ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 5, Issue 4, pp: (11-18), Month: October - December 2017, Available at: www.researchpublish.com

Comparison of Otoliths and Cleithra for Age and Growth Studies of the Grunt Haemulon steindachneri (Teleostei: Haemulidae) from Southwest Margarita Island, Venezuela

Misael D. Morales[1], Leo W. González[2]

[12]Área de Biología y Recursos Pesqueros, Instituto de Investigaciones Científicas, Universidad de Oriente, Nueva Esparta, Venezuela

Abstract: **Age and growth estimation in fishes using bony structures usually presents some disadvantages in the analysis of growth rings due to the morphologic characteristics of the species. Therefore it is recommended to examine more than one structure through direct observation and by applying statistical methods. The objective of this study was to compare otoliths and cleithra of the grunt *Haemulon steindachneri* to validate adequate structures for determining the age and growth of the species. Two hundred and fifty specimens were used; the sample was captured by the handcrafted fleet of Southwest Margarita Island between September 2005 and April 2006. Otoliths and cleithra were removed from the animals and analysed with a magnification binocular glass and reflected light. The percentage of bony structures with clearly legible and non-legible growth rings was verified. Partial and total radiuses were measured, and the relation between the total length of the fish and the total radius of the structure was established. These parameters were used later to estimate the back-calculated lengths for each structure. A Fisher test was used to statistically compare the back-calculated average lengths of each group of age from otoliths and cleithra. The back-calculation did not present significant statistical differences (*p> 0.05*) between the four groups of age observed. The study concluded that both bony structures are statistically similar for age and growth studies of *H. steindachneri.* Otoliths are recommended as the appropriate structure for presenting easily removable and clearly visible growth rings without the need for any special treatment.**

Keywords: **Otolith, Cleithrum, Age, Growth, Haemulon steindachneri, Margarita Island, Venezuela.**

I. INTRODUCTION

Age and growth studies are essential for fish stock assessment and fisheries management. These parameters together with mortality and abundance are fundamental to implement the sustainable use of marine resources [1-4]. It is important to know the minimum age at which a species should be caught and how long it takes for fish to reach that minimum length [4,5]. Applying direct methods for age determination of fishes have some limitations, for example, in tropical ecosystems establishing the relation between growth rings and environmental variables. Bony structures might provide evidence for traumatic events such as a drop in the salinity during a windy season or spawning, which have a direct relation to changes in the surface temperature and intense upwelling, for example, in the case of the sardine *Sardinella aurita* in Venezuela [6,7].

Haemulon steindachneri is a sandy and rocky bottom fish from the Haemulidae family usually found up to 50 m depth. This grunt grows around 20 cm total length (TL), and it is very abundant in sandy shallow waters and *Thalassia sp* meadows. As with other grunts, it is a carnivorous fish from the tropical sea that feeds mainly on invertebrates [8]. Its distribution comprises the western Atlantic Ocean from Guatemala to Brazil and the eastern Pacific Ocean from the Sea of Cortez to Peru [9].

In Venezuela, age and growth studies have been conducted using bony structures from *H. Steindachneri* [10,11]. This methodology was valid in other Venezuelan marine species, such as *Sardinella aurita* [12,13], *Cynoscion sp* [14], *Eugeres plumieri* [15], *Lutjanus analis* [16], *Opisthonema oglinum* [17], *Lutjanus purpureus* [18,19] and *Dactylopterus volitans* [20]. The purpose of this study was to compare the lengths at previous ages back-calculated from otoliths and cleithra and to establish the bony structure that is adequate to determine the age and growth of the grunt *H. steindachneri*.

II. MATERIALS AND METHODS

Monthly samplings of *H. steindachneri* were carried out from September 2005 to April 2006 in Boca del Rio, Margarita Island, Venezuela. Samples were provided by the local fishing community consisting of a handcraft fleet of 69 vessels operating in the southwestern region of the island (10º 53' N - 64º 12' W; Fig. 1) [21]. A total of 250 fishes sampled from the commercial catch were measured (TL) using an Ichthyometer Fish Measuring Board *Wilco 118*.

