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MORPHOMETRIC RELATIONSHIPS AND BASIC MORPHOLOGY OF SAGITTA OTOLITHS OF THE PACIFIC BEARDED BROTULA (*Brotula clarkae*) IN THE COLOMBIAN PACIFIC OCEAN

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Abstract
Based on sagitta otoliths of the Pacific bearded brotula *Brotula clarkae*, Hubbs, 1944, morphometric relationships were estimated among the length and weight of fishes, with length, weight, and radius of otoliths. The best fit was with power models for Total length - Otolith length, Total length - Otolith weight, Total weight - Otolith length, Total weight - Otolith weight, Total Length - Otolith Radius, and Total weight - Otolith Radius. Morphological descriptions showed otoliths as spindle-shaped, slightly curved, laterally elongated (tusk-like), and slightly concave from the distal surface with no similarities with other otoliths of the family Ophidiidae. There is an ontogenic dimorphism where sagitta otoliths of small specimens showed several protuberances at the distal surface, while those of larger specimens showed only two-to-three protuberances.

Keywords: Otolith description, otolith morphometric relationship, ontogenic dimorphism, Pacific Ocean, Colombia

Introduction

Otoliths are small bones made of calcium carbonate (mainly in the form of aragonite), organic matter, and other elements present in the auditory capsule of bony fishes and related to the mechanisms for equilibrium and hearing. Although fishes have three pairs of symmetrical otoliths (sagitta, lapillus, and asteriscus), most studies are carried out with the sagittae (Campana, 2004; Moyle & Cech Jr, 2004; Rossi-Wongtschowski et al., 2014; Callicó et al., 2017; Altin & Ayyildiz, 2018). Otoliths are interest of study since the 18th Century (Koken, 1891; Rossi-Wongtschowski et al., 2014) because they are important in the understanding of the life cycle of fish, and their populations. Otoliths are used in different kinds of studies such as age, growth, paleontology, taxonomy, stock identification and chemistry (Rossi-Wongtschowski et al., 2014; Callicó et al., 2017; Oré-Villalba, 2017; Puentes et al., 2019; Kızılkaya et al., 2021).

Studies about the morphology of otoliths and their morphometric relationships were carried out for several species in different countries (e.g., Volpedo & Echavarria, 2000; 2003; García-Godos, 2001; Curin-Osorio et al., 2012; Rivera et al., 2013; Carvalho & Correa, 2014; Williams et al., 2015; de La Cruz-Agüero et al., 2016; Nimesh & Jaim, 2018; Ayyildiz et al., 2020). Identifying growth rings and the best way to count and measure them in otoliths sections are important to establish if these sections are useful in growth and age studies (e.g. Morales–Nin, 1989; Fawler, 1990; Martínez-Tovar et al., 2006; Wiff et al., 2007).

Two species are reported for the genus Brotula in the Colombian Pacific Ocean: *Brotula clarkae* Hubbs, 1944 and *Brotula ordwayi* (Hildebrand & Barton, 1949), and the first one is the most important for fisheries (Franke & Acero, 1995; Puentes, 1995; Rodriguez et al., 2020). The Pacific Bearded Brotula, *B. clarkae*, is distributed from the Gulf of California to Peru, and it inhabits rocky reefs and soft bottoms in depths ranging 1 and 650 m (Robertson & Allen, 2015). Small scale long line ("espinel") capture range in Colombia is between 27.1 and 107 cm TL (Rojas et al., 2004). The mean size at initial sexual maturity of females in the Colombian Pacific is between 61.3 and 62.9 cm in total length (Lt) with several spawn pulses between May and October (Acevedo et al. 2007; Rodriguez & Rueda, 2017); in Costa Rica, size at sexual maturity is even higher at 71.9 cm Lt (Herrera et al. 2016). Around 66% of captures with "espinel" are below sexual maturity; its higher levels of capture per unit effort are between February and April, and its feeding habits include mainly crustaceans (mantis shrimps, crabs, and shrimps) and fishes (Rojas et al., 2004). In an industrial longline fleet during the 1990s, it was captured in deep rocky or soft bottoms (50 to 120 fathoms), where an oscillatory (through time) and isometric growth was estimated (Puentes, 1995). Rodríguez & Rueda (2017) reported better conditions in rocky bottoms than soft bottoms with a healthy population since the mean capture size was over the mean size at maturity in the Northern Colombian Pacific Ocean.

