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Potential use of dorsal fin spines of the roosterfish for age estimation

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The roosterfish (*Nematistius pectoralis* Gill, 1862) is the only species in the genus *Nematistius* (family Nematistiidae). The most distinguishable external feature of this species is the presence of 7 elongated (comb-shaped) dorsal spines, which give rise to its common name (Rosenblatt and Bell 1976) and allow it to be easily identified from other species (Niem 1995). This species does not present sexual dimorphism.

The roosterfish is distributed in the Pacific Ocean, from San Clemente in southern California to San Lorenzo Island in Peru, including the Gulf of California and the Galapagos Islands (Love et al. 2005). It is a coastal pelagic species that plays a dominant role as a nearshore predator in the waters of Mexico and Central America (Love et al. 2005, Sepulveda et al. 2015). It inhabits mainly coastal areas, with juveniles often found along the shoreline and larger individuals commonly distributed in areas close to reefs and subsurface features (Niem 1995, Sepulveda et al. 2015).

Roosterfish can reach up to 191 cm in total length and weight over 50 kg (Robertson and Allen 2015). In terms of feeding habits, it is considered a specialist predator that commonly feeds at depths of 3 to 4 meters, mainly on coastal pelagic species such as mojarras (e.g., *Eucinostomus gracilis* and *E. dowii*) and anchovies (e.g., *Anchoa ischana* y *A.* spp.) (Rodríguez-Romero et al. 2009).

In Mexico, the roosterfish is reserved for recreational fishing activities, with commercial protection offered within a coastal strip of 50 nautical miles from shore, and daily retention limits set at two organisms per day per fisher (NOM-017-PESC-1994, DOF 1995[1],

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[1] DOF. 1995. Diario Oficial de la Federación (DOF). Norma Oficial Mexicana NOM-017-PESC-1994, para regular las actividades de pesca deportiva en las aguas de la jurisdicción federal de los Estados Unidos Mexicanos, Tomo 15-19, México, D.F.

2013[2]). The roosterfish is considered a prized species by the sport fishing community, which provides direct and indirect economic benefit to the tourism commerce along the coast of the Baja California peninsula and the Sea of Cortez. In 2007 these activities generated income of over 633.6 million dollars in the region of Los Cabos alone (Ditton et al. 1996, Sosa-Nishizaki 1998, Southwick et al. 2010). The roosterfish is also caught incidentally by artisanal fleets that operate along the coast, but this species in not directly targeted due to its relatively dark myotomal musculature and low food-value compared to other inshore species (Sepulveda et al., 2015).

Despite the ecological and economic importance of the roosterfish, there are few studies on its biology, ecology, and movement patterns (Sepulveda et al. 2015, Ortega-Garcia et al. 2017). Parameters such as age and individual growth are essential for fisheries management and are the basis of most stock assessment models (Goldman 2005). Age and growth can be determined by various methods including: growing fish in confinement, examining hard parts, which encode age information, and through biochemical testing. The feasibility and use of different methods depends on accessibility, habitat and life history. In most instances, it is necessary to determine the age of wild fish through the examination of calcified structures, from which age can be estimated. The structures, which have been shown to encode age information include bones (fin rays, vertebrae, cleithra, opercular bones), scales and otoliths (Stevenson and Campana 1992). These structures tend to sequentially accumulate calcified growth material as they age, thus producing concentric areas that often have characteristics reflecting the time of year (season) in which the material is being deposited (Calliet et al. 2006). Fin spines and soft rays have been used to estimate the age structure and growth rate of a wide variety of freshwater and marine fishes (Kopf et al. 2010). These calcified structures can be removed and processed easily and quickly (Beamish and Fournier 1981), often provide good legibility (Hill et al. 1989) and may exhibit growth bands that reflect the different seasons of the year (Kopf et al. 2011).

To date, there is a single study focused on age and individual growth of roosterfish by Ortega-Garcia et al. (2017). These authors analyzed daily growth increments in sagittal otoliths from individuals caught in El Golfo Dulce, Costa Rica, and the southern Baja California Sur peninsula, Mexico and concluded that daily growth increments in sagittal otoliths can only be used to estimate age in individuals measuring up to 66 cm fork length (~1 year of age). Aging efforts from otoliths of larger specimens were largely unsuccessful because daily incremental marks were difficult to differentiate, and annuli were not readily visible. Thus, sagittal otoliths were not considered a suitable structure for age estimates of individuals greater than one year. The current study focused on identifying alternative hard structures that may be used to estimate age and individual growth of larger individuals (>1 yr). The primary objective of this study was to assess which sections of roosterfish dorsal fin spines have the most legible indicators of growth for use in future aging studies.

