

INDIRECT VALIDATION OF DAILY INCREMENTS IN WHITEMOUTH CROAKER (*Micropogonias furnieri*) LARVAE OTOLITHS*

by

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RESUMEN

Validación indirecta de incrementos diarios en otolitos de larvas de corvina rubia (*Micropogonias furnieri*). La validación indirecta en el conteo de incrementos (anillos) por un análisis de precisión permitió determinar el patrón diario de depósito en otolitos de corvina rubia (*Micropogonias furnieri*) durante el estadio larval y juvenil temprano. Para comparar el número de anillos en cada individuo se utilizaron ambos otolitos *sagittae* (n = 71) y las lecturas realizadas por dos lectores independientes (n = 27). Una leve variación del foco óptico permitió eliminar los incrementos subdiarios y obtener el mejor plano de lectura del patrón de crecimiento diario. Entre los incrementos 28 y 35, aproximadamente, los otolitos en crecimiento mostraron una zona algo difusa en la que aparecieron núcleos secundarios alrededor del núcleo central. A partir del incremento 35 se observó otro patrón de depósito marcadamente distinto al de los núcleos secundarios. Las pendientes de las regresiones lineales ajustadas entre ambos otolitos del par y entre las observadas por los lectores independientes sobre el mismo otolito no mostraron diferencias significativas respecto de 1. El análisis replicado en cada individuo y la intervención de dos lectores independientes experimentados constituyó un método consistente para identificar el patrón diario de depósito en otolitos de larvas y juveniles tempranos de la especie.

SUMMARY

The indirect validation in increments (rings) counting by a precision analysis allowed to determine the daily deposition pattern in whitemouth croaker (*Micropogonias furnieri*) otoliths during the larval and early juvenile stages. To compare the number of rings in each individual both *sagittae* otoliths (n = 71) and the readings by two independent readers (n = 27) were used. A slight variation of the optic focus allowed to eliminate sub-daily increments and obtain the best reading plane of the daily growth pattern. Between the 28th and 35th increment, approximately, the growing otoliths showed a rather diffuse zone where accessory nuclei around the main nucleus appeared. From the 35th increment onwards another deposition pattern markedly different from that of the accessory nuclei was noticed. The slopes of the

linear regressions fitted between the otoliths of the pair and between the ones observed on the same otolith by the independent readers did not show significant differences with respect to 1. The analysis replicated on each individual and the intervention of two skilled independent readers constituted a consistent method to identify the daily deposition pattern in otoliths of the species larvae and early juveniles.

Palabras clave: Otolitos, larvas de peces, microestructura, anillos de crecimiento, incrementos diarios, *Micropogonias furnieri*.

Key words: Otoliths, fish larvae, microstructure, growth rings, daily increments, *Micropogonias furnieri*.

INTRODUCTION

The interpretation of an otolith microstructure constitutes a powerful tool to determine age and growth of fish larvae and juveniles (Thorrold *et al.*, 1997; Rooker *et al.*, 1999; Power *et al.* 2000). During those stages, otoliths develop at a daily rhythm (Pannella, 1971) through deposition of a structure of *annuli* or increments with a translucent band (incremental zone) and an opaque band (discontinuous zone). The finding triggered the emergence of a large number of methods based on otoliths microstructure applied worldwide (Campana and Neilson, 1985; Jones, 1986; Campana and Moksness, 1991; Campana, 1992, 2001; Stevenson and Campana, 1992).

A previous step to apply said methods is to validate the daily deposition pattern which implies rearing of individuals and dying of otoliths with different substances at the laboratory (Geffen, 1992). Among the most widely used, Alizarin Complexone (Reichert *et al.*, 2000), Oxytetracycline (McFarlane and Beamish, 1987; McBride, 2002) and Calcein (Bumguardner and King, 1996) are to be mentioned. The method entails knowledge of age and/or time between marking events (dying) and allows direct identification of daily increments. However, the analysis of the same otoliths by different experienced readers could be considered as an indirect method to validate increment periodicity. That type of analysis minimizes the sources of error produced by readers' subjectivity (Campana, 1992). A commonly used criterion to detect daily increments is to

slightly vary the microscope focusing of the otolith microstructure. Such slight variation results particularly useful to eliminate sub-daily increments from the visual field (Campana and Jones, 1992; Morioka and Machinandiarena, 2001). In summary, image analysis provides manipulation advantages (Campana, 1992).

