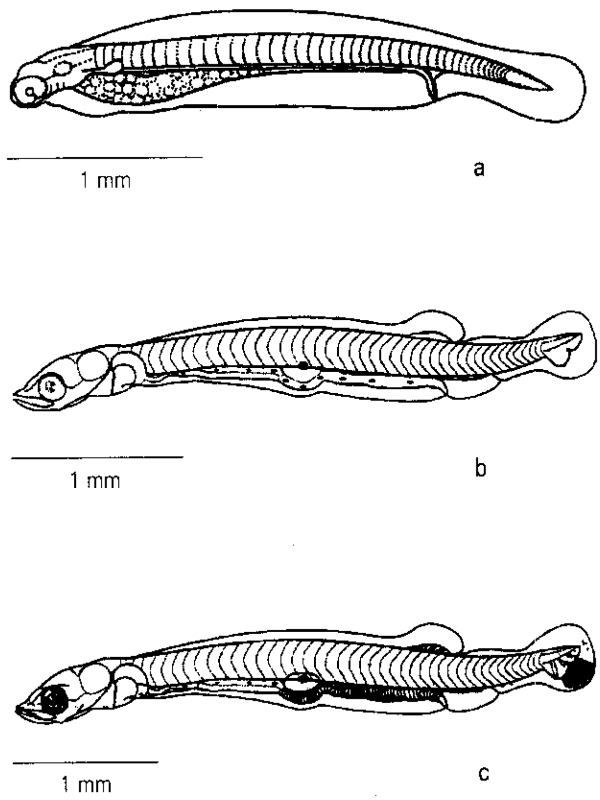

Figure 5. a: *E. purpurea* belonging to age group "0"; b: *E. purpurea* belonging to age group "1"; c: *E. purpurea* belonging to age group "2".

Table 2. Morphological characteristics of the three stages of the development of *E. purpurea* (Age groups "0", "1", and "2").

	Age group "0"									Age group "1"			Age group "2"
			*		**						
Yolk sac	present		present		traces		absent		absent		absent
Pectoral fin	absent	→	absent	→	implantation visible	→	1*	→	1*	→	2*
Eye pigmentation	absent		absent		absent		absent		traces		present

*Recent hatched larvae, bended anteriorly.

**Distended larvae.

1* Diameter of pectoral fins implantation ≈ pupil diameter.

2* Pectoral fin rays visible.

pigmentation, larvaes (notochord length ≤4 mm) can be classified into three age groups.

Separation of larvae based on those features was not a difficult task. Larvae pertaining to the same group were similar and there were no intermediate categories. Nevertheless, larvae larger than 4 mm could not be assigned to any age group based only on developmental characteristics or size.

When larvae reach the first 12 hours of life (age group "0"), yolk sac disappears, and pectoral fin buds come to a similar diameter to that of the eye (Table 2, figure 5a). Average diameter of the nucleus of *sagittae* in larvae pertaining to this age group is 0.0202 mm. At this point, the first growth ring has not been formed yet.

At 24 hours after hatching, larvae could be grouped into age group "I". Morphological characteristics used to distinguish individuals one-day-old are shown in Table 2 and figure 5b. In this stage, larvae present, for the first time, food items in their digestive tract. During the first day of life the first growth ring is formed, making possible to accurately age young individuals by counting growth rings in the otolith. In Table 2, morphological characteristics of organisms belonging to age group "2", are shown. 48 hours after hatching, larvae exhibit a completely developed mouth (Figure 5c). *Sagittae* have two growth rings.

Struhsaker and Uchiyama (1976) accomplished periodical capture-and-sacrifice experiments, and concluded that there is a high correlation between the average increment in the number of rings and the days that had passed between consecutive experiments. This fact, as well as the results obtained in this work, confirm the existente of daily growth increments in this species.

Relation between otolith growth and fish growth.

Figure 6 shows the relationship between total length and postrostral length of the otolith as a function of standard fish length. Total length of otoliths present two important inflection points. The first one corresponds to 20 mm SL organisms. At this point metamorphosis occurs. The second inflection point happens when individuals reach 30 mm SL. Sexual maturity occurs at this time, thus there might be differences in the calcium carbonate and otolin protein deposition rates on *sagittae* related to this phenomenon.