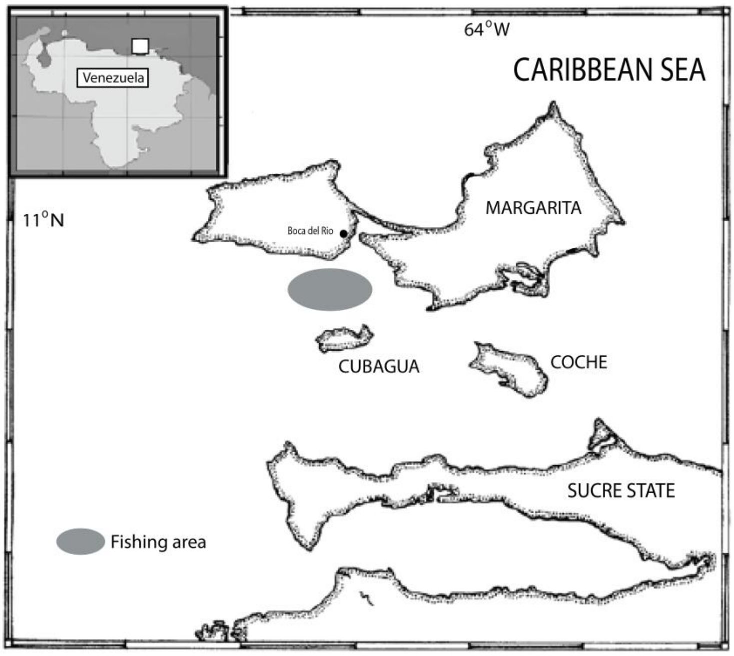

Fig 1. Fishing area of Boca del Rio's handcraft fishing fleet from Southwest Margarita Island, Venezuela

Otoliths: Sagittae otoliths were extracted from the fish by making a ventral cut with a scalpel and breaking the basioccipital bone and the auditory capsule to expose the pieces, later they were removed using tweezers [2,22]. Subsequently, the parts were cleaned with warm water, regular soap, and a soft brush to remove the rest of the tissue. Once dried, they were stored in labelled envelopes. A Magnification Binocular Glass *Wild* was used to read the otoliths. The structures were immersed in a petri dish previously filled with glycerine, placed on a dark background, and reflected with light [1]. The percentage of readable and non-readable otoliths was established. A micrometrical scale was adapted to a 16X objective and used to measure the partial and total rings (Fig. 2).

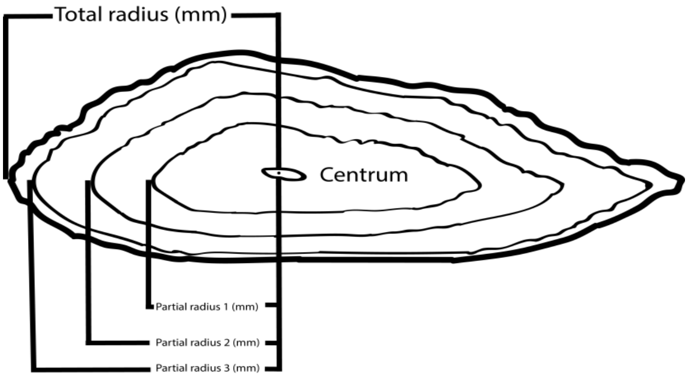

Fig 2. Sagitta otolith, showing the Centrum and the measurements for the analysis: partial and total radius (mm).

Cleithra: Cleithra were removed from the pectoral girdle by making a cut with a scalpel from the gular region to the joint between the post-temporal bone and the supracleithrum [10,17]. The parts were cleaned with warm water and a hard brush to separate the rest of the muscle and tissue. Once dried, the pieces were stored in labelled envelopes. The same methodology used with otoliths was performed to read the growth rings. The percentage of readable and non-readable cleithra was established. A micrometrical scale was adapted to a 6.4X objective and used to measure the partial and total rings (Fig. 3).