Whitemouth croaker (*Micropogonias furnieri*, Desmarest 1823) age and growth studies were performed in Argentina and Uruguay (Cotrina and Lasta, 1986; Cotrina and Carozza, 1997; Borthagaray *et al.*, 2011). Nevertheless, they were focused on adults and provide scarce information on early stages of the species (Albuquerque *et al.*, 2009). Survival of early life stages may be affected by the impact of temperature on growth and development, food availability, predation and physical processes such as retention, drift, and micro-turbulence, among others. Even small changes in growth rates produced by high mortality levels may reduce or extend the duration of larval stages with the consequent serious effects on recruitment (Houde, 1989). The documentation gathered shows that a fishery resource biomass fluctuation is influenced by the events occurred during the early life stages of the species (Houde, 2008).

Daily increments in *M. furnieri* larvae from Lagoa dos Patos, Brazil, reared for 29 days since hatching were directed validated (Albuquerque *et al.*, 2009). However, no studies were performed to determine the daily pattern of older or wild individuals. The aim of this study is to analyze precision in increment counting using an indirect validation method to determine *M. furnieri* otoliths deposition pattern during the larval and early juvenile stages.

MATERIALS AND METHODS

A total of 71 *M. furnieri* individuals 4.3-34.9 mm SL younger than a year old were used. Specimens derived from the CC-03/2006 "Whitemouth croaker larvae ecology" survey carried out on board of the R/V "Capitán Cánepa" from 10th through 20th March 2006 along the turbidity front of the Río de la Plata area. Samples were taken with a Motoda net (200 microns mesh size) in plankton tows at different depth levels and with an epibenthic sampler (500 microns mesh size) in bottom tows. Larvae and juveniles were separated on board and kept frozen for conservation. At the laboratory, individuals were measured to the nearest mm and the *sagittae* otoliths extracted for microstructure analysis.

To proceed to tissue disintegration of both otoliths, extracted from the heads, sodium hypochlorite was used. Later, they were rinsed with distilled water to eliminate tissue remains and crystals of sodium hypochlorite. After drying, using dissecting needles each pair was mounted in a glass slide with transparent resin (PRO-TEX or nail polish) and fixed in the *sagittal* plane. To carry out the procedures a binocular dissecting microscope was used.

The increments were observed under a binocular optical microscope (200X or 400X with immersion oil) using transmitted light connected to a computer equipped with an image analyzing system consisting of a video camera and a measuring software (Kontron, KS 300). When the increments were not visible, otoliths were polished using 12, 9 and 3 μm lapping film paper. At the microscope, improved visualization and contrast of increments were obtained with filters of polarized light.

Images of otoliths microstructure were permanently taken interacting with the focal distance. Counting of increments in each otolith of the pair and fitting of a linear model to the number of

increments of both otoliths were performed. Later, 27 otoliths randomly taken were counted by two independent readers who did not have any information on the specimens age or size (blind readers). Finally, a second linear model was fitted to the number of increments determined by both readers. In both cases the statistical significance of the slope was tested under the null hypothesis that it was equal to 1.

RESULTS

The daily growth pattern became visible changing the microscope focusing. With a poor focus sub-daily rings were eliminated and more intense and wider increment structures observed (Figure 1 A and B). The comparison established with an otolith of a *M. furnieri* larvae reared at the laboratory and experimentally marked in a previous study (Albuquerque *et al.*, 2009) confirmed the presence of daily and sub-daily increments (Figure 1 C).

