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Research Note

Validations of the daily periodicity of increment deposition in rocky intertidal fish otoliths of the south-eastern Pacific Ocean

Validación de la periodicidad diaria de los incrementos en otolitos de peces intermareales del Pacífico sudoriental

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Abstract.- Daily deposition of otolith increments was validated for juveniles of 8 intertidal fish species in central Chile, *Helcogrammoides chilensis*, *Helcogrammoides cunninghami* (Tripterygiidae), *Auchenionchus microcirrhis*, *Auchenionchus crinitus* (Labrisomidae), *Hypsoblennius sordidus* (Blenniidae), *Gobiesox marmoratus*, *Sicyaces sanguineus* (Gobiesocidae) and *Myxodes viridis* (Clinidae). Validation was performed by Alizarin Red-S labelling followed by 6 days of growth.

Key words: Otolith daily increments, alizarin red S, intertidal fishes

Introduction

The increment patterns of otoliths have permitted great progress in studies on the growth dynamics of fish. In addition to age and growth, fish otoliths may also record life history events such as metamorphosis, settlement and migration (Victor 1982, Sponaugle & Cowen 1994, Jenkins *et al.* 1996, Wilson & McCormick 1997, Thorrold *et al*. 2001). Age estimates can be obtained by the enumeration of growth increments observed in the otoliths. These increments can be deposited annually (*e.g.*, Fowler 1990), daily (*e.g.*, Campana & Neilson 1982, Schmitt 1984, Iglesias *et al.* 1997), or even sub-daily (*e.g.*, Pannella 1971, Campana & Neilson 1982).

Because of the potential variation in the temporal pattern of increment formation, it is important to validate otolith increment periodicity when using otoliths to age fishes. The experimental manipulation of temperature, light or food ration can produce a natural structural mark in the otolith which may be used in age validation (*e.g.*, Victor 1982). However, a chemically induced mark is preferable because it is more easily distinguished from naturally occurring variation in increment structure. Therefore, validation of increment periodicity is commonly achieved by inducing a mark in the otolith, then sacrificing the fish after a number of days, months or years. The number of growth increments deposited between the induced artificial mark and the otolith edge is then compared to the known time period the fish was alive since mark induction (Hernaman *et al.* 2000).

Fluorescent chemicals such as tetracycline and calcein have been widely used to mark otoliths of species from many families of tropical and temperate fishes (*e.g.*, Schmitt 1984, Monaghan 1993, Wilson & McCormik 1997). Recently Alizarin Red S has been used as an effective chemical marker in a number of studies (Skov 2001, Fitzpatrick *et al.* 2010). This fluorescent compound is assimilated and incorporated into the calcareous structure of the otoliths and can be detected by epifluorescence microscopy. Used in combination with light microscopy, the staining technique reveals the number of rings deposited after they have been marked (Campana 2001).

The central coast of Chile comprises a large diversity of fish, including the 44% of the all endemic species recorded in southeast Pacific (Ojeda *et al*. 2000). Typically, fish assemblages observed in rocky intertidal zones are characterized by resident and transient species. While residents remain in the intertidal area after recruitment, transient species migrate to the subtidal once they reach a certain body size (Varas & Ojeda 1990, Muñoz & Ojeda

1997). Numerous studies have focused on the biology and ecology of the most conspicuous species found in the rocky intertidal, primarily on the juvenile and adult phases of life (Varas & Ojeda 1990, Cáceres & Ojeda 2000, Fariña *et al*. 2000, Muñoz & Ojeda 2000, Williams & Springer 2001, Hernández-Miranda *et al*. 2009, Hernández-Miranda & Ojeda 2006, Pulgar *et al*. 2006). However, in recent years, research has addressed pre-recruitment biology and dynamics (Plaza-Pasten *et al*. 2003, Hernández-Miranda *et al*. 2009, Palacios-Fuentes *et al*. 2012, Contreras *et al*. 2013). Most of the research conducted on pre-recruits heavily relied on the information obtained from reading otoliths, usually assuming the number of observed rings corresponded with a daily frequency of deposition. To confirm this assumption, the current study aimed to validate the periodicity of increment deposition in post-settlement juveniles of 8 intertidal fish species of central Chile using Alizarin Red S as the fluorescent marker.