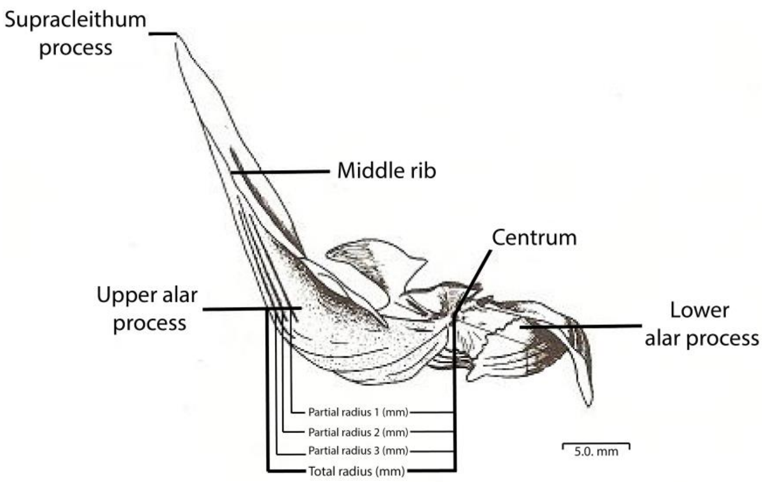

Fig 3. Cleithrum, showing the supracleithrum process, upper alar-process, lower alar-process, central rib, centrum, and the measurements for the analysis: partial and total radius (mm).

Data Analysis: The sampling information was entered into a Microsoft Excel sheet containing the following: date, TL, partial radius and total radius (R) of both structures, and the back-calculation of the lengths. A linear regression established the relation between the TL and the R of the structures, which was then used for the back-calculation according to the Lea model [23,24]:

$$L_i = r_i / R\ (TL - a) + a$$

Where *Li* is the back-calculated length, *ri* is the partial ring, *R* is the radius of the bony structure, *TL* is the fish total length, and *a* is the y-intercept from the linear regression. Subsequently, the average length of each group of age was calculated [25]. The comparison of the back-calculated TL by the group of age was made by applying a t_s test, and the variance homogeneity was previously proved by an *F-test* with an $\alpha=0.05$ confidence level [26-28].

III. RESULTS

From the observation analysis, 96.2% of otoliths and 80.4% of cleithra were legible (TABLE I). These proportions along with the statistical results allowed determining the bony structure that was adequate for age and growth studies of *H. steindachneri*.

TABLE I. Percentage of bony structures (otoliths and cleithra) from *H. steindachneri* with legible and non-legible growth rings.

	Growth Rings	
Bony structure	**Legible (%)**	**Non-legible (%)**
Otoliths	96.2	3.8
Cleithra	80.4	19.6

There was no significant difference between the back-calculated lengths in both structures (TABLE II).

TABLE II. Statistical comparison (*F-test; p>0.05*) between the average back-calculated lengths of the groups of age (L_1, L_2, L_3, L_4) through the analysis of growth rings (otoliths and cleithra) from *H. steindachneri*.

	Average Back-Calculated Length by Group of Age (mm)							
	L_1		**L_2**		**L_3**		**L_4**	
Statistic	**Otolith**	**Cleithrum**	**Otolith**	**Cleithrum**	**Otolith**	**Cleithrum**	**Otolith**	**Cleithrum**
n	199	143	181	134	121	77	21	16
Mean	65.26	124.06	106.32	146.52	133.71	163.26	150.33	171.68
Test	F= 0.84 *p*> 0.05		F= 1.43 *p*> 0.05		F= 0.90 *p*> 0.05		F= 1.02 *p*> 0.05	

The length distribution was composed of four group of age presented by the same number of hyaline growth rings. The length structure in otoliths was from 39 to 107 mm (Group 1^+), from 65 a 149 mm (Group 2^+), from 83 to 182 mm (Group 3^+), and from 113 to 189 mm (Group 4^+) (Fig. 4).