As the otolith developed the image analysis showed an increased microstructure complexity. The otolith grew three-dimensionally adopting a lenticular shape. Bi-dimensionally (through microscopic observation) it could be seen that along the anterior-posterior axis it changed from a circular to a more elongated shape. In older individuals, between the 28th and 35th increment, approximately, accessory nuclei around the central nucleus appeared (Figure 2 A). The accessory nuclei zone, fairly diffuse, (Figure 2 B) showed a discontinuous increment pattern and a change in the direction of deposition. From the 35th increment onwards, a marked different deposition pattern with increments positioned in the same direction of those near the nucleus was observed. Although they were not as clear as those from the nucleus, identification was easier than that of the accessory nuclei. It was difficult to maintain a single focus plane throughout the major axis of

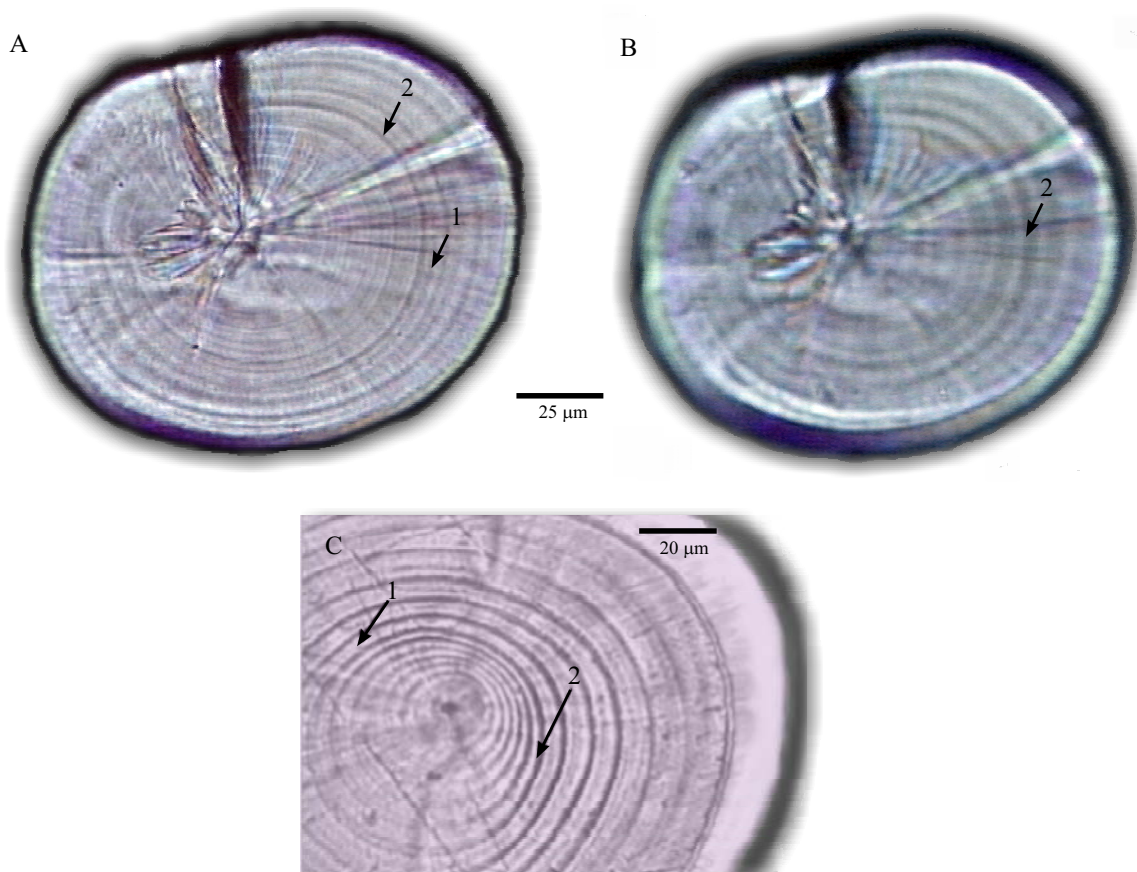


Figure 1. Sub-daily (1) and daily (2) increments in a 5 mm TL *Micropogonias furnieri* larva otolith observed before (A) and after (B) the slight variation of the image pursuant to Campana and Jones (1992) and Morioka and Machinandiarena (2001) criterion. Image of a similar otolith taken from Albuquerque and Muelbert (2004) (C).

Figura 1. Incrementos subdiarios (1) y diarios (2) en el otolito de una larva de *Micropogonias furnieri* de 5 mm de LS observado antes (A) y después (B) del leve desenfoque de la imagen según el criterio de Campana y Jones (1992) y Morioka y Machinandiarena (2001). Imagen de un otolito similar tomada de Albuquerque y Muelbert (2004) (C).

the otolith, mainly in those from larger individuals. Consequently, readings were performed with different focuses.

