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Skeletal Development and Mineralization Pattern of the Vertebral Column, Dorsal, Anal and Caudal Fin Complex in *Seriola Rivoliana* (Valenciennes, 1833) Larvae

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Abstract

Bone and fins development in *Seriola rivoliana* were studied from cleared and stained specimens from 3 to 33 days after hatching. The vertebral column began to mineralize in the neural arches at 4.40 ± 0.14 mm Standard Length (SL), continued with the haemal arches and centrums following a cranial-caudal direction. Mineralization of the caudal fin structures started with the caudal rays by 5.12 ± 0.11 mm SL, at the same time that the notochord flexion occurs. The first dorsal and anal fin structures were the hard spines (S), and lepidotrichium (R) by 8.01 ± 0.26 mm SL. The metamorphosis was completed by 11.82 ± 0.4 mm SL. Finally, the fin supports (pterygiophores) and the caudal fins were completely mineralized by 16.1 ± 0.89 mm SL. In addition, the meristic data of 23 structures were provided. Results from the present study might be used as a practical guide for future studies on this field with *S. rivoliana* or in related species.

Introduction

Longfin yellowtail, *Seriola rivoliana* (Valenciennes, 1833) is one of the species proposed for marine aquaculture diversification, mostly due to its fast growth rate [1,2] and worldwide distribution. This species belongs to Carangidae family, along with other popular species like *Seriola dumerili* (greater amberjack), *Seriola lalandi* (yellowtail king fish) and *Seriola quinqueradiata* (Japanese yellowtail). Even though *S. rivoliana* is commercially produced [3], studies about its biology are scarce and only few reports on larval rearing have been conducted in Ecuador [4-6], Hawaii [7] and more recently in the Canary Islands [8]. In contrast, numerous studies of the genus *Seriola* have been published related to the feeding requirements and nutrition [9-17], reproduction biology [2,18-21] and culture needs [8,22-26].

Regarding osteology studies, previous reports illustrate the bone structure development for other *Seriola* species. The osteological development of the greater amberjack have been described by different authors [27,28] These authors obtained distinct results probably associated to different environmental conditions and/or the number of samples. Also, the caudal skeleton development of the *S. lalandi* has been reported [29]. In addition, numerous studies have described the osteological development of other marine finfish species, such as *Sparus aurata* [30,31], *Pagrus pagrus* [32,33], *Solea senegalensis* [34,35], *Dentex dentex* [36], *Argyrosomus regius* [37], *Epinephelus septemfasciatus* [38] or *Dicentrarchus labrax* [39].

The objective of the present study was to chart the ossification of the vertebral column, dorsal, anal and caudal fin complex in *S. rivoliana* larvae cultured under semi intensive system conditions (mesocosms, [8]). Larvae culture under this type of system usually performed better than those cultures under intensive conditions [32]. The identification of bony structures and mineralization pattern will serve as a tool for future studies, where different factors (zootechnical, nutritional, environmental parameters, etc) may affect the apparition of skeletal abnormalities.

Material and Methods

S. rivoliana eggs were obtained from induced spawning (hormonally injection, GnRHa; Sigma-Aldrich™), based on the reported dosage [8]. Larvae were reared under mesocosms rearing system (4.5 eggs.l⁻¹ in two 40 m³ tanks [8]), kept under natural photoperiod and filtered natural sea water with 37 g/L salinity and temperature of 23.0 ± 0.9°C. Green water technique was used adding live phytoplankton (*Nannochloropsis* sp.) to maintain a concentration of 250000 cells ml⁻¹ in the rearing tanks. From 2 to 25 days after hatching (dah) rotifers, *Brachionus* sp., L-strain enriched with DHA Protein Selco (INVE™), were added twice a day (08:00 and 14:00 h). Artemia feeding starts at 15 dah, and were enriched with A1 Easy Selco (INVE™). Weaning protocol included manual feeding from 20 dah (Genma Micro, Skretting ™) to 25 dah and automatic feeding afterwards.