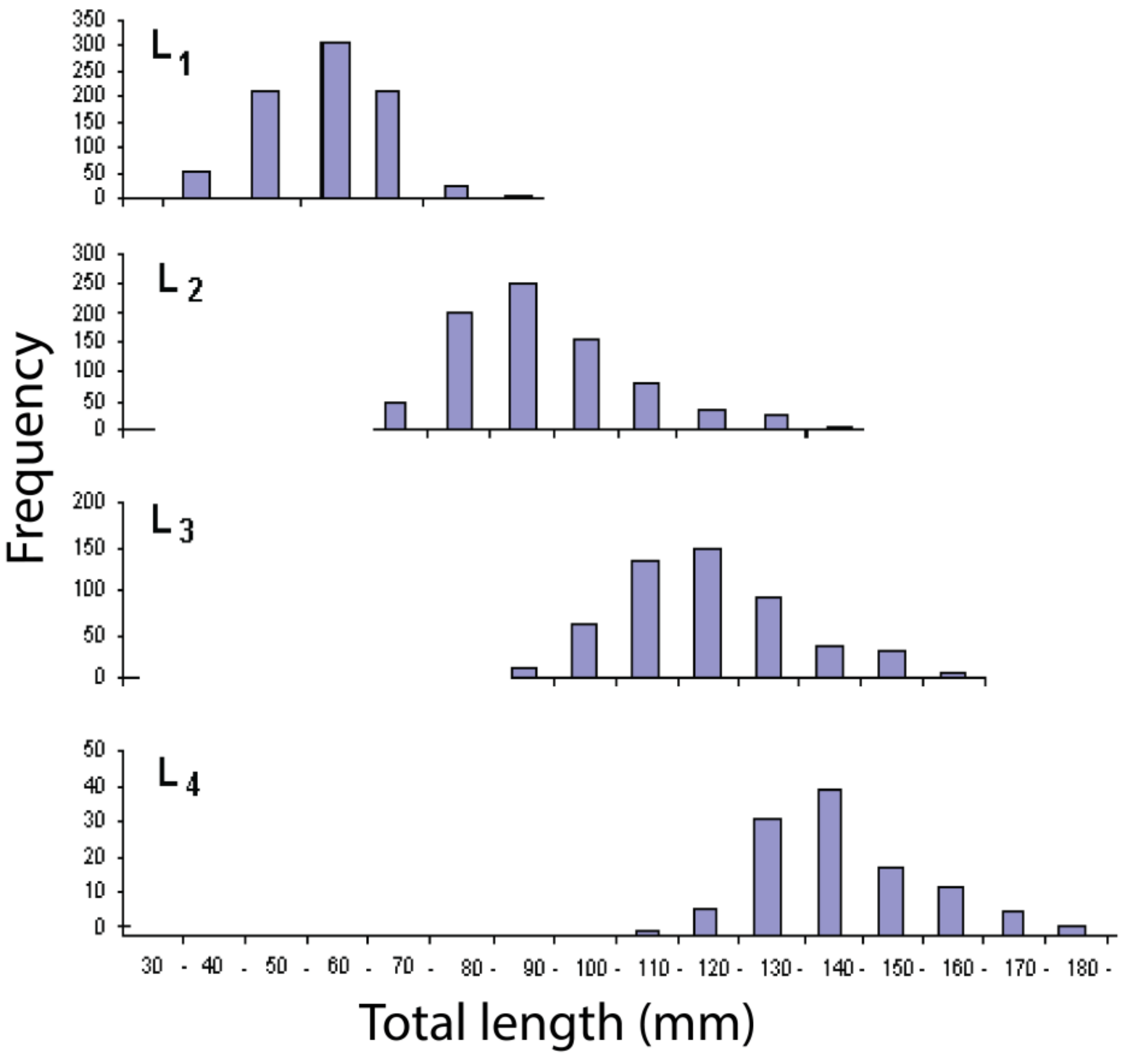

Fig 4. Length frequency by group of age through the analysis of otolith growth rings from *H. steindachneri*.

The length structure in cleithra was from 86 to 161 mm (Group 1^+), from 111 to 190 mm (Group 2^+), from 125 to 202 mm (Group 3^+), and from 140 a 211 mm (Group 4^+) (Fig. 5).

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 5, Issue 4, pp: (11-18), Month: October - December 2017, Available at: www.researchpublish.com