Increment readings of both otoliths of the pair were similar (Figure 3) and the slope of the linear model did not result statistically different from 1 ($p > 0.6$; $n = 71$). Likewise, the information provided by both independent readers did not differ in the number of increments counted ($p > 0.14$; $n = 27$) (Figure 4). Both analyses indicated consistency as regards identification of *M. furnieri* larvae otoliths daily deposition pattern.

DISCUSSION

M. furnieri larvae otoliths daily increment validation, conducted by Albuquerque *et al.* (2009), comprised individuals of a narrow age range (not beyond 29 days old). The results derived from this study on older and larger larvae that includes wild early juveniles up to 90 days old, confirm the experimental observations made by Albuquerque *et al.* (2009) and increase the observation

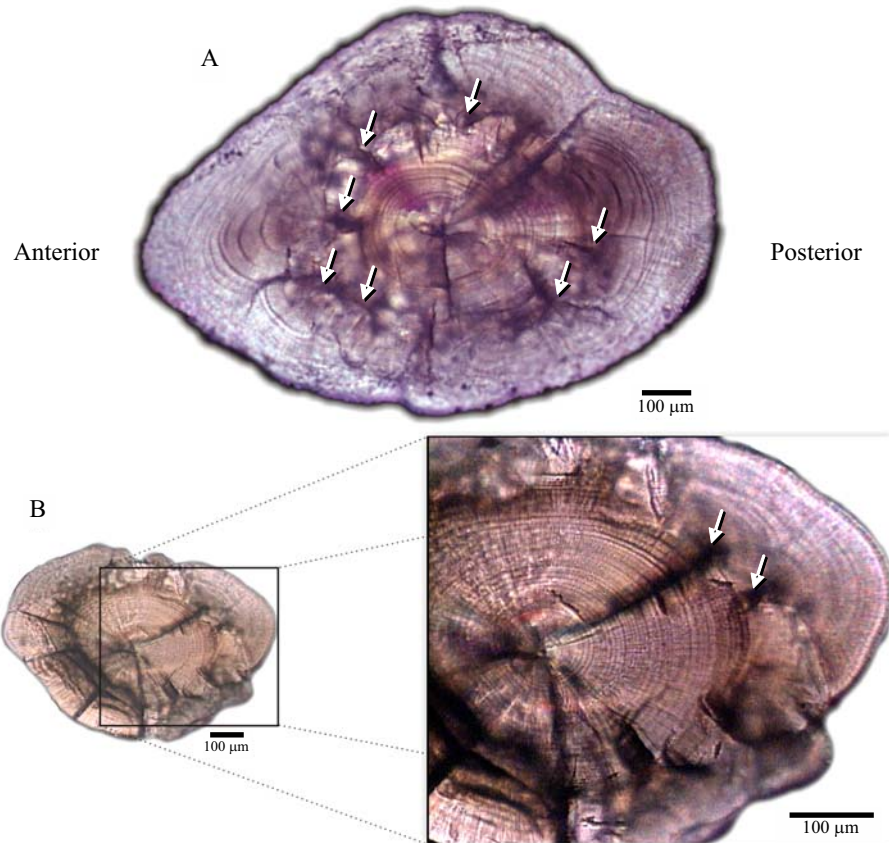


Figure 2. A) Otolith of a *Micropogonias furnieri* 18 mm SL (61 increments) early juvenile showing the presence of growth accessory nuclei (white arrows) around the central nucleus. B) Detail of an otolith corresponding to a 15 mm SL individual in which recent accessory nuclei appear showing the change in the deposition pattern.

Figura 2. A) Otolito de juvenil temprano de *Micropogonias furnieri* de 18 mm LS (61 incrementos) donde se observa la presencia de núcleos secundarios de crecimiento (flechas blancas) alrededor del núcleo central. B) Detalle de un otolito correspondiente a un individuo de 15 mm LS, en el que aparecieron recientes núcleos secundarios mostrando el cambio de patrón de depósito.

interval. Even though the procedure used in this work is not a direct validation method, that would include larvae rearing and marking of otoliths through immersion in fluorescent dye (Morioka and Machinandiarena, 2001; Brown and Fuentes, 2010), the interpretation and counting of daily increments by experienced readers demonstrates to be a reliable method developed in other fish species (Campana and Moksness, 1991). One of the advantages of said validation method is that no reared larvae but wild individuals are used; therefore, as shown by Tsuji and Aoyama (1982),

increments are more clearly observed than those under laboratory conditions. Thus, the usefulness of the indirect validation method is based on the fact that larvae and juveniles age determination is performed on wild individuals.