There are six studies published using fish bony structures in Colombia. Reina et al. (1995) used pectoral spines, Arévalo et al. (2004) used urohial bones, Zapata & Herron (2002) used lapillus otoliths, Mejia-Falla et al. (2014), Torres-Palacios et al. (2019) used vertebra, and Puentes et al. (2019) used sagitta otoliths. The present study pretends to contribute new knowledge about the Pacific bearded brotula otolith morphometric relationships, age determination, and describing the otolith morphology.

Material and Method

A total of 49 pair of otoliths of *B. clarkae* were collected from fishes captured in the National Natural Park (NNP) Gorgona (78˚ 09', 78˚14' W and 2˚56', 2˚59' N) and neighboring areas (Figure 1).

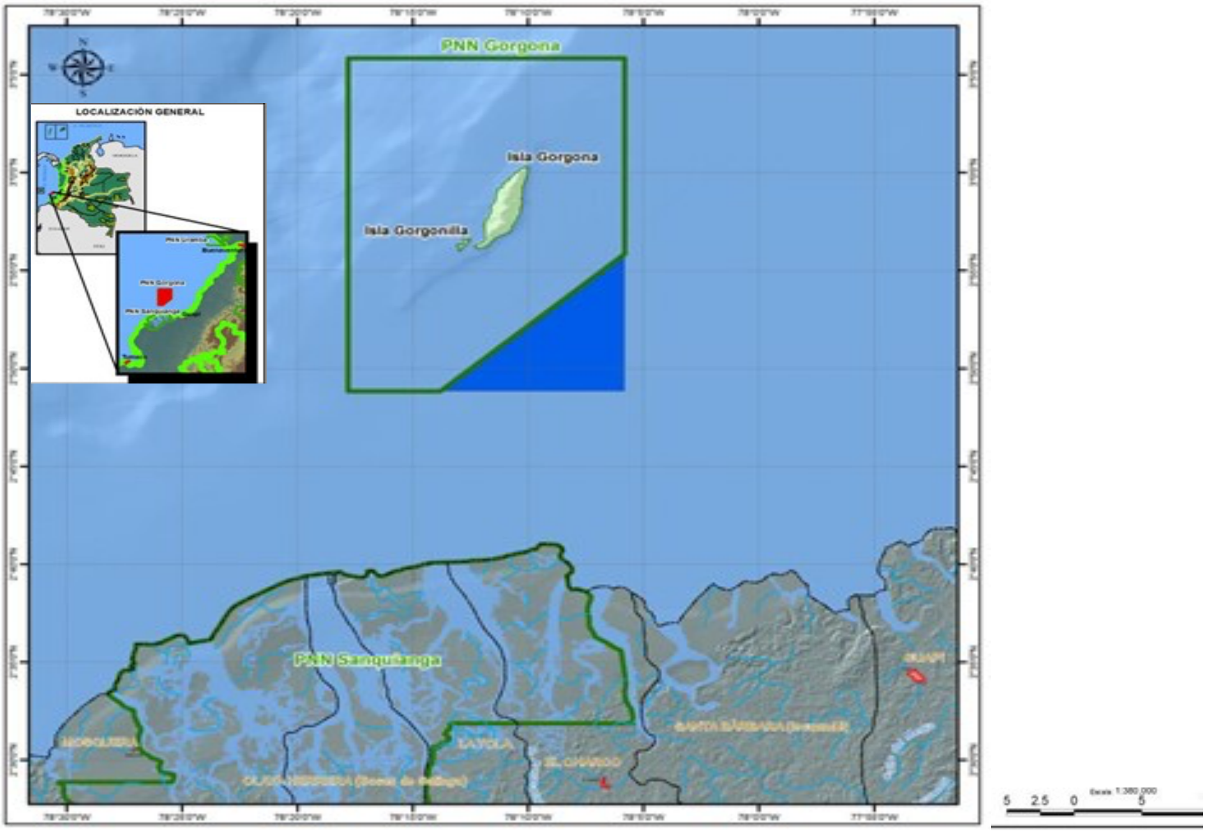


Figure 1. National Natural Park Gorgona and its neighboring area in the Southern Colombian Pacific. Source: National Natural Parks, Pacific Division, Management Group. 2014.