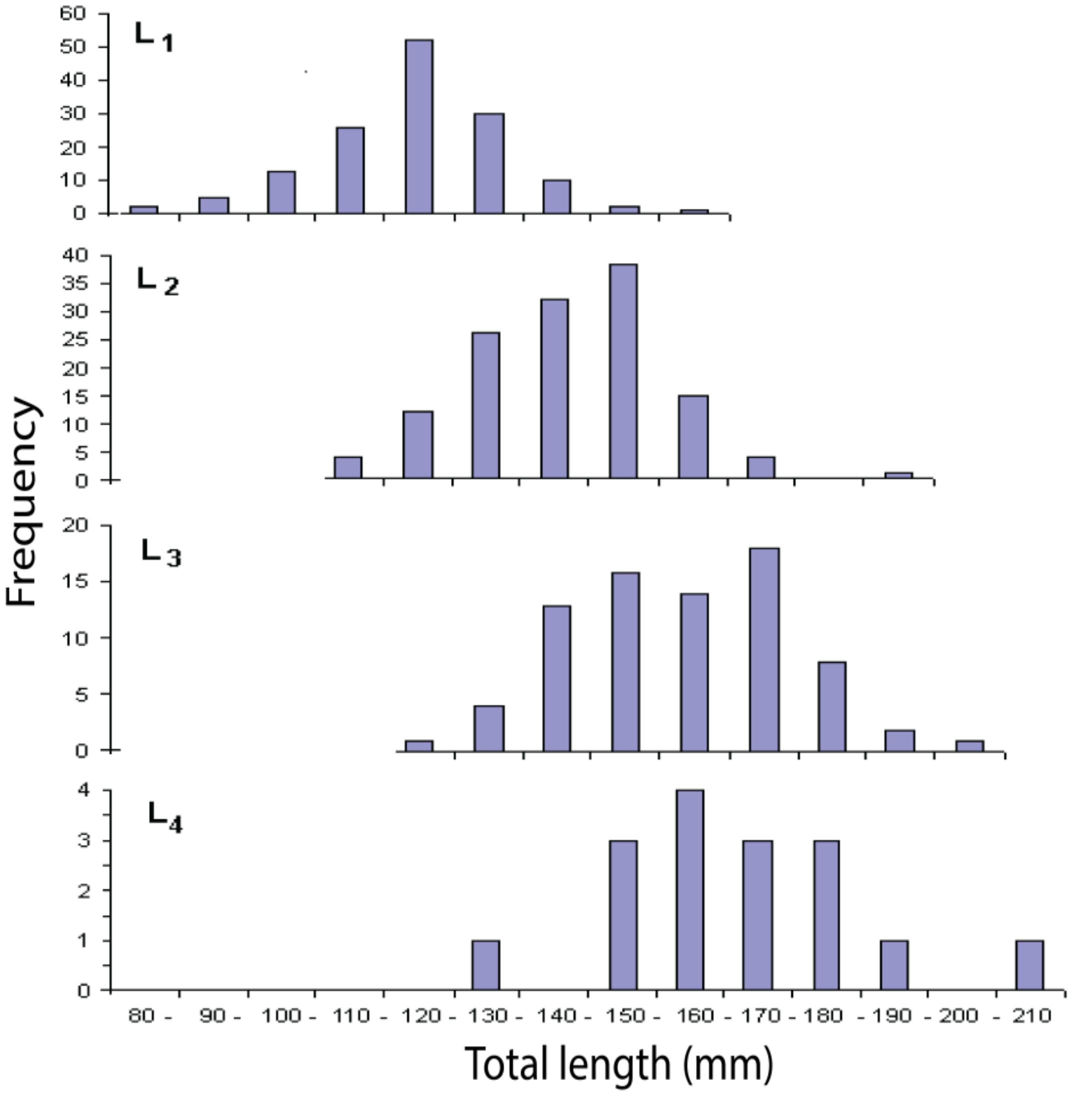

Fig 5. Length frequency by group of age through the analysis of cleithra growth rings from *H. steindachneri*.

The Centrum and actual growth rings were clearly visible in both structures. It was possible to distinguish the overlapping of false rings, which allowed to quickly establish the groups of age with minimum error [29]. The correlations between the TL and the R were linear in otolith and cleithra (Fig. 6,7).

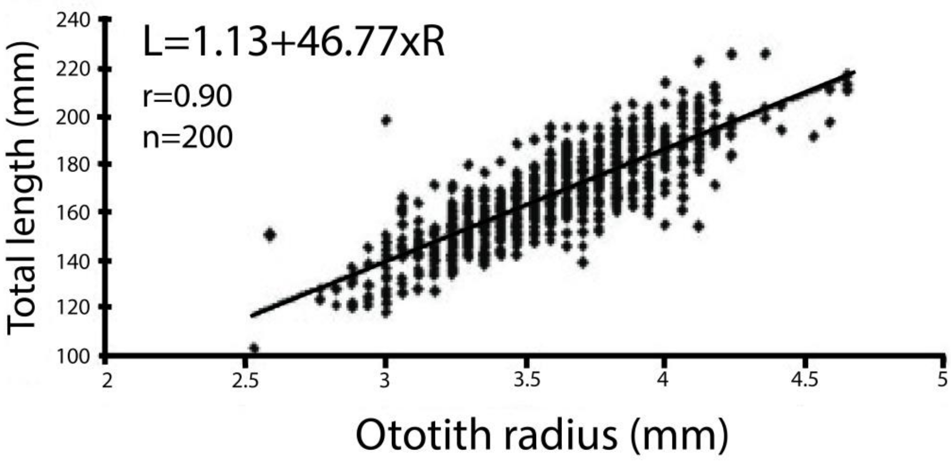

Fig 6. Relation between the fish total length (mm) and the otolith total radius (mm) from *H. steindachneri*