A poor focus of increments improves identification of *M. furnieri* otoliths daily growth pattern. As observed by Campana (1992), Campana and Jones (1992) and Morioka and Machinandiarena (2001), the optical method is an effective tool to observe the increment continuity pattern along the otolith surface. On the contrary, a

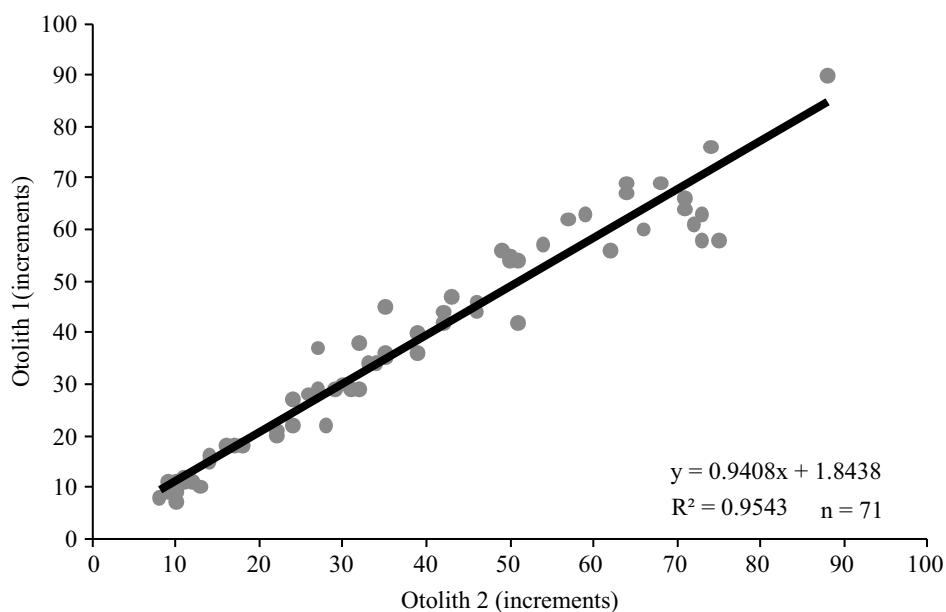


Figure 3. Relation among the daily increments counted in both otoliths of each individual.

Figura 3. Relación entre los incrementos diarios contados en ambos otolitos de cada individuo.

good focus shows sub-daily increments thus troubling distinction of daily growth, specially if high magnification levels causing loss of resolution are used. Sub-daily rings were identified in larvae and juveniles of different species (Palomera *et al.*, 1988; Wright *et al.*, 1991; Albuquerque *et al.*, 2009; Buratti and Santos, 2010). To consider such increments as daily would overestimate individual ages. Therefore, the correct identification of sub-daily increments is of the outmost importance to perform a precise aging analysis. The use of high magnification levels leads to confuse daily and sub-daily increments and does not allow visualization of the otolith as a whole. Hence, instead of high magnifications (1,000X), those 200X and 400X were combined.

Subjectivity in microstructure analysis increases when working with complex otoliths such as those of Sciaenidae. The intervention of two independent and experienced readers constitutes a consistent interpretation method. Powell *et al.* (2000), who worked on *Cynoscion nebulosus*

larvae age determination, recommend separated validation studies for each reader. Those on Sciaenidae otoliths daily increments are scarce due to the difficulties encountered when trying to understand microstructure as the otolith develops. In this work, the basis to interpret *M. furnieri* larvae and juveniles otoliths is provided.