Larval growth was assessed measuring the standard length (SL) of 25 larvae, every 2 days using a profile projector (Nikon V-12A, NIKON™). A total of 75 specimens were individually stained (3.28 ± 0.15-16.1 ± 0.89 mm SL). To study the bone ossification, all specimens were fixed in 10% buffered formalin, from hatching to 33dah. Fixed larvae were cleared and stained with alizarin red [40]. Larvae were individually examined using stereomicroscopy. Drawings of the different developmental stages were made using the Adobe Photoshop CS3-10.0 (1990-2007 Adobe System Incorporated, United States) directly from digital photographs. Bone description, followed the terminology suggested by different authors [41-43], and their abbreviations, and are illustrated in the Table 1. The angles of the spine

Region	Skeletal elements	Abbreviations
Vertebral Column	Vertebra centra	Ce
	Notochord	No
	Urostyle	Ur
	Neural Arch	Na
	Neural Spine	Ns
	Haemal Arch	Ha
	Haemal Spine	Hs
	Dorsal Ribs	Eb
	Pleural Ribs	Plr
	Parapophyses	Pp
	Anterior neural zygapophysis	Anz
	Posterior neural zygapophysis	Pnz
	Anterior haemal zygapophysis	Ahz
	Posterior haemal zygapophysis	Phz
Caudal Fin	Hypurals	Hy
	Parhypural	Ph
	Epurals	Ep
	Uroneurals	Un
	Caudal lepidotrichia	PCR
	Caudal Dermatotrichia	SCR
Dorsal Fin	Predorsal	Pd
	Hard Spines	S
	Lepidotrichium	R
	Proximal Pterygiophores	Pr
	Distal Radial	Dr
Anal Fin	Hard Spines	S
	Lepidotrichium	R
	Proximal Pterygiophores	Pr
	Distal Radial	Dr

Table 1: Skeletal elements and their abbreviations.

were measured from the beginning of the vertebral body to the tip of the spine.

A total of 10 *S. rivoliana* reared juveniles were soft X-ray monitored (Mod. Senographer-DHR, General electric's, USA) for meristic counts.

Results

Vertebral column

In the present study, *S. rivoliana* vertebral column mineralization was initiated with the neural arches (Na_1-Na_3) by 4.40 ± 0.14 mm SL (Figure 1A), followed by the haemal arches (Ha_1-Ha_3) and the cephalic vertebrae (Ce_{1-4}) by 4.74 ± 0.27 mm SL (Figure 1B). The ossification of the vertebral column followed a cranial-caudal direction, being totally ossified by 11.82 ± 0.4 mm SL (Figure 1G). This size marked the end of metamorphosis. The notochord flexion was initiated at 5.12 ± 0.11 mm SL (Figure 1C), at the same time that the caudal complex mineralization was initiated. Initially, urostyle was formed by two independent structures (Ur_1-Ur_2) that fused by 10.23 ± 0.26 mm SL (Figure 1F). The neural spine (Ns_{23}), the haemal spine (Hs_{13}) and the Ce_{22}-Ce_{23} were the last structures that mineralized. At 11.82 ± 0.4 mm SL (Figure 1G), four types of articulation processes were mineralized: anterior neural zygapophyses (Anz), posterior neural zygapophyses (Pnz), anterior haemal zygapophyses (Ahz) and posterior haemal zygapophyses (Phz), (Figure 1G).

The vertebral bodies mineralization in the cephalic (without parapophyses) and prehaemal (with parapophyses) region (Ce_{1-4} and Ce_{5-10} respectively) proceeded from dorsal to ventral direction and

from the surface to internal bone layers (Figures 2A and 2B), whereas in the haemal (with Hs) and caudal region (Ns and Hs modified to support caudal fin complex) the mineralization pattern proceeds in both directions dorsal and ventrally (Ce_{11-21} and Ce_{22-23} respectively), joining in the middle of the centra (Figure 2C). Exceptionally, in Ce_9 and Ce_{10}, the mineralization of the vertebral bodies differed from other vertebral structures of the prehaemal region, proceeding dorsally first and ventrally later on.

The Na and Ha developed from centrum and fused in the middle, forming the neural and haemal canals with rounded shape, later developing into the Ns and Hs (Figures 2A-2C). The Ns and Hs angle in relation to the vertebral body varied along the vertebral column, increasing in cranial-caudal direction (Figure 1G). The Hs developed according to the angle of the first anal pterygiophore, and decreasing afterward.