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 5, Issue 4, pp: (11-18), Month: October - December 2017, Available at: www.researchpublish.com

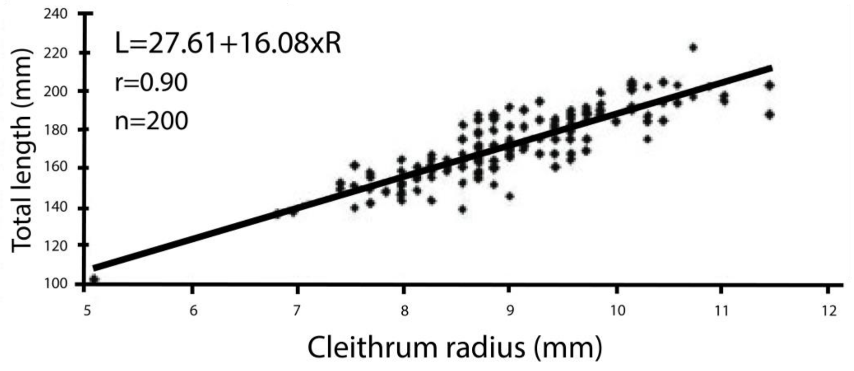

Fig 7. Relation between the fish total length (mm) and the cleithrum total radius (mm) from *H. steindachneri*

IV. DISCUSSION

Calculating the percentage of legible and non-legible growth rings in otoliths and cleithra was the qualitative approach to establish the adequate bony structure for age and growth studies of the grunt *H. steindachneri*. However, it was important to apply statistical methods to biometrics measures to optimise the results obtained from the growth ring analysis and therefore establish an accurate age-length key [29].

The high correlation coefficient evidenced proportionality between the fish TL and the R of both bony structures. Values of $a>0$ demonstrated a linear allometric growth, suggesting that the structures are not formed in the larval stage; instead, they only appear when the fish has reached a post-larval length [3,4].

A previous similar study using scales and cleithra described three groups of age (1^+, 2^+, 3^+) for *H. steindachneri* [10]; in the present study, four groups of age (1^+, 2^+, 3^+, 4^+) were found. This is an interesting outcome because the TL average is similar in both studies. However, a possible explanation of this difference might be the sample size and period, which was bigger and longer, respectively, in the present study, allowing us to achieve a representative sample of the population. No individual from a group of age 0^+ was tested in this study most probably because these small fishes are not a target of commercial handcraft catches from Southwest Margarita Island [21,22].

The similarity of the length frequency distributions of the groups of age found in the analysis of both bony structures and the fact that no significant statistical difference ($p>0.05$) was observed in the back-calculation, indicate that both structures are statistically valid to determine the age and growth of *H. steindachneri*. However, qualitatively, the otolith was found to be an adequate bony structure for age and growth studies of the species. These structures are easy to extract, to clean, and to analyse [1,2,22,29,30]. Therefore, it is recommended to use sagittae otoliths for determining the age and growth of *H. steindachneri* due to the clearly visible growth rings that do not require special treatment for analysis (e.g., cutting, staining, burning and polishing) [1,2]. Nevertheless, otoliths of *H. steindachneri* have an excellent size that facilitates their location and handling.

The disadvantage of the cleithrum is the difficulty in the cleaning process. These bones present high lipid content, and the muscular tissue is attached, complicating their lecture. These are structures that support the animal's pectoral fins, and therefore they keep an intense lipid activity related to their function and chemical composition. That is the case of *Haemulon album* [31]. However, similarity with another study was found in the percentage of legible cleithra [10], which increases the confidence of the qualitative approach.

Although knowing the formation of growth rings is beyond the scope of this study, it is relevant to mention that the species growth ratio is most probably influenced by metabolic changes during the reproduction [22] or by the seasonal variations that affect the availability of food as well as the sea mean temperature and salinity [6,14].