For each specimen, sex, maturity stage, total length (Lt), and total weight (Wt) were recorded. Morphometric relationships were carried out measuring each otolith to the nearest 0.1 mm (Figure 2) and recording each otolith's weight with a precision of 0.001 g. The following relationships were estimated: Total length (Lt) - Otolith length (Ol), Total length (Lt) - Otolith weight (Ow), Total weight (Wt) - Otolith weight (Ow), Total weight (Wt) - Otolith length (Ol), Otolith radius (Or) - Total length (Lt), and Otolith radius – Total weight (Wt). Linear, power, and exponential models were tested to get the best fit for getting the significance ($p < 0.05$), coefficient of determination (r^2), and Akaike's Information Criterion (AIC). The model with lowest AIC was chosen to fit the morphometric relationships using the software R (R Core Team, 2018).

Morphological descriptions of sagitta otoliths covered the whole range of otolith sizes; basic descriptions of the otolith were done using the left otolith of each specimen based on Tuset et al. (2008), with a stereoscope LEICA MZ12.5 (Leica Microsystems, Heerburg, Switzerland).

A total of 42 otoliths (other 7 otoliths were not viable) sections were cut, in which otoliths were embedded in epoxy resin for 48 hours. Longitudinal and transversal sections were made with a Microcutter type MC-201 (Maruto Co. Ltd. Tokyo, Japan), and polished with a polisher type 9 820 (Makita Co., Ltd, Tokyo, Japan), getting a 0.2 mm otolith section, which was mounted on a slide properly labeled. Sections were analyzed with a stereoscope LEICA MZ 12.5 to identify which type of section (longitudinal or transversal) was the best to describe otolith section morphology, type of light (direct or reflected), and the best area to count and measure growth rings (Figure 2B). Since there was no visible nucleus distinguished by the naked eye, transversal sections were performed in different parts of the otolith to establish the best part for a transversal section.

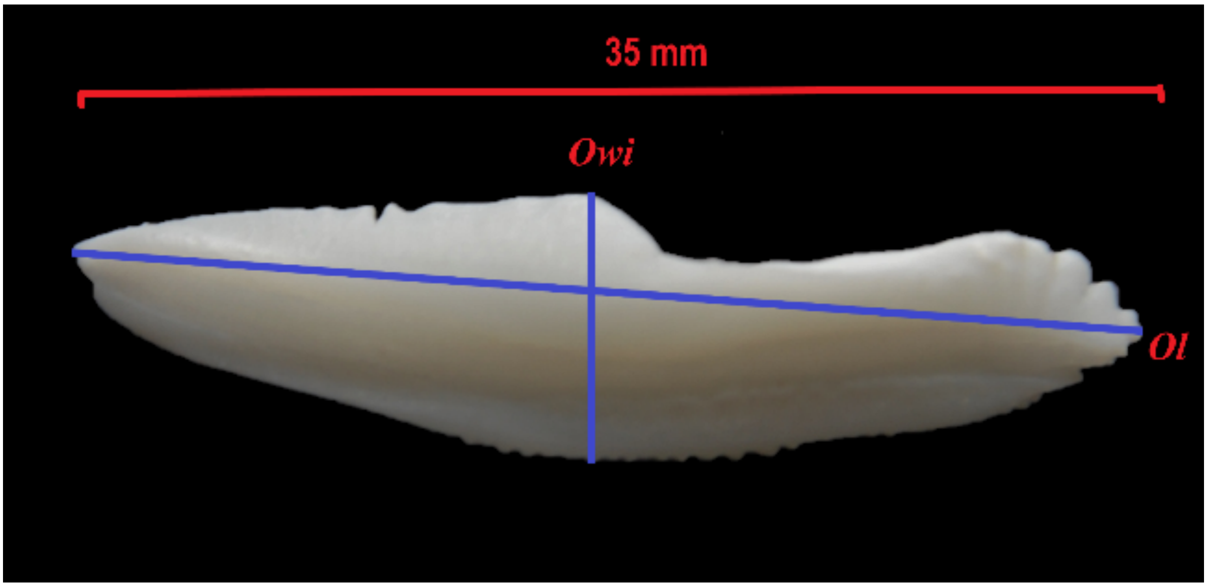


Figure 2. Otolith length (Ol) and Otolith width (Owi) of a *B. clarkae*'s sagittal otolith.