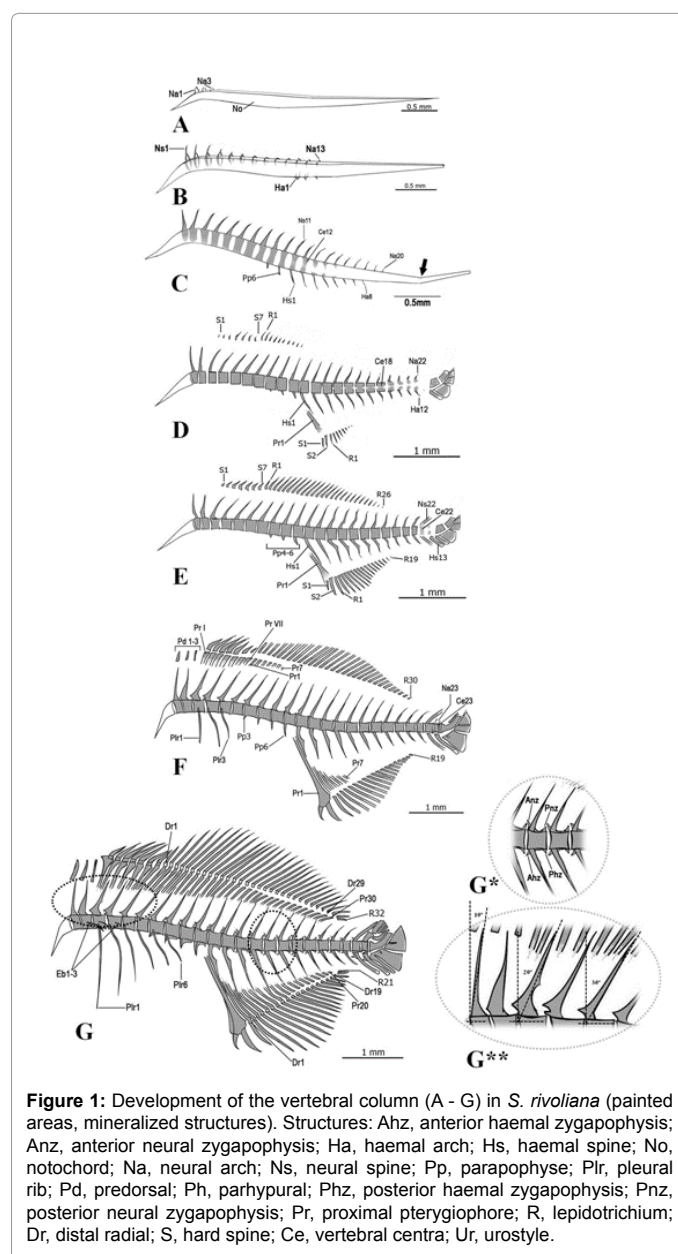The parapophyses (Pp) was first observed with the mineralization

Figure 1: Development of the vertebral column (A - G) in *S. rivoliana* (painted areas, mineralized structures). Structures: Ahz, anterior haemal zygapophysis; Anz, anterior neural zygapophysis; Ha, haemal arch; Hs, haemal spine; No, notochord; Na, neural arch; Ns, neural spine; Pp, parapophyse; Plr, pleural rib; Pd, predorsal; Ph, parhypural; Phz, posterior haemal zygapophysis; Pnz, posterior neural zygapophysis; Pr, proximal pterygiophore; R, lepidotrichium; Dr, distal radial; S, hard spine; Ce, vertebral centra; Ur, urostyle.