V. CONCLUSION

Otoliths and cleithra are bony structures that are statistically adequate for age and growth studies of the grunt *H. steindachneri*, and we can recommend the use of sagittae otoliths since they are easily extractable, have clearly legible growth rings, and do not require any special treatment for its analysis.

ACKNOWLEDGEMENTS

The authors thank Mrs Ramona Marin† President of the *Asociación de Pescadores Artesanales de Boca del Río*, and her husband Mr Jesus Marcano for facilitating the samples used in this study. Also, the authors extend their thanks to Prof Nora Eslava *Universidad de Oriente, Venezuela* for her statistical advice and Francisco Guevara *Laboratorio de Biología Pesquera del IIC-UDONE* for his logistic support at the laboratory.

REFERENCES

[1] Pascual A. Métodos para el estudio de la edad y crecimiento en peces. Technical report VECEP ALA 92/43. Programa de Pesca Instituto Nacional de Pesca. Guayaquil, Ecuador. 1996.

[2] Morales-Nin B. Review of the growth regulation processes of otolith daily increment formation. Fish Res. 2000; 46: 53-67. doi: 10.1016/S0165-7836(00)00133-8.

[3] Cadima EL. Fish stock assessment manual. FAO Fish Tech Pap 393. Rome. 2003. Available from: http://www.fao.org/3/a-x8498e.pdf.

[4] Kilduff P, Carmichael J, Latour R. Guide to fisheries science and stock assessments. NOAA Atlantic States Marine Fisheries Commission. Technical Report No. NA05NMF4741025. Virginia. 2009.

[5] Tresierra A, Culquichicon Z. Biología pesquera. First edition. Libertad Editorial. Trujillo, Peru. 1993.

[6] Gassman J, Eslava N, Gonzalez, LW. Reproducción de la sardina, Sardinella aurita (Cupleiformes: Clupeidae) del sureste de la Isla de Margarita, Venezuela. Jour Trop Bio. 2008; 56(4): 1813-1824. Available from: http://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S0034-77442008000400018.

[7] González LW, Eslava N, Troccoli L, Guevara, F. Analysis of the artisanal sardine (Sasrdinella aurita) fishery in Venezuela. Tecnociencia. 2016; 10: 81-89.

[8] Cervigón F. Los peces marinos de Venezuela Vol IV. Segunda edición. Fundación Científica Los Roques, Caracas. 1993.

[9] Carpenter K. The living marine resources of the western central Atlantic, bony fishes part 2 (Opistognathidae to Molidae), sea turtles and marine mammals. FAO Species Identification Guide for Fishery Purposes and American Society of Ichthyologists and Herpetologists. Special Publication. 2002; 5: 1375-2127. Available from: http://www.fao.org/docrep/009/y4162e/y4162e00.HTM.

[10] Eslava N. Comparación del uso de escamas y cleitra para estudios de edad y crecimiento del Chere-Chere, Haemulon steindachneri (Jordan and Gilbert, 1882) (Teleostei: Haemulidae). Mem La Salle. 1991; 5: 97-107. Available from: http://www.fundacionlasalle.org.ve/userfiles/men_1991_51_135-136_97-107.pdf.

[11] Barreto C. Edad y crecimiento de Haemulon steindachneri, capturados en la costa norte del estado Sucre, Venezuela. BS Thesis, Universidad de Oriente. Cumaná, Venezuela. 1996.

[12] Heald E, Griffiths R. The age determination from scale reading of the sardine, Sardinella anchovia of the gulf of Cariaco, eastern Venezuela. Inv Pesq. 1967; 1: 374-446.

[13] González LW. Determinación de edad y crecimiento de la sardina, Sardinella aurita (Veleciennes, 1847) (Pisces: Clupeidae) de la Región nororiental de Venezuela. Bol Inst Oce Vzla. 1985; 24: 111-128.

[14] González LW. Aspectos técnicos de preparación de otolitos para estudios de edad de algunas especies del género Cynoscion (Pisces: Sciaenidae). Lagena. 1977; 39: 43-48.

[15] Romero M, González LW. Edad y crecimiento de la Mojarra, Eugerres plumieri (Cuvier en CandV, 1830) (Pisces: Guerridae), de La Laguna de La Restinga, Isla de Margarita, Venezuela. Bol Inst Oceanogr Vzla. 1986; 25: 21-34.