Results

Morphometric Relationships

Minimum total length was measured as 37 cm and maximum was 97 cm (mean length was 66 cm). The minimum total weight was 350 g and maximum 7000 g (mean weight was 2400 g). Morphometric relationships between total length and total weight with otolith length, otolith weight, and otolith width are described; equations showed the best fit (power models) with its respective residual analysis (Figure 3A, B, C, D, E, F) as follows:

$L_t = e^{0.940 \times O_l^{0.938}}$ (Power model), r^2=0.726; P<0.05; AIC=-65.792 (1)

$L_t = e^{4.212 \times O_w^{0.374}}$ (Power model), r^2=0.793; P<0.05; AIC=-74.784 (2)

$W_t = e^{-2.342 \times O_l^{2.888}}$ (Power model), r^2=0.771; P<0.05; AIC=24.819 (3)

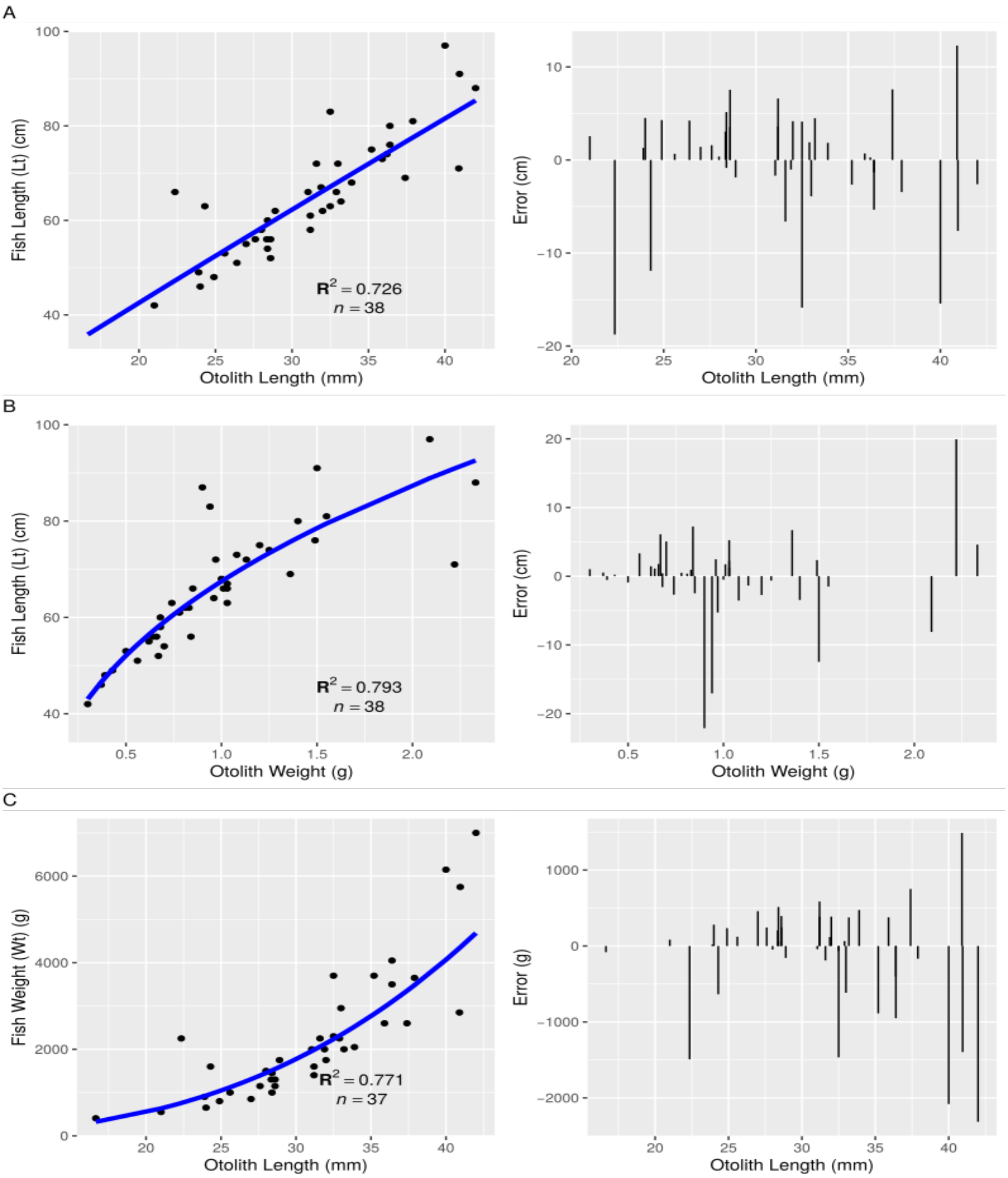
$W_t = e^{7.729 \times O_w^{1.207}}$ (Power model), r^2=0.881; P< 0.05; AIC=14.536 (4)