Species	Regions																								References
	Vertebral column							Caudal fin						Dorsal Fin						Anal Fin				
	Vertebra centra	Urostyle	Neural spine	Haemal spine	Dorsal Ribs	Pleural Ribs	Parapophyses	Hypurals	Parhypural	Epurals	Uroneurals	Lepidotrichia	Dermatotrichia	Predorsal	Hard Spines	Proximal Pterygiophores	Lepidotrichia	Proximal Radial	Distal Radial	Hard Spines	Lepidotrichia	Proximal Radial	Distal Radial	
S. rivoliana	10+13	1+1	23	13	6+6	8+8	6	5	1	3	1+1	10+9	10+9	3	VII+I	7	30/34	30/34	30/34	II+I	19/21	19/22	19/22	Present Study
S. dumerili	10+13	1+1	23	13	6+6	8+8	6	5	1	3	1+1	9+9	12+10	3	VII-VIII	08-Jul	31/34	35	32	III	19/21	20/23	20/23	[28]
S. fasciata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	VIII+I	-	28/31	-	-	II+I	18/20	-	-	[60]
C. crysos	-	1+1	-	-	-	-	-	5	1	3	1+1	-	-	-	-	-	-	-	-	-	-	-	-	[44]
S. fasciata	-	-	-	-	-	-	-	-	-	-	-	-	-	-	VIII+I	-	28/31	-	-	II+I	18/20	-	-	[61]
S. rivoliana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	VII+I	-	30	-	-	II+I	21	-	-	[62]
S. rivoliana	-	-	-	-	-	-	-	-	-	-	-	-	-	-	VII+I	-	32	-	-	II+I	22	-	-	[63]
S. dumerili	10+13	1	22	13	-	8+8	-	5	1	3	1+1	9+7	12+11	3	VII-VIII		31/32	38	-	II-III	20	21	-	[27]
S. lalandi	-	1+1+1	-	-	-	-	-	5	1	04-Mar	1+1	-	-	-	-	-	-	-	-	-	-	-	-	[29]
S. setapinnis	10+14	-	24	14	-	-	-	5	1	-	-	9+8	5+6	-	-	-	-	-	-	-	-	-	-	[45]
H. amblyrhynchus	10+14	-	24	14	-	-	-	-	-	-	-	9+9	8+8	-	VII+I	-	28	-	-	II+I	25	-	-	[55]
T. japonicus	10+14	1	24	14	7+7	10+10	6	5	1	2	1	17	-	3	8	8	27/35	27/35	27/35	II+I	25/31	25/31	25/31	[43]

Table 2: Meristic counts in different carangid species. (-) no data; (+) and; (/) between.

of the Pp_6-Pp_5 by 5.12 ± 0.11 mm SL (Figure 1C). These structures had a caudal-cranial development and were fully ossified at 11.82 ± 0.4 mm SL (Figure 1G). The Pp structures become larger form Ce_4 to Ce_{10}.

The pleural ribs (Plr) were observed for the first time at 10.23 ± 0.26 mm SL (Plr_1-Plr_3) with the caudal development (Figure 1F). Plr_4-Plr_7 developed at 11.82 ± 0.4 mm SL (Figure 1G). The dorsal ribs (Eb) were first seen at 11.82 ± 0.4 mm SL (Figure 1G), with the mineralization of the Eb_1-Eb_3, following the caudal development.

Dorsal and anal fins development

The formation and mineralization of the dorsal and anal fins of the long fin yellowtail followed a cranial-caudal direction. The first dorsal fin structures were the hard spines (S) and lepidotrichium (R) by 8.01 ± 0.26 mm SL (Figure 1D), which initiated its mineralization from the base to the tip of the structure. Predorsal bones (Pd_1-Pd_3) and proximal pterygiophore (Pr) had a dorsal-ventral mineralization pattern (Figure 1F).

In the anal fin, the two hard spines (S_1-S_2) were first seen at 8.01 ± 0.26 mm SL (Figure 1D), same as the anal lepidotrichia (R) and Pr. The S_1-S_2 fused into the Pr1 by 9.92 ± 0.84 mm SL (Figure 1E). The S_1-S_2 and R mineralized from the base to the tip of the structure, whereas the Pr followed a ventral-dorsal pattern (Figure 1F).