From January 2010 to December 2012, roosterfish were sampled from the sport fishing fleet in Cabo San Lucas, Baja California Sur, Mexico. Fork length (FL) to the nearest 0.1 cm and weight to the nearest 0.01 kg were recorded from each roosterfish sampled. The dorsal fin was removed from the base with a knife and stored for further laboratory preparation.

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[2] DOF. 2013. Diario Oficial de la Federación (DOF). Modificación a la norma oficial mexicana NOM-017-PESC-1994, para regular las actividades de pesca deportivo-recreativa en las aguas de jurisdicción federal de los Estados Unidos Mexicanos, publicada en el diario oficial de la federación el 9 de mayo de 1995, Tomo 15-19, México, D.F.

The first five spines of each dorsal fin were cleaned; and the distance between the inferior apophyses of the base of each spine was measured with a caliper (Helios Vernier) to the nearest 0.05 mm (Figure 1). This measurement was used as a reference unit to ensure uniform sampling for the transverse sections. The spines were marked, orientation was noted, and the sections were labelled from the base and along the primary axis, at 100%, 200% and 300% of the reference unit (Figure 1). This was done to provide a consistent transverse section location across samples.

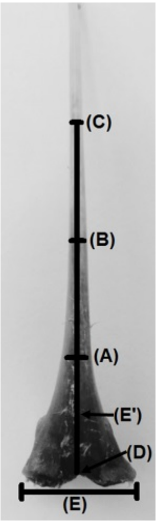

Figure 1.— Front view of the fourth spine of the dorsal fin of roosterfish (*Nematistius pectoralis*) showing the section levels (A): 100%, (B): 200% and (C): 300%. E is the distance between lower apophysis and corresponds to the same distance as E' measured from the base (D) of the spine.

To select the most appropriate spine and section level for observing and counting growth marks in the roosterfish, the linear relationship between each of the diameters measured in each spine and fork length was analyzed using the following equation:

$$FL = a + b(D)$$

where: FL is fork length (cm), a is the intercept with the Y axis, b is the slope of the regression line, and D is spine diameter (cm).

This relationship was determined for each spine and section level, resulting in 15 linear relationships (5 spines x 3 section levels). The spine and section level selected through this procedure corresponded to the relationship that yielded the highest linear trend (proportional growth) between fish length (FL) and spine diameter (D), denoted by the highest coefficient of determination (r^2) (Daniel 2012).

Cross-sections of different thicknesses (0.25 mm, 0.45 mm and 0.5 mm) were sliced from each section level (Figure 1) in each spine using a low-speed saw (Buehler brand, model 11-1280-160) with a Diamond Wafering Blade (15HC series). Digital images of the cross-sections were obtained with a camera (Carl Zeiss brand, model AxioCam MRc 5) adapted to a stereoscope (Carl Zeiss brand, model Stemi SV11).

Digital images of the spine cross-sections were evaluated to determine the thickness at which growth marks were observed with greatest clarity, and consequently could be readily enumerated by two independent readers. Each growth mark consisted of an opaque band followed by a translucent band. Readings were made directly on the digital images of sections to avoid the direct manipulation of the spine sections due to their fragility.

To evaluate the precision between counts made by each reader, the coefficient of variation (Chang 1982) was calculated:

$$\mathrm{CV}_j = 100\% * \frac{\sqrt{\sum_{i=1}^{R} \frac{(\mathrm{X}_{ij} - \mathrm{X}_j)^2}{\mathrm{R} - 1}}}{\mathrm{X}_j}$$

where: CV_j is the estimated coefficient of variation for the *j*th fish, X_{ij} is the *i*th age estimation of the *j*th fish, X_j is the mean age estimation for the *j*th fish, and *R* is the number of times each fish is aged. The relationship between number of growth marks and fork length was analyzed through a linear regression.

Given that organisms were not sampled in all months of the year, the validation of whether the marks observed in cross-sections have an annual formation period was beyond the scope of this study.

All organisms sampled (n= 93) were obtained from the retained catch from sport fishing activities based off the southern portion of Baja California Sur. Samples sizes ranged from 14 to 133 cm FL, with most individuals (76% of the total organisms sampled) falling between 50 and 90 cm FL. Because exceptionally small and large individuals are typically released by the recreational fleet, samples < 40 cm and above 100 cm FL were scarce.