$L_t = e^{0.402 \times O_r^{1.183}}$ (Power model), r^2=0.722; P<0.05; AIC= - 43.4 (5)

$W_t = e^{-4.143 \times O_r^{3.681}}$ (Power model), r^2 = 0.722; P <0.05; AIC = 35.394 (6)

Otolith morphology

Sagitta otoliths of the Pacific bearded brotula are spindle-shaped and look like a slightly curved tusk. At the proximal surface (Figure 4A), otoliths of any size are smooth, laterally elongated, and slightly concave from the distal surface. Margins are entire, but some may have a soft crenate margin at the ventral side. Sulcus acusticus is inframedian, archaesulcoid, and pseudo-ostiocaudal-like, going along almost the whole otolith. In bigger specimens (60 cm Lt and longer), a kind of the second sulcus, parallel to the main one in the median part is formed starting in the middle region, going to the posterior side, and ending towards the dorsal margin. In middle size and smaller specimens (under 60 cm Lt), this structure is not that evident, but still noticeable.



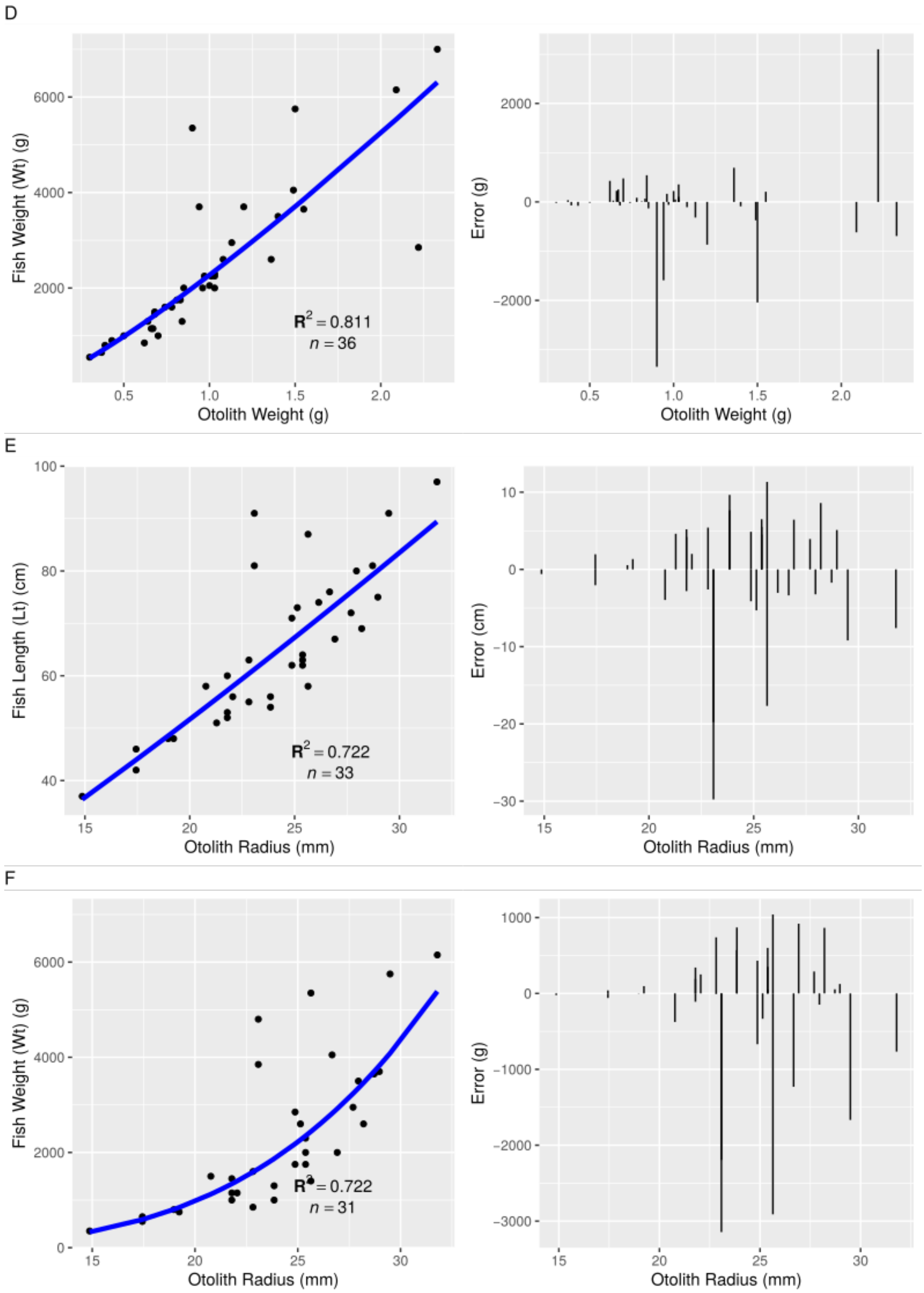


Figure 3. Morphometric relationships estimated for *B. clarkae* with its respective residual analysis A. otolith length-fish length, B. otolith weight-fish length. C. otolith length-fish weight. D. otolith weight-fish weight, E. Otolith radius – fish length. F. Otolith radius – fish weight

There is no ostium, colliculum, or esccisura ostii, and the cauda is straight. The rostrum, if distinguishable, is very small and peaked, usually in bigger specimens; in middle size and small specimens, it is hard to distinguish. Antirostrum is usually difficult to identify, and it may be part of some lobules in the tip of the anterior part of the otolith.

As for the distal surface, it is smooth with a soft dent from the anterior to the posterior part, at the ventral side with two to three protuberances at the dorsal anterior side in bigger specimens (Figure 4B). For smaller specimens (< 60 cm Lt), protuberances increase according to smaller sizes until having several ones in specimens around 40 cm Lt (Figure 4C). As for the proximal surface, some otoliths may show protuberances at the anterior part making a dentate margin (Figure 4D). No growth rings were observed by the naked eye in any otolith size.