Age-length relationship.

The average length for each age group of *Encrasicholina purpurea* has been determined from the analysis of the daily growth rings on the *sagittae* (Table 3). Equation resulting

Table 3. Age-length relationship for *Encrasicholina purpurea*.

Age group (months)	Standard Length (mm)
0	2.18*
1	18.16
2	29.92
3	36.04
4	40.20
5	43.35
6	45.67
7	47.78

*Notochordal length.

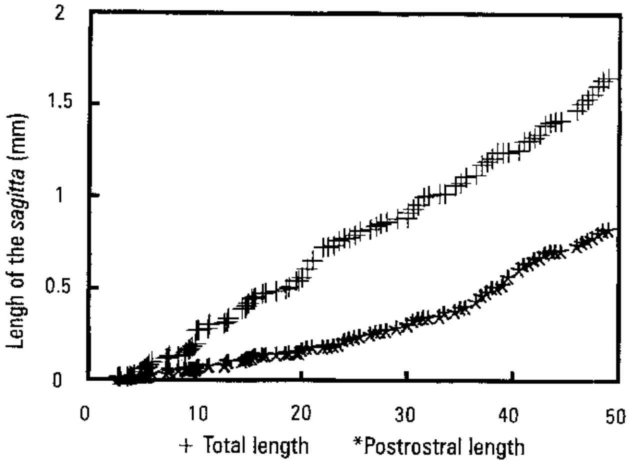

Figure 6. Total length and postrostral length of otolith as a function of *E. purpurea*'s SL.

from the nonlinear parameter estimation (Prager *et al.*, 1989) of the von Bertalanffy growth function is:

$$SLt \approx 50mm[1- e^{-0.4(t+0.1)}] \qquad (p < 0.05)$$

CONCLUSIONS

The first growth ring in the *sagitta* is formed during the first day of life of fish. Therefore it is possible to accurate age young individuals by counting daily growth increments. Estimates of age of organisms after sexual maturity is been reached might be underestimated if based upon enumeration of rings alone.

Nuclei of *sagittae* are eccentric on each surface plane, the rostral side grows faster than postrostral. The ventral section grows faster than the dorsal one.

Width of concentric daily growth rings formed around the nucleus decreases as the fish grows. Fast-growth rings are wider than slow-growth ones.

The relationship between total length and width of *sagittae* shows four stages. The first stage represents an isometric growth (individuals ≤15 mm SL). The second stage shows a negative allometric growth in fish 15 + to 32 mm SL. This decrease is related to the moment in which sexual maturity is achieved. The third stage keeps a smoother negative allometric relationship (fish up to SL ≈40 mm), while values corresponding to the last phase (individuals ≥ 40 mm SL) are close to isometry.

The relationship between otolith growth and fish growth shows two inflection points, corresponding to metamorphosis and gonadic maturation of this species, respectively.

Ageing by daily growth increments provided the means to establish eight age groups.

ACKNOWLEDGMENTS

Authors are grateful to T.A. Clarke, of the Hawaii Institute of Marine Biology, for his comments and technical support during the field work.

REFERENCES

Brothers, E.B., 1978. Exogenous factors and the formation of daily and subdaily growth increments in fish otoliths. *Am. Zool., 18*: 631.

Campana, S.E., and J.D. Nelson, 1982. Daily growth increments in otoliths of starry flounder (*Platichthys stellatus*) and the influence of some environmental variables in their production. *Can. J. Fish. Aquat. Sci., 39*: 937-942.

Clarke, T.A., 1987. Fecundity and spawning frequency of the Hawaiian anchovy or nehu, *Encrasicholina purpurea*. *Fish. Bull., 85*: 127-138.