Putative growth marks were identifiable within the dorsal spine cross-sections of all roosterfish examined, with opaque-translucent bands most clearly legible in the 0.45-mm-thick cross-sections of the dorsal spines. The 0.25 mm sections were extremely fragile and brittle and determined to be unsuitable for consistent quantification of growth marks, while it was difficult to distinguish between opaque-translucent bands in 0.5 mm sections (Figure 2).

The fourth dorsal fin spine yielded the highest r^2 values (Table 1) during linear regression analysis between spine diameter at different section levels and fork length. Although the 300% section level of the fourth dorsal fin spine had a slightly higher r^2 (r^2= 0.947, Table 1), when compared to the 200% section level (r^2= 0.932, Figure 2b, Table 1), the latter provided increased growth mark legibility. Given the importance of growth mark differentiation, the 200% section was chosen for the cross-sections analysis and the counting of putative growth marks.

Most cross-sections (93%) at the 200% section level of the fourth dorsal fin spine of the roosterfish, allowed observing and counting growth marks, which were clearly visible. The precision in growth-mark counts performed by two independent readers was high, as indicated by the low coefficient of variation (*CV*= 9.67%); therefore, there was consistency between the counts by both readers. The count of growth marks on cross-sections ranged from 0 to 4 opaque-translucent bands. Individuals with one growth mark were the most abundant, followed by individuals with 0 and 2 marks, whereas individuals with 3 and 4 marks were scarce (Figure 3).

The number of growth marks (GM) increased with fork length and there was a significant relationship between the number of growth marks and roosterfish fork length (FL= 47.33 + 20.95 * GM, r^2= 0.569, $P < 0.05$). Roosterfish with a single growth mark ranged

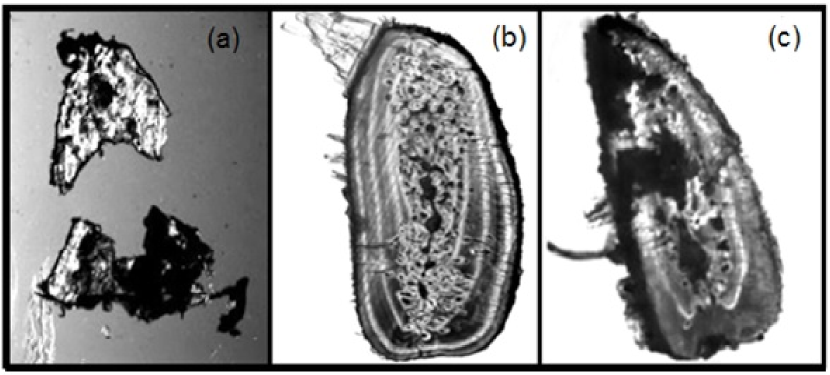

Figure 2.— Photographs of cross-sections of different thicknesses a) 0.25 mm, b) 0.45 mm and c) 0.5 mm of spines of the dorsal fin of roosterfish (*Nematistius pectoralis*) landed by the sport fleet of Cabo San Lucas, B.C.S. in the period 2010-2012.

Table 1.— Coefficients of the linear regressions for each spine in each section level for roosterfish (*Nematistius pectoralis*) caught by the sport fishing fleet of Cabo San Lucas, B.C.S. between 2010-2012.

Spine #	Section level	*a*	*b*	r^2	*P*
1	100%	9.55	24.78	0.913	< 0.05
	200%	15.53	29.59	0.908	< 0.05
	300%	20.79	35.21	0.691	< 0.05
2	100%	4.83	21.93	0.917	< 0.05
	200%	13.93	24.28	0.913	< 0.05
	300%	14.85	31.22	0.911	< 0.05
3	100%	5.05	24.25	0.871	< 0.05
	200%	12.89	33.44	0.902	< 0.05
	300%	10.43	42.95	0.902	< 0.05
4	100%	4.16	24.82	0.897	< 0.05
	200%	14.71	34.26	0.932	< 0.05
	300%	8.31	48.21	0.947	< 0.05
5	100%	12.34	24.77	0.751	< 0.05
	200%	10.31	44.38	0.864	< 0.05
	300%	6.27	55.81	0.875	< 0.05

in size from 43 to 92 cm FL and fish with two growth marks varied from 57 to 118 cm FL (Figure 3).