Caudal fin development

The first caudal complex structures in mineralized were the upper and lower caudal lepidotrichia (PCR) by 5.12 ± 0.11 mm SL (Figure 3A). Then, hypurals (Hy) initiated their mineralization as fused structures, first $Hy_1 + Hy_2$, continues with $Hy_3 + Hy_4$ and finally parhypural (Ph) by 5.38 ± 0.11 mm SL (Figure 3B). At the same time, the first upper caudal dermatotrichia (SCR) started to mineralize, following a base-tip mineralization pattern. The last hypural (Hy_5) delayed its mineralization to 8.01 ± 0.26 SL (Figure 3C). By 9.92 ± 0.84 mm SL uroneurals ($Un_1 + Un_2$) arted to mineralize and fused forming a single structure (Uroneural) (Figures 3D and 3E). Finally, the last
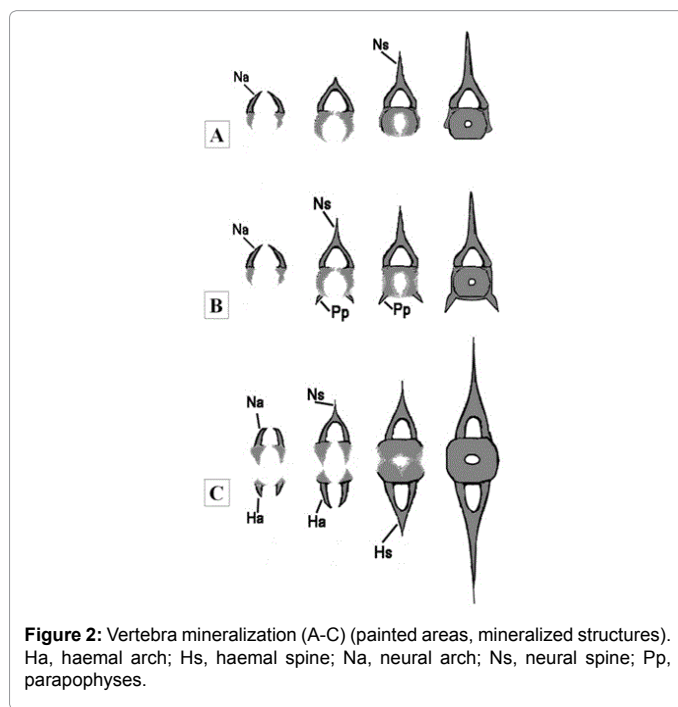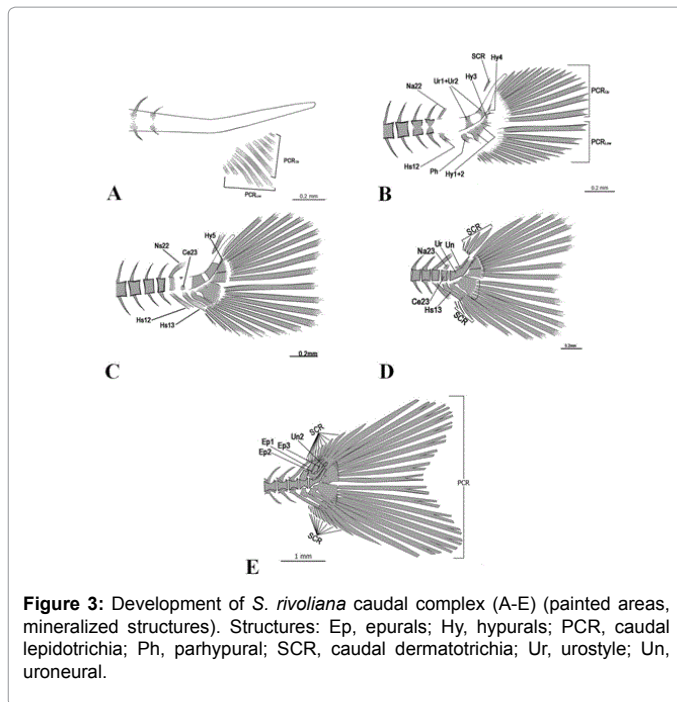

Figure 2: Vertebra mineralization (A-C) (painted areas, mineralized structures). Ha, haemal arch; Hs, haemal spine; Na, neural arch; Ns, neural spine; Pp, parapophyses.

caudal complex structures in mineralized were the epurals (Ep_{1-3}) by 11.82 ± 0.4 mm SL (Figure 3E).