Clarke, T.A., 1989a. Species Profiles: Life histories and environmental requirements of coastal vertebrates and invertebrates, Pacific Ocean region. Report 4, The Hawaiian anchovy or nehu, *Encrasicholina purpurea* (Engraulidae). *Tech Rep. EL-89-10, USA Army Eng. Wat. Exp. Sta, Vicksburg, MS*, 20 p.

Clarke, T.A., 1989b. Seasonal differences in spawning, egg size, and early development of the Hawaiian anchovy or nehu, *Encrasicholina purpurea*. *Fish. Bull. 87*: 593-600.

Chiappa-Carrara, X., 1993. Análisis de la edad, crecimiento, dieta, tasas de digestión y preferencias tróficas de la anchoveta hawaiana *Encrasicholina purpurea* Fowler (Pisces: Engraulidae). *Doctoral thesis, UACPYP-CCH, UNAM*, 142 p.

Comitini, S., 1977. An economic analysis of the state of the Hawaiian skipjack tuna fishery. *Sea Grant Tech. Rep. UNIHI-SEAGRANT-TR-78-01, Univ. Hawaii, Honolulu*, 46 p.

Degens, E.T., W.G. Deuser and R.L. Haedrich, 1969. Molecular structure and composition of fish otoliths. *Mar. Biol. 2*: 105-113.

Gallardo-Cabello, M., 1979. Características biológicas de *Phycis blennoides* (Brünnich, 1768) con especial referencia a algunas modificaciones debidas al efecto de la explotación pesquera. *Doctoral thesis, Univ. Central de Barcelona,* 420 p.

Gallardo-Cabello, M., 1985. Determinación de la edad de la anchoveta *Engraulis mordax* Girard, en aguas de Baja California Norte (Pisces: Engraulidae). *An. Inst. Cienc. del Mar y Limnol., UNAM 12*: 221-234.

NAKAMURA, E.L.,1970. Synopsis of biological data on Hawaiian species of *Stolephorus*. *In*: J.C. MARR (Ed.), *The Kuroshio: a symposium on the Japan current*. East-West Center Press, Honolulu, pp. 425-446.

PANNELLA, G., 1971. Otoliths daily growth layers and periodical patterns. *Science 173*: 1124-1127.

PANNELLA, G., 1974. Otolith growth patterns: an aid in age determination in temperate and tropical fishes. *In*: T.B. BAGENAL (Ed.), *The Ageing of Fish*, Unwin Bro. Ltd., Surrey, pp. 28-39.

PANNELLA, G., 1980. Growth patterns in fish sagittae. *In*: *D.C. Rhoads* y R.A. LUTZ (Eds.), *Skeletal Growth of Aquatic Organisms. Biological Records of Environmental Changes*. Plenum Press, NY and London, pp. 519-560.

PRAGER, M.H., S.B. SAILA and C.W. RECKSLEK, 1989. Fishparm: a microcomputer program for parameter estimation of nonlinear models in fishery science. *Old Dominion University, Oceanography Tech. Rep. 87-10*,18 p.

STRUHSAKER, P., W.J. BALDWIN and G.I. MURPHY, 1975. Environmental factors affecting stress and mortality of the Hawaiian anchovy (*Stolephorus purpureus*) in captivity. *Univ. Hawaii Sea Grant Tech. Rep., UNIHI-SEAGRANT-TR-75-02*, Honolulu, 124 p.

STRUHSAKER, P., and J.H. UCHIYAMA, 1976. Age and growth of the nehu, *Stolephorus purpureus* (Pisces: Engraulidae), from the Hawaiian Islands as indicated by daily growth increments of *sagittae*. *Fish. Bull. 74*: 9-17.

TAUBERT, B.D., and D.W. COBLE, 1977. Daily rings in otoliths of three species of *Lepomis* and *Tilapia mossambica*. *J. Fish. Res. Bd. Can., 34*: 332-340.

WILSON, K.H. and P.A. LARKIN, 1980. Daily growth rings in the otoliths of juvenile sockeye salmon (*Oncorhynchus nerka*). *Can. J. Fish. Aquat. Sci. 37*: 1495-1498.

Recibido: 21 de noviembre de 1995.

Aceptado: 14 de marzo de 1996.