This study provides information on potential use of dorsal spines in determining age and growth parameters for the roosterfish, a valuable coastal resource of the eastern Pacific. Because previous work identified difficulties in aging roosterfish beyond 1yr (66 cm FL) using otoliths, this study examined the first five dorsal spines to assess the presence

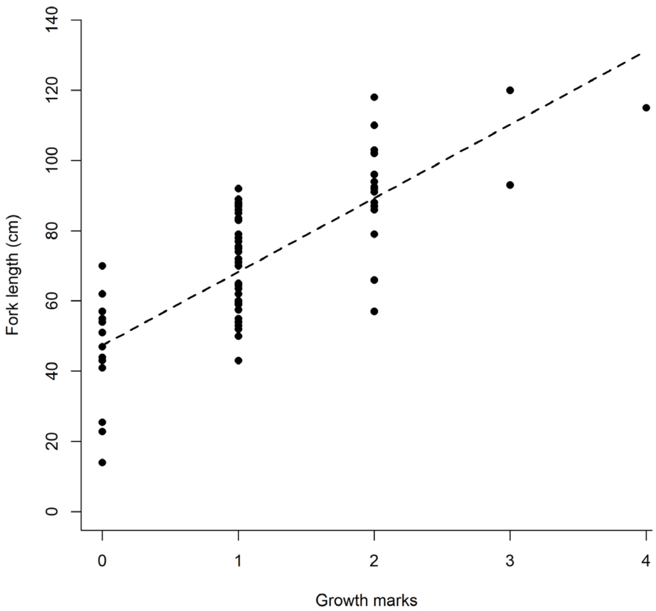

Figure 3.— Linear regression (straight line) between the fork length and the number of growth marks derived from counting in cross-sections at the section level of 200% of the fourth dorsal fin spine of the roosterfish (*Nematistius pectoralis*) landed by the sport fleet of Cabo San Lucas, B.C.S in the period 2010-2012.

of putative growth marks and their potential in future aging efforts. From the specimens examined, it was determined that the fourth dorsal spine provides clearly legible growth marks that align with previous growth hypotheses (Ortega-Garcia et al. 2017). Although additional field validation is needed to determine whether dorsal fin growth marks can be used to accurately estimate roosterfish age and growth parameters, this work provides the initial basis from which future ageing studies can be conducted.

Although it was not possible to obtain samples from individuals outside of the typical range of fish landed in sport fishing operations that occur along Southern Baja California, the size range did encompass and surpass the sizes of individuals for which ageing was not possible (> 66 cm FL) for previous roosterfish growth estimates (Ortega-Garcia et al. 2017). Most of fish sampled ranged from 50-90 cm FL because roosterfish under 40cm are rarely landed in the sport fishery and individuals in excess of 100cm FL are considered “trophy fish” and typically released following capture. Despite the limited size range of individuals sampled in this work, it was possible to identify consistent readings from potentially valuable hard structures for use in future ageing work.

In this study, the thickness of the roosterfish dorsal spine section that yielded the optimal platform for observing putative growth marks was 0.45 mm. Similarly, optimal thickness for ageing blue marlin (*Makaira nigricans*) was identified to be 0.45 mm (Jakes-Cota 2008) and 0.6 mm for sailfish (*Istiophorus platypterus*, Ramírez-Pérez 2005). Optimal section thickness has been shown to vary from one species to another, as dorsal spine morphology varies greatly between various groups of fishes. In the yellowfin tuna (*Thunnus albacares*) and little thunny (*Euthynnus alletteratus*), the most suitable thickness proposed for counting growth marks ranges from 0.75 mm to 1 mm (Lessa and Duarte-Neto 2004; Alcaraz-García 2012), while swordfish (*Xiphias gladius*) has been shown to have an optimal thickness that ranges between 1 mm to 1.5 mm (Chong and Aguayo 2009).

For several species of large pelagic fish, fin spines have been shown to be the most suitable structure for determining age (Kopf et al. 2010). Spines are more suitable for non-lethal examination and processing relative to other hard structures, such as otoliths and vertebrae (Sun et al. 2002; Kopf et al. 2010). One of the basic assumptions in age determination is the growth proportionality between the hard structure used and fish size (Bagenal 1974). This study found the strongest linear relationship between fish FL and the diameter of the fourth dorsal fin spine, relative to other dorsal spines.

The spines used for counting growth marks varies by species, since the growth of bones shows specific sensitivity to internal (e.g., physiological and pathological) and external (e.g., climatic) factors (Panfili et al. 2002). In several species, such as tuna (yellowfin tuna and little thunny), aging analyses have focused on the use of the first dorsal fin spine (Lessa and Duarte-Neto 2004; Alcaraz-García 2012); while for swordfish, the second dorsal fin spine (Chong and Aguayo 2009); and in blue marlin and sailfish, the fourth spine (Ramírez-Pérez 2005; Jakes-Cota 2008).