Materials and methods

Young-of-the-year (YOY) of triplefins *Helcogrammoides chilensis*, *Helcogrammoides cunninghami* (Tripterygiidae), labrisomids *Auchenionchus microcirrhis*, *Auchenionchus crinitus* (Labrisomidae), blenny *Hypsoblennius sordidus* (Blenniidae), clingfishes *Gobiesox marmoratus*, *Sicyases sanguineus* (Gobiesocidae) and clinid *Myxodes viridis* (Clinidae) were collected from rocky intertidal pools at 3 localities separated by approximately 10 km of sandy beaches along the central Chilean coast: Las Cruces (LC: 33°30'S; 71°37'W), El Tabo (ET: 33°31'S, 71°40'W), and Isla Negra (IN: 33°24'S, 71°43'W). Fish were captured with hand nets aided by the use of 20% benzocaine (BZ-20) between April 2010 and December 2011. Captured YOY were placed in labelled plastic bags and transported to the laboratory, where total length (TL) was measured to the nearest mm. The collected specimens (Table 1) were transferred to the laboratory in 'Estación Costera de Investigaciones Marinas' (ECIM). To acclimatize the fishes, they were kept in 15-L aquaria for 7 days under natural light photoperiod, with constantly aerated, circulating water, and fed *ad libitum* with TetraMarine Saltwater Flakes® food nutritional supplement on a daily basis.

The Alizarin Red S (LobaChemi®) was dissolved in seawater in two 15-L tanks at a concentration of 150 mg l^{-1}. The tanks remained strongly aerated to maintain the pH near 7. Individuals were kept in the Alizarine Red S tanks for 24 h before transferring them to small aquaria (5-L),

Table 1. Alizarin Red S marking experiment. Survival during the experiment (% sur.), number of analysed sagittae (n), average total length in mm (TL ± SD), number of rings/sagitta (r ± SD)) and chi-square statistical results (χ^2), degrees of freedom (df), probability (*P*) of the 8 species evaluated / Porcentaje de sobrevivencia resultante del experimento (% sur.), número de sagitta analizados (n), longitud total promedio de los individuos en milímetros (TL ± DE), número de anillos/sagitta (r ± DE) y resultados estadísticos chi-cuadrado (χ^2), grados de libertad (df), probabilidad (*P*), de las 8 especies evaluadas

Family/ Species	% Survival	n	TL (mm)	r	χ^2	df	*P*
Labrisomidae							
A. crinitus	81.81	9	75.11 ± 17.17	5.22 ± 0.44	1.17	8	0.997
A. microcirrhis	81.81	9	135.39 ± 36.33	5.22 ± 0.44	1.17	8	0.997
Tripterygiidae							
H. chilensis	88.63	15	37.60 ± 12.40	5.20 ± 0.68	2.67	14	0.999
H. cunninghami	83.33	5	32.17 ± 2.96	6.00 ± 0.0	0.21	4	0.977
Blenniidae							
H. sordidus	87.18	15	38.50 ± 5.95	3.87 ± 0.83	13.00	18	0.527
Clinidae							
M. viridis	45.45	4	91.75 ± 2.30	4.75 ± 0.50	1.17	3	0.761
Gobiesocidae							
G. marmoratus	87.50	7	40.91 ± 9.47	4.71 ± 0.49	2.17	6	0.904
S. sanguineus	14.29	5	24 ± 5.21	4.60 ± 0.89	2.17	4	0.705