Meristic characters

Meristically, *S. rivoliana* had a total number of 23 vertebrae (urostyle not included), 23 neural spines, 13 haemal spines, 16 pleural ribs, 12 dorsal ribs and 6 parapophyses. In the dorsal region, 3 predorsal spines, VII+I hard spines and a variable number (30-34) lepidotrichia were identified. Besides, VII hard spine proximal pterygiophores, 30 to 34 distal radial and proximal pterygiophores were also observed.

Figure 3: Development of *S. rivoliana* caudal complex (A-E) (painted areas, mineralized structures). Structures: Ep, epurals; Hy, hypurals; PCR, caudal lepidotrichia; Ph, parhypural; SCR, caudal dermatotrichia; Ur, urostyle; Un, uroneural.

Within the anal region, II+I hard spines and 19-21 lepidotrichia, same number of distal radial and proximal pterygiophores were identified. Finally, in the caudal complex, 1 parahypural, 5 hypurals, 3 epurals, 2 uroneurals, 10+9 caudal lepidotrichia and 10+9 caudad dermatotrichia were observed (Table 2).

Discussion

This study reported for first time *S. rivoliana* skeletal development and mineralization. The comparison of present results with other species from the same family and genus, such as *S. dumerili*, suggest some correspondence. Thus, [27] describes first mineralized structure in the vertebra centra for *S. dumerilli* at 6.6 mm (NL) while other result for the same specie [28] identified Neural Spine (NS) and centra at 4.8mm (TL). This pattern agrees with present data for *S. rivoliana*, where similar mineralization was obtained (4.74 ± 0.27 mm SL). The differences between vertebra centra mineralization timing for *S. dumerili* could be explained by the different environmental conditions, such as temperature, or rearing protocols applied in those studies [32]. In fact, mineralization pattern is more accurately described when larval growth is used as reference instead of larval age [33].

Also, the present study showed a similar vertebra centra mineralization timing in comparison with other carangid species, such as *Caranx crysos* [44] and *Selene setapinnis* [45,46], suggesting that some developmental events during mineralization process may be common for many species. For instance, the dorsal flexion at the posterior end of the notochord could be an external indicator of the initiation of the internal column mineralization for this and other species. In fact, these events also occur in other species such as *S. aurata* larvae (5.7-6.0 mm, SL) [30], *Solea senegalensis* larvae (4.7 mm, SL) [35], *Pagrus pagrus* larvae (6.0 ± 0.5 mm, TL) [32] or *Argyrosomus regius* larvae (5.42-6.01 mm, TL) [47].

In most Perciforms, the vertebral column follows a bidirectional mineralization pattern (Pagrus major, [42]; *S. aurata*, [30]; *Dentex dentex*, [36]; *Diplodus sargus*, [47]; *Pagrus pagrus*, [32]). However, in *S. rivoliana* the vertebral column followed a unidirectional mineralization pattern, in agreement with data reported in *S. dumerili* [28] and in *A. regius* [46].

According to the centrum mineral deposition, three complementary models occur in the vertebral region: in a dorsal-ventral direction (D-V), in a ventral-dorsal direction (V-D) or simultaneously (DVS). In *S. aurata* [30] and *D. sargus* [47], two centra mineral deposition models occur the first one takes place in a D-V direction from the Ce_1 to Ce_{21} and the second one in a V-D direction in Ce_{22} and Ce_{23}. In *S. rivoliana*, the mineralization expands in a D-V direction from Ce_1 to Ce_{23}, following the same pattern as *S. dumerili* between the Ce_1-Ce_{19} [28]. Additionally, *S. rivoliana* had simultaneously DVS mineralization from Ce_8 to Ce_{23}, whereas *S. dumerili* [28] presents this simultaneous DVS mineralization pattern from Ce_{20} to Ce_{23}. Other marine finfish such as *A. regius* [47] showed a D-V mineral deposition from Ce_1-Ce_4, while the remaining vertebrae had simultaneous DVS mineralization.