The 200% section location was found to be the most suitable for the observation and counting of putative growth marks. At this location the marks are clearly visible, in contrast with other section levels, hence increasing the possibility of counting and measuring them. The selection of the fourth dorsal fin spine and the 200% section level are consistent with the criteria of greatest legibility of growth marks (Panfili et al. 2002) and growth proportionality with fish length (Hill et al. 1989).

Few previous studies have evaluated which spine and section level are most suitable for the observation and counting of growth marks. The importance of standardizing age determination methods when using fin spines has been emphasized by Kopf et al. (2010), who recommend that after selecting the most appropriate spine to estimate age, at least some form of standardization should be applied, so cross-sections are obtained from the same level using consistent criteria. This type of analysis can be used to develop a standardized methodology for the estimation of age in roosterfish, so that future studies on this species can be comparable.

When using the fourth spine, the putative growth marks examined in this study were clearly visible and legible in nearly all cases (93%), with good agreement between individual readers (CV= 9.67%). There is no *a-priori* CV value that can be used for reference because it can be influenced by factors other than the reader, including the fish species and the nature of the hard structure (Campana 2001). In studies of shark age determination, which were based on counting growth marks in the vertebrae, it is common to find CV values higher than 10% (Campana 2001). In large pelagic fish, CV values between 10 and 15% are common (DeMartini et al. 2007), while for fish species of moderate longevity some authors suggest that a 5% CV may serve as a reference point (Campana 2001). The CV calculated in this study suggests relatively high precision in the counts performed independently by the readers and confirms that the fourth dorsal fin spine may offer future studies an opportunity to accurately determine roosterfish age.

Although field validation is necessary for accurately determining the age of roosterfish, this work was compared to previous aging hypotheses for reference (Ortega-Garcia et al. 2017). Ortega-Garcia et al. (2017) found that otoliths were not useful for accurately ageing fish over 66 cm FL or ~1-year-old, as daily ring differentiation decreased with increasing size. Roosterfish with one growth mark on dorsal spines ranged in size from 43-92 cm FL, a size range spanning the mean size (66 cm FL) of age-1 roosterfish estimated from daily otolith ring counts (Ortega-Garcia et al. 2017). The wide size range of fish with one dorsal fin growth mark was likely due to the potential inclusion of individuals that may be slightly older than one year and also the intermittent sampling protocol used in this work. Although we acknowledge the need for future validation and the inclusion of a larger size range of individuals, these data support the use of the fourth dorsal spine in future aging work for this species.

In general, opaque bands are wider than translucent bands, and as the number of marks in a section increases, the width of each band decreases (Jakes-Cota 2008). A complete growth mark consists of a broad opaque band (rapid growth) followed by a narrow translucent band (slow growth) when viewed under transmitting light (Fablet 2006); the presence and characteristics of such marks (opaque and translucent) were observed in all sections of rooster fish spines. The size, shape and clarity of marks can vary between individual fish, populations, and species, as well as between different methods of preparation of the hard structures (Fablet 2006).

The interpretation of growth marks to determine age is not straightforward, since it can be misled by the presence of false marks and the vascularization of the nucleus of fin spines (Kopf et al. 2010). False growth marks have the potential to be an important source of error in age determination studies. False growth marks have been described as partial segments that do not extend around the sections of fin spines (Speare 2003). False growth marks are sometimes, but not always, thinner and less clear than true marks. Although false growth marks were occasionally found in cross-sections of fourth dorsal fin spine in the roosterfish, these were easily detected because they were very diffuse and did not form a complete ring around the focus of the cross-sections of the spine.

The vascularization of the nucleus of fin spines is known as bone remodeling, a process that has been reported for different fish species (Panfili et al. 2002). Vascularization can lead to underestimating age and overestimating growth because it tends to obscure the first growth marks. Vascularization occurs to some extent in all fin spines, and the vascularized proportion increases directly with fish length (Franks et al. 2000; Drew et al. 2006). In the roosterfish, it was found that the vascularized area is small and does not obscure the first growth marks; therefore, it is not a factor that likely influenced the counting of said marks in this study.

The correlation between the number of growth marks and fork length for roosterfish in this study was high and supports the findings presented in previous studies on this species (Ortega-Garcia et al. 2017). The number of growth marks increased with length for all individuals examined, further suggesting its potential use as an age estimation tool in future studies. The accurate estimation of age and growth in the roosterfish will largely depend on the successful validation of the periodicity of formation of growth marks on cross-sections of dorsal fin spines or other hard structures. The mark-recapture of chemically-tagged (i.e., oxytetracycline) wild fish is one of the best methods that can be used for such validation studies. Nonetheless, this work provides the necessary foundation for future testing of age and growth hypotheses for the roosterfish.

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