The appearance of accessory nuclei as the otolith develops complicates microstructure analysis. Such nuclei, observable in 8-16 mm SL larvae, could introduce errors in readings as the zones where they appear, fuzzy in many otoliths, require constant variation of the focus plane to identify increments. Interestingly enough, those observed in the accessory nuclei area were wider than the ones in the center. It was proved that the changes that occur in otoliths are related to important moments in the fish life (Campana and Neilson, 1985). The processes related to the impact caused by the environment or ontogeny can be identified as 'checks' in the otolith microstructure (Brothers and McFarland, 1981; Campana, 1984; Campana and Nielson, 1985). In different species, the

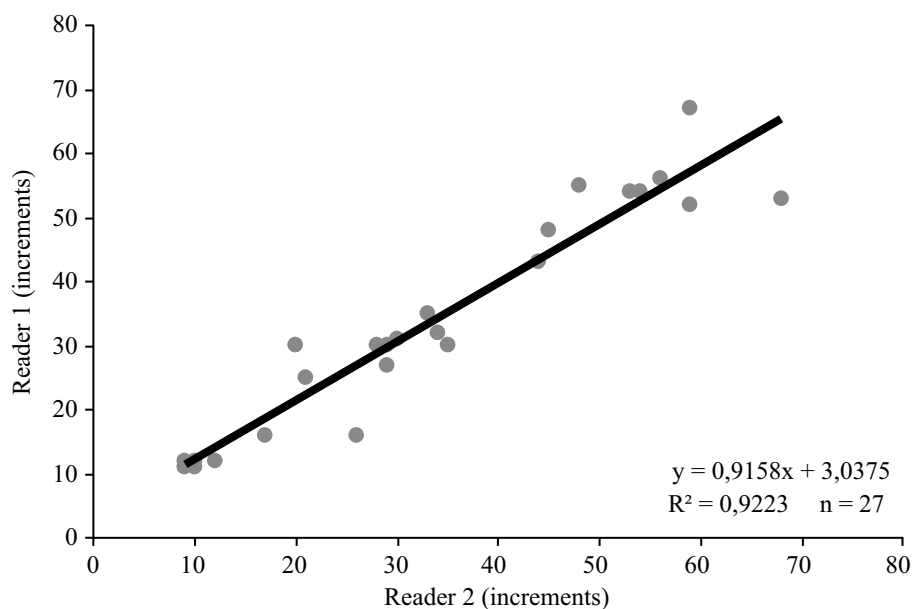


Figure 4. Relation among the number of daily increments counted by two independent readers.
 Figura 4. Relación entre el número de incrementos diarios contados por dos lectores independientes.

appearance of accessory nuclei during the otolith development or sudden changes in the increment width are related either to the larva-juvenile transition (metamorphosis) (Nishimura and Yamada, 1984; Xie *et al.*, 2005) or to a habitat change (Wilson and McCormick, 1997; 1999; Buratti and Santos, 2010). Accessory nuclei are specially reported in demersal species from temperate waters that experience habitat changes during their early development. Examples are *Theragra chalcogramma* (Nishimura and Yamada, 1984), *Merluccius hubbsi* (Buratti and Santos, 2010), flatfish *Pseudopleuronectes americanus* (Sogard, 1991) and *Solea solea* (Lagardere and Troadec, 1997). The presence of similar nuclei in *M. furnieri* otoliths could be attributable to metamorphosis or habitat changes (settlement). Weiss (1981) described the position of the mouth as being totally horizontal at 20 mm TL. In this study it was found that *M. furnieri* otoliths had all the accessory nuclei formed at the same fish size. Probably, both morphological events are associated and further studies are needed to check hypotheses.

REFERENCES

- ALBUQUERQUE, C.Q. & MUELBERT, J.H. 2004. The use of haematoxylin stain to improve contrast of growth increments in otoliths of larval whitemouth croaker. *J. Fish Biol.*, 64: 794-798.
- ALBUQUERQUE, C.Q., MUELBERT, J.E. & SAMPAIO, L.A.N. 2009. Early developmental aspects and validation of daily growth increments in otoliths of *Micropogonias furnieri* (Pisces, Sciaenidae) larvae reared in laboratory. *Pan-Am. J. Aquat. Sci.*, 4 (3): 259-266.
- BORTHAGARAY, A.I., VEROCAI, J. & NORBIS, W. 2011. Age validation and growth of *Micropogonias furnieri* (Pisces-Sciaenidae) in a temporally open coastal lagoon (South-western Atlantic-Rocha-Uruguay) based on otolith analysis. *J. Appl. Ichthyol.*, 27: 1212-1217. doi: 10.1111/j.1439-0426.2011.01778.x
- BROTHERS, E.B. & MCFARLAND, W.N. 1981. Cor-