[16] Palazón JL, González LW. Edad y crecimiento del pargo cebal, Lutjanus analis (Cuvier, 1828) (Teleostei: Lutjanidae) en la Isla de Margarita y alrededores, Venezuela. Inv Pesq. 1986; 50: 151-165.

[17] González LW, Mengual A. Edad y crecimiento del machuelo Opisthonema oglinum (Le Sueur, 1818) (Teleostei: Clupeidae) de la Isla de Margarita, Venezuela. Cien Mar. 1995; 21: 387-399. Available from: http://www.redalyc.org/pdf/480/48021403.pdf.

[18] González LW, Eslava N, Silva C. Edad, crecimiento y mortalidad de Lutjanus purpureus Poey, 1867 (Pisces: Lutjanidae) de la región de Guayanas. Bol Inv Mar Cos. 1998; 27(1): 7-20. Available from: http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S0122-97611998000100001.

[19] González LW, Eslava N. Edad y crecimiento del pargo colorado Lutjanus purpureus Poey, 1867 (Teleostei: Lutjanidae) de la región oriental de Venezuela. Bio Mar Oce. 1999; 34(1): 99-107. Available from: http://www.revbiolmar.cl/escaneados/341-99.pdf.

[20] Longart Y, Lanza N, Lista M, Prieto A, Acosta V. Age estimation using analysis of otoliths in a population Dactylopterus volitans. Zootec Trop. 2015; 33(1): 47-60. Available from: http://www.scielo.org.ve/scielo.php?script=sci_arttext&pid=S0798-72692015000100005&lng=es&nrm=iso&tlng=es.

[21] González LW, Eslava N, Guevara F. Catálogo de la pesca artesanal del estado Nueva Esparta, Venezuela. Universidad de Oriente, Radoca Editorial C.A. Cumaná. 2006.

[22] Morales M, González, LW. Age and growth of the grunt, Haemulon steindachneri (Perciformis: Haemulidae), from the Southwest area of Margarita Island, Venezuela. Jour Trop Bio. 2010; 58: 299-310. Available from: http://www.redalyc.org/articulo.oa?id=44918951022.

[23] Tresierra A, Culquichicon Z, Veneros B. Dinámica de poblaciones de peces. First edition. Libertad Editorial. Trujillo, Peru. 1995.

[24] Roemer ME, Oliveira K. Validation of back-calculation equations for juvenile bluefish (Pomatomus saltatrix) with the use of tetracycline-marked otoliths Fish Bul. 2007; 105: 305-309.

[25] Sparre P, Venema S. Introduction to tropical fish stock assessment. Part 1. Manual. FAO Fisheries Technical Paper 306. Rome. 1998. Available from: http://www.fao.org/docrep/W5449E/W5449E00.htm.

[26] Sokal R, Rohlf J. Biometría: principios, métodos estadísticos en la investigación biológica. Blume Editorial, Madrid. 1979.

[27] Zar JH. Biostatistical Analysis. Fifth edition. Pearson Prentice Hall. New Jersey, USA. 2010.

[28] McDonald JH. Handbook of Biological Statistics. Third edition. Sparky House Publishing. Baltimore, USA. 2014.

[29] Khan A, Khan S. Comparison of age estimates from scale, opercular bone, otolith, vertebrae and dorsal fin ray in Labeo rohita (Hamilton), Catla catla (Hamilton) and Channa marulius (Hamilton). Fish Res. 2009; 100: 255-259. doi:10.1016/j.fishres.2009.08.005.

[30] Casselman J. Analysis of hard tissue of pike, Exo lucius L. with special reference to age and growth, 13–27 pp. In: Bagenal, T, editors. Ageing of fish proceedings of an international symposium. Unwin brothers Ltd. England. 1974.

[31] Bustamante G. Características ecofisiológicas de algunos peces de la plataforma suroccidental de Cuba: cibí carbonero, Caranx rubber (Bloch) (Carangidae); jallao, Haemulon album (Cuvier) (Haemulidae) y sardina de ley, Harengula humeralis (Cuvier) (Clupeidae). PhD Thesis, Instituto de Oceanología, Academia de Ciencias de Cuba. La Habana, Cuba. 1986.