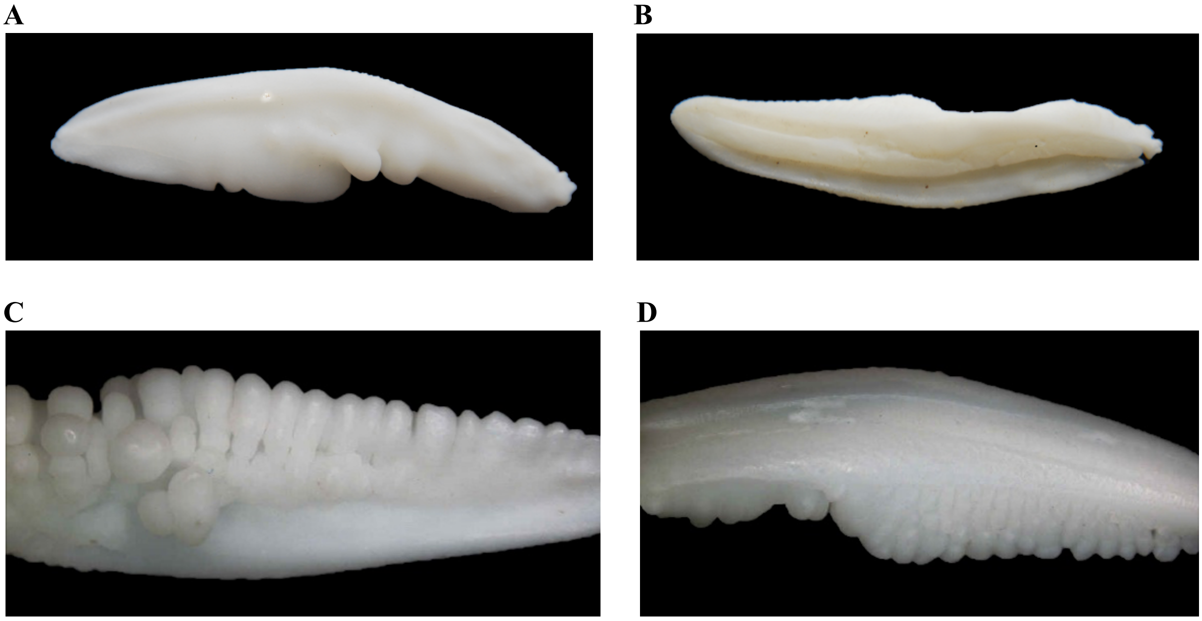


Figure 4. **A**: Proximal surface of *B. clarkae*´s otolith (specimen of 70 cm Lt, and 2 700 g). **B**: Distal surface of an otolith showing two protuberances in a big specimen (70 cm Lt, and 2550 g). **C**: Distal surface of an otolith showing several protuberances at the anterior part in a small specimen (42.5 cm Lt, and 450 g). **D**: Proximal surface of an otolith showing some protuberances making a dentate margin at the anterior part in a small specimen (38 cm Lt, and 350 g).

Various difficulties were encountered while working on the otolith sections (Figure 5). Otoliths were quite fragile to get an appropriate section, and they frequently broke down. It was not clear to distinguish the otolith cross-section structures (e.g., growth rings) in 8 otoliths (19%).

Otoliths were big to perform successful longitudinal sections, so few otoliths were chosen to prove these sections, dividing the otolith into two parts, performing one section on the anterior side and another on the posterior side with no successful sections. On the other hand, the best part to perform a transversal section was in the widest part of the otolith from the dorso-ventral perspective, in which the otolith width was measured (Figure 2).

Figure 5. Transversal section of a *B. clarkae*'s otolith indicating the best area for radius measurement and growth rings counting.

Discussion

No otolith studies were found for *B. clarkae* in Colombia so this study is the first one regarding morphometric relationships, otolith morphology, and otolith sections for this species in the country. Oré-Villalba (2017) is the only one who reported the sagitta otoliths of *B. clarkae* in his photographic catalog of otoliths of marine and freshwater fishes from Peru, but only one picture is shown with no further description. Other otoliths of the Ophidiidae family were included (*Genypterus maculatus*, *Lepophidium negropinna*, *Lepophidium pardale*, and *Lepophidium prorates*).

Acevedo et al. (2007) showed specimens of both sexes at all length classes recorded, except for the largest one (107.5 cm). Determination coefficients for length and/or weight of otoliths with length and/or weight of fishes for *B. clarkae* are considered satisfactory (over 0.70). Otoliths are slightly curved by their distal surface, and this could introduce some errors that could affect the models based on otolith length since otoliths were measured in a straight line from the posterior side to the anterior side. Measures along the surface are recommended to improve the models obtained. The power model showed the best fit for all relationships estimated in *B. clarkae*: fish length (Lt) - otolith length, fish length - otolith weight, fish weight (Wt) - otolith length, and fish weight - otolith weight, otolith radius – fish length, and otolith radius – fish weight. Models with otolith radius were not as good as those with either otolith length or otolith weight, as they showed larger errors, although, for small fish, results were acceptable. Residual analyses for all relationships expressed better correlations of individual sizes and otolith sizes when data are in a middle range (i.e., medium size according to the minimum and maximum size found), as expected.

Otoliths of *B. clarkae* are quite particular and there are no other species within the Ophidiidae family with sagitta otoliths like these ones. Oré-Villalba (2017) showed some otoliths of other species for the family, and only the otoliths of *L. negropinna* share some similarities to those of *B. clarkae*, but they are still very different. Other species' otoliths of different families such as *Merluccius* gayi (Merluccidae), *Physiculus talarae* (Moridae), *Cynoscion squamipinnis* (Sciaenidae), *Sphyraena ensis* (Sphyraenidae), and even Arapaima gigas (Arapaimidae – freshwater fish) may have some similarities with those of *B. clarkae* but only in general shape features since in detail, they are distinctively different.