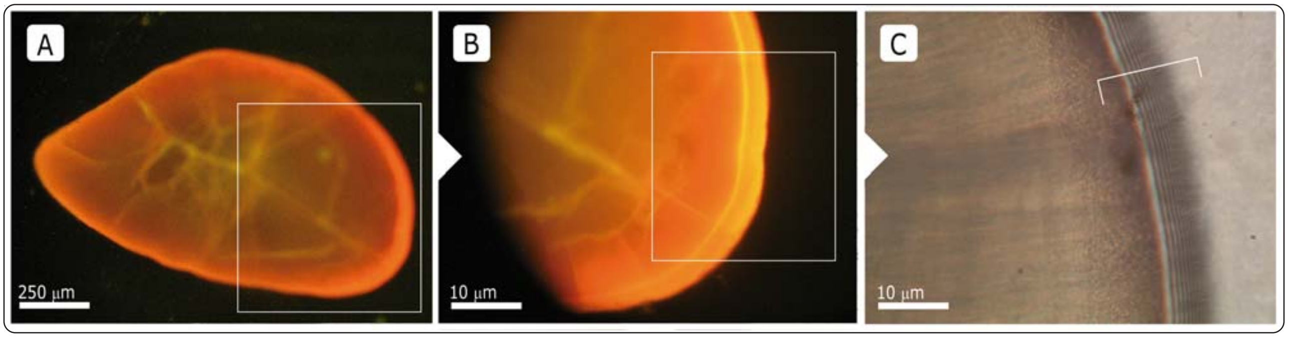

Figure 1. Photographs of sagittae used to validate the daily periodicity of otolith increments in *H. chilensis*. A-B: fluorescence microscope, C: light microscope / Fotografías del otolito sagitta de *H. chilensis* utilizado para validar la periodicidad de los incrementos. A-B: Imágenes en microscopio de fluorescencia, C: Imagen en microscopio óptico

with constantly aerated, circulating water. Each tank had five fish that were fed *ad libitum* for 6 days. After the sixth day, the fish were subject to fasting one day, followed by the second 24-h staining treatment with Alizarin Red-S was then carried out. The second staining was performed in order to have a specific fluorescent ring and test the hypothesis that if the dynamics of deposition is daily, 6 rings must be found between the 2 fluorescent rings.

The surviving fish were sacrificed with BZ-20, measured and fixed in 96% ethanol. Sagitta, asteriscus and lapillus otoliths were removed and kept in bidistilled water. The sagitta otoliths were mounted on slides with epoxy resin, polished with 30 ìm and 1 ìm grit paper and photographed with white light and UV under an epifluorescence microscope (Nikon® Eclipse E4000, blue filter Nikon B-2A). Images were obtained with an Olympus® Camedia C-5050 digital camera. The numbers of rings in the images were counted between the Alizarine Red S marks with the aid of the Image Pro-Express® Software, version 4.5, 2002 (Media Cybernetics) (Fig. 1). The number of observed rings observed was compared with the expected number of rings (6) and analyzed with the chi square test using STATISTICA 6.0 (Zar 1999).

Results and discussion

Survival rates to the Alizarin Red S treatment were high for six of the eight species under study (≥ 85%). The lowest survival rates were recorded for *M. viridis* (45%) and *S. sanguineus* (15%; Table 1). A fluorescent mark was clearly visible under UV light in the sagittae of all fishes. Daily increments following the fluorescent mark were clearly discernible in all of the sagittae examined. There was a generally good agreement between the expected and observed numbers of daily increments between the Alizarin Red S marks for all species (Table 1).

The present research confirmed that primary increments in otoliths of YOY of 8 intertidal fish species studied were deposited on a daily basis. The results are consistent with what is known to date. The daily periodicity of otolith increments has been confirmed in the larvae and juveniles of many teleost fishes (Hernaman *et al.* 2000, Joh *et al.* 2005, Parkinson *et al.* 2012) including some intertidal species (Hernaman *et al.* 2000) including 4 species of New Zealand intertidal triplefins that were validated using injected tetracycline (Kohn & Clements 2011). In the current research, Alizarin Red S appeared as clear fluorescent marks in all of the YOY analyzed, which agrees with recent studies demonstrating this compound to be a very effective chemical marker for validating growth bands in larval, juvenile and adult teleost fishes. It had a similar performance to alizarin complexone, calcine and other tetracycline-derived markers (Bashey 2004, Meisfjord *et al.* 2006, Crook *et al.* 2007, Liu *et al.* 2009, Fitzpatrick *et al.* 2010).

Finally, this work demonstrates a simple technique to validate the increment patterns of otoliths in pre-recruits of the most common species inhabiting the rocky intertidal coast of central Chile. Moreover, our research validates results of previous studies and provides a basis for future investigations of growth of pre-recruits in intertidal fish assemblage in central Chile.

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