About to urostyle (Ur) structure of *S. rivoliana* larvae and other marine finfish such as *P. major, C. crysos and S. dumerili* [28,44,48], this was formed by the fusion of two elements (Ur_1+Ur_2). In contrast, at least three elements were necessary to form this structure in *S. lalandi* [29]. The results of the present study suggest that the fusion of different structures to form the urostyle is nonspecific of the genus *Seriola* sp.

The development of the parapophyses (Pp) of *S. rivoliana* followed a caudal-cranial development, in concordance with *S. dumerili* [28] and many other perciforms such as *S. aurata* [30], *Lates calcarifer* [49], *Diplodus sargus* [47] or *Pagrus pagrus* [32]. The correlation between the present study and many other marine finfish suggest that the developmental patter for the parapophyses may be common in perciforms [49].

In many marine finfish species, the mineralization of the anal and posterior dorsal fins starts prior to the anterior dorsal fin [31,32,50-52]. Unlike this developmental pattern, but in accordance with *S. dumerili* [28], *S. rivoliana* dorsal and anal structures followed a cranial-caudal development, developing the anterior dorsal fin prior to posterior dorsal fin. However, despite *S. rivoliana* and *S. dumerili* had the same developmental pattern in dorsal, anal and caudal fins; some differences in structures development have been observed. For instance, in *S. rivoliana*, firsts structures in mineralized were hard spines (S) and lepidotrichium (R) (present study), whereas in *S. dumerili* [28] the dorsal fins development starts with the mineralization of the proximal pterygiophore.

During the process of the caudal complex mineralization of *S. rivoliana*, the fusion of hypurals (Hy_1+Hy_2 and Hy_3+Hy_4) was observed. This developmental pattern is common in other carangid species such as *S. lalandi* [29], *S. setapinnis* [47], *C. crysos* [44] and *S. dumerili* [27,28]; as well as other perciforms such as *Coryphaena equiselis* [53], *P. major* [42], *S. aurata* [30] and *D. dentex* [36]. The development of three distinct structures (Hy_1+Hy_2, Hy_3+Hy_4, Hy_5) could remain as a characteristic of carangids and Coryphaena [44].

In the present study, the development of three epurals and two uroneurals were observed. The number of epurals in the caudal complex of *Carangoidei* species varies between species [44]: 3 epurals in *S. dumerili* [28], between 3-4 epurals (usually 3 epurals) in *S. lalandi* [29], 2 independent epurals that fused during ontogeny in *C. equiselis* [53] and 3 epurals for *S. rivoliana* (present study). Other authors [54] considered that the presence of uroneurals is a characteristic of the Teleost. The presence of two uroneurals in *S. rivoliana* caudal fin complex is in concordance with other species from the same genus

such as *S. lalandi* [29] and *S. dumerili* [28].

Meristically, the vertebral column of longfin yellowtail (*S. rivoliana*) was characterized in this study. Similar results have been reported in *S. dumerili* [28]. Nevertheless, in other carangid species such as *S. setapinnis* [45], *Hemicaranx amblyrhynchus* [55] or *Trachurus japonicas* [43] a total number of 24 vertebral structures were observed, and the first haemal arch was observed at the 10th vertebra [45] instead of at the 11th vertebrae in *S. dumerili* [27,28] and *S. rivoliana* (present study), indicating that this could be a conserved feature among the genus Seriola (Table 2).

Concerning the caudal complex, *S. rivoliana* presented similar results than those observed in *S. lalandi* [29], *S. setapinnis* [45], *C. crysos* [44] and *S. dumerili* [27,28], although the number of caudal fin rays is a characteristic for each species. Thus, in this study, *S. rivoliana* had 10+9 caudal lepidotrichia and 10+9 caudad dermatotrichia, while 9+9 caudal lepidotrichia and 11-13+10 caudal dermatotrichia where reported in *S. dumerili* [28] or 9+8 caudal lepidotrichia and 6+5 caudad dermatotrichia were observed in *S. setapinnis* [45].

The importance of the meristic characterization is widely known for the identification not only for marine finfish species, [56,57] but also in cultured fish species [52,58,59].

Results from the present study might be used as practical guide for future studies on this field with *S. rivoliana* or in related species.

Acknowledgement

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