- relations between otolith microstructure, growth, and life history transitions in newly recruited french grunts [*Haemulon flavolineatum* (desmarest), haemulidae]. Rapp. p.v. Reun., 178: 369.
- BROWN, D.R. & FUENTES, C.M. 2010. Daily increments in otoliths of sabalo (*Prochilodus lineatus*) larvae. J. Appl. Ichthyol., 26: 123-125.
- BUMGUARDNER, B.W. & KING, T.L. 1996. Toxicity of oxytetracycline and calcein to juvenile striped bass. Trans. Am. Fish. Soc., 125: 143-145.
- BURATTI, C.C. & SANTOS, B.A. 2010. Otolith microstructure and pelagic larval duration in two stocks of the Argentine hake, *Merluccius hubbsi*. Fish. Res., 106: 2-7.
- CAMPANA, S.E. 1984. Interactive effects of age and environmental modifiers on the production of daily growth increments in otoliths of plainfin midshipman, *Porichthys notatus*. Fish. Bull., 82 (1): 165-177.
- CAMPANA, S.E. 1992. Measurements and interpretation of the microstructure of fish otoliths. In: STEVENSON, D.K. & CAMPANA, S.E. (Eds.). Otolith microstructure examination and analysis. Can. Spec. Publ. Fish. Aquat. Sci., 117: 59-71.
- CAMPANA, S.E. 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. J. Fish Biol., 59 (2): 197-242.
- CAMPANA, S.E. & JONES, C.M. 1992. Analysis of otolith microstructure data. In: STEVENSON, D.K. & CAMPANA, S.E. (Eds.). Otolith microstructure examination and analysis. Can. Spec. Publ. Fish. Aquat. Sci., 117: 73-100.
- CAMPANA, S.E. & MOKSNESS, E. 1991. Accuracy and precision of age and hatch date estimates from otolith microstructure examination. ICES J. Mar. Sci., 48: 303-316.
- CAMPANA, S.E. & NEILSON, J.D. 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci., 42: 1014-1032.
- COTRINA, C. & CAROZZA, C.R. 1997. Edad y crecimiento de corvina rubia (*Micropogonias furnieri*) del sector costero de la provincia de Buenos Aires, Argentina. In: COLACMAR 7°, Congreso Latinoamericano sobre Ciencias do Mar, Sao Paulo, Brasil: 210-212.
- COTRINA, C.P. & LASTA, C. 1986. Estudio preliminar de la determinación de edad en la corvina (*Micropogonias furnieri*). Public. Com. Téc. Mix. Fr. Mar., 1 (2): 311-318.
- GEFFEN, A.G. 1992. Validation of otoliths increment deposition rate. In: STEVENSON, D.K. & CAMPANA, S.E. (Eds.). Otolith microstructure examination and analysis. Can. J. Fish. Aquat. Sci., 117: 101-113.
- HOUDE, E.D. 1989. Comparative growth, mortality, and energetics of marine fish larvae: temperature and implied latitudinal effects. Fish. Bull., 87: 471-495.
- HOUDE, E.D. 2008. Emerging from Hjort's Shadow. J. Northw. Atl. Fish. Sci., 41: 53-70.
- JONES, C.M. 1986. Determining age of larval fish with the otolith increment technique. Fish. Bull., 84 (1): 91-104.
- LAGARDÈRE, F. & TROADEC, H. 1997. Age estimation in common sole *Solea solea* larvae: validation of daily increments and evaluation of a pattern recognition technique. Mar. Ecol. Prog. Ser., 155: 223-237.
- MCBRIDE, R.S. 2002. Spawning, growth, and overwintering size of searobins (Triglidae: *Prionotus carolinus* and *P. evolans*). Fish. Bull., 100: 641-647.
- MCFARLANE, G.A. & BEAMISH, R.J. 1987. Selection of dosages of oxytetracycline for age validation studies. Can. J. Fish. Aquat. Sci., 44: 905-909.
- MORIOKA, S. & MACHINANDIARENA, L. 2001. Comparison of daily increment formation pattern between sagittae and lapilli of ling (*Genypterus blacodes*) larvae and juveniles collected off Argentina. N. Z. J. Mar. Freshw. Res., 35: 111-119.
- NISHIMURA, A. & YAMADA, J. 1984. Age and