There are no sexual differences found in the Pacific Bearded Brotula´s otoliths; this study and former studies (Puentes, 1995; Acevedo et al. 2007; Rodriguez & Rueda, 2017) have not reported any external or size sexual dimorphism for the species. However, Portilla (2017) reported that males may be bigger and more robust than females in the southern Colombian Pacific, and internally, besides the obvious gonad difference, there is an additional sexual dimorphism in the morphology of the swim bladder, where females swim bladder has a kind of tube directed towards the anus, while in males the swim bladder has no such tube. Schwarzhans (1994) on the other hand, found external differences in some Ophidiidae species such as those of the genus *Neobythites*, *Spectrunculus*, and *Hoplobrotula*.

There was an ontogenic dimorphism in *B. clarkae*´s sagitta otoliths; otoliths of small specimens showed several protuberances at the distal surface while larger specimens showed only two-to-three protuberances. There is a soft dent from the anterior to the posterior part in larger otoliths at the dorsal side while there is a noticeable channel in the same part in small otoliths, going along and next to the protuberances, but they may be present only at the dorsal-anterior part of the otolith in some otoliths. Otoliths of *B. clarkae* reported by Oré-Villalba (2017) showed an otolith of a small specimen (42 cm Lt) with several protuberances, like the ones examined in this study (Figure 4C). Schawarzhans (1994) found ontogenic dimorphism in otoliths of the genus *Spectronculus* and *Pycnocraspedum* (Ophidiidae) and related it with the pelagic lifestyle of juveniles and more benthic lifestyle of adults.

Processing otolith sections was difficult, but it was possible to perform acceptable transversal sections that allowed seeing better otolith section structures (e.g., growth rings). A maximum of 13 growth rings was counted in a 91 cm specimen, and the minimum was 4 rings in a 37 cm specimen. It was not easy to decide on a proper location for growth ring counting; this study shows a preliminary area where the counting was possible for these otolith sections (Figure 2B).

The periodicity of growth rings was not validated, and recently formed growth rings were detected in only 4 otolith sections of specimens captured in January-February (2) and July-August (2). Few studies on age and growth have been carried out on the species belonging to Ophidiidae family, showing annual rings in *G. blacodes* (Horn, 1993; Wiff et al., 2007) in New Zealand and Chilean waters, respectively.

The sulcus acusticus in otolith sections appears as if divided into three parts. The first one lies right next to the ventral tip, which looks like a long narrow dent that seems like the beginning of a second sulcus observed mostly in larger specimens, but also in some smaller ones (this study; Oré-Villalba, 2017). Next to it, comes the other part of the sulcus acusticus, which is slightly divided by a small tip from the third part that goes up to the well-formed dorsal tip of the sulcus acusticus.

Conclusions

This is the first approach to the analysis of sagitta otoliths of *B. clarkae*, and future studies should include more otoliths and other methods that may include shape indices, digital image processing and multivariate analysis, allometry, and geometric relationships (Lombarte & Lleonart, 1993; Tuset et al., 2003; Monteiro et al., 2005; Bervian et al., 2006).

The relationships between length or weight of otoliths with length or weight of fish, or otolith radius with fish length or weight for *B. clarkae* are power relationships; these relationships will surely improve with more data. *B. clarkae*'s sagitta otoliths morphology is very particular and easy to identify. It was possible to establish an ontogenic dimorphism related to growth, in which small otoliths showed several protuberances while larger otoliths presented only two to three protuberances. Transversal sections showed otolith structures and it is worth trying growth and age studies so that it is necessary to validate the periodicity of growth rings. Otolith studies are useful to get more knowledge about *B. clarkae*'s life history, and they can give more reliable information for their fishery management.

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Ethical approval

Does not apply

Informed consent

Not available

Data availability statement

The authors declare that data are available from authors upon reasonable request. In case of unavailable data due to conditionals of funding organizations, etc., a clear explanation should be given.

Conflicts of interest

There is no conflict of interest in publishing this study.

Funding organizations

No funding available.

Contribution of authors

V. Puentes: Conceptualization, formal analysis, writing original draft, supervision, validation, editing.
G. Pavolini: Formal analysis, investigation, manuscript review, software analysis, validation.
P. Rojas: Data gathering, investigation, analysis contribution, draft review.
C.F. Gutierrez: Data gathering, analysis contribution, draft review.
A.A. Villa: Data gathering, analysis contribution, draft review.

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