- growth of larval and juvenile walleye pollock, *Theragra chalcogramma* (Pallas), as determined by otolith daily growth increments. *J. Exp. Mar. Biol. Ecol.*, 82: 191-205.
- PALOMERA, I., MORALES-NIN, B. & LEONART, J. 1988. Larval growth of anchovy, *Engraulis encrasicolus*, in the Western Mediterranean Sea. *Mar. Biol.*, 99 (2): 283-291.
- PANNELLA, G. 1971. Fish otoliths: daily growth layers and periodical patterns. *Science*, 173: 1124-1127.
- POWELL, A.B., LABAN, E.H., HOLT, S.A. & HOLT, G.J. 2000. Validation of age estimates from otoliths of larval and juvenile spotted seatrout, *Cynoscion nebulosus*. *Fish. Bull.*, 98: 650-654.
- POWER, M., ATTRILL, M.J. & THOMAS, R.M. 2000. Temporal abundance and growth of juvenile herring and sprat from the Thames estuary 1977-1992. *J. Fish Biol.*, 56: 1408-1426. doi: 10.1006/jfbi.2000.1264.
- REICHERT, M.J.M., DEAN, J.M., FELLER, R.J. & GREGO, J.M. 2000. Somatic growth and otolith growth in juveniles of a small subtropical flatfish, the fringed flounder, *Etropus crossotus*. *J. Exp. Mar. Biol. Ecol.*, 254: 169-188.
- ROOKER, J.R., HOLT, S.A., HOLT, G.J. & FUIMAN, L.A. 1999. Spatial and temporal variability in growth, mortality, and recruitment potential of postsettlement red drum, *Sciaenops ocellatus*, in a subtropical estuary. *Fish. Bull.*, 97: 581-590.
- SOGARD, S.M. 1991. Interpretation of otolith microstructure in juvenile winter flounder (*Pseudopleuronectes americanus*): ontogenetic development, daily increment validation, and somatic growth relationships. *Can. J. Fish. Aquat. Sci.*, 48: 1862-1871.
- STEVENSON, D.K. & CAMPANA, S.E. 1992. Otolith microstructure examination and analysis. *Can. Spec. Publ. Fish. Aquat. Sci.*, 117, 126 pp.
- THORROLD, S.R., JONES, C.M. & CAMPANA, S.E. 1997. Response of otolith microchemistry to environmental variations experienced by larval and juvenile Atlantic croaker (*Micropogonias undulatus*). *Limnol. Oceanogr.*, 42 (1): 102-111.
- TSUJI, S. & AOYAMA, T. 1982. Daily Growth Increments Observed in Otoliths of the Larvae of Japanese Red Sea Bream *Pagrus major* (Temminck et Schelegel). *Bull. Jap. Soc. Sci. Fish.*, 48 (11): 1559-1562.
- WEISS, G. 1981. Ictioplankton del estuario de Lagoa dos Patos, Brasil. Tesis de Doctorado, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, 164 pp.
- WILSON, D.T. & MCCORMICK, M.I. 1997. Spatial and temporal validation of settlement marks in the otoliths of tropical reef fishes. *Mar. Ecol. Prog. Ser.*, 153: 259-271.
- WILSON, D.T. & MCCORMICK, M.I. 1999. Microstructure of settlement-marks in the otoliths of tropical reef fishes. *Mar. Biol.*, 134: 29-41.
- WRIGHT, P.J., ROWE, D. & THORPE, J.E. 1991. Daily growth increments in the otoliths of Atlantic salmon parr, *Salmo salar* L., and the influence of environmental factors on their periodicity. *J. Fish Biol.*, 39 (1): 103-113.
- XIE, S., WATANABE, Y., SARUWATARI, T., MASUDA, R., YAMASHITA, Y., SASSA, C. & KONISHI, Y. 2005. Growth and morphological development of sagittal otoliths of larval and early juvenile *Trachurus japonicus*. *J. Fish Biol.*, 66: 1704-1719. doi: 10.1111/j.0022-1112.2005.00717.x.

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