

Fat-Soluble Nutrient Contents in Normal and with Cranial Abnormalities Juveniles of Palm Ruff, *Seriolella violacea* (Guichenot, 1848)

¹Wilfrido Argüello-Guevara, ²Milton Bohórquez-Cruz and ³Alfonso Silva

¹Doctorado en Acuicultura, Departamento de Acuicultura, Facultad de Ciencias del Mar, Universidad Católica del Norte, Larrondo 1281. Coquimbo, Chile

²Magíster en Acuicultura, Departamento de Acuicultura, Facultad de Ciencias del Mar, Universidad Católica del Norte. Larrondo 1281. Coquimbo. Chile

³Departamento de Acuicultura. Facultad de Ciencias del Mar, Universidad Católica del Norte, Larrondo 1281. Coquimbo. Chile

Abstract: Larvae of *Seriolella violacea* were reared at 14°C (T14) and 18°C (T18) during 80 days, following the protocol of production of Universidad Católica del Norte (Chile). Fat-soluble nutrient contents in tissues of Normal and with obvious cranial skeletal anomalies juveniles were analyzed. Temperature had a significant effect ($P<0.05$) on fatty acid content but not on vitamin content in tissues of juveniles, while the normal condition (NC) and with cranial anomalies (deformed condition DC) significantly influenced vitamin A (VA) and E (VE) contents being higher in normal juveniles. This study determined that fat-soluble nutrient content into tissues of juveniles fish reflect the composition of diet, but are not the causative factor of cranial skeletal abnormalities observed, even when VA and VE concentrations in normal fish (VA = 19.68 ± 1.91 $\mu\text{g}/100\text{g}$ y VE= 173.92 ± 39.83 $\mu\text{g}/100\text{g}$) were higher than deformed fish (VA= 15.23 ± 3.88 $\mu\text{g}/100\text{g}$ y VE= 121.47 ± 46.89 $\mu\text{g}/100\text{g}$), due to the occurrence of cranial anomalies still in early stages of development, indicating the need for further studies of embryonic development stages, egg handling and broodstock nutrition.

Key words: Cranial abnormalities • Temperature • Fatty acids • Vitamin

INTRODUCTION

Skeletal abnormalities, especially in both cranial and spinal axis, have been reported in almost all species of farmed fish with varying incidence and severity, developed mainly during ontogeny to metamorphosis [1]. Among the most common causative factors that have been associated with skeletal anomalies are found: intensive culture, unsuitable abiotic conditions, nutritional imbalances, diseases and genetic factors [1-3]. Larval nutrition, particularly in relation to the content of fatty acids and/or certain vitamins in diet, has been implicated in the onset of cranial abnormalities [2, 4]. Northern palm ruff, *Seriolella violacea*, is an important species positively projected for aquaculture diversification in northern Chile and Peru [5]. Laboratory

of Fish Culture at the Universidad Católica del Norte (UCN) has obtained several spontaneous spawning and performed experiments in larvae and juveniles, showing animals with cranial anomalies in later stages of development [6]. Likewise, experiences with different culture temperatures have preliminarily found improved larval growth without affecting survival or increasing the presence of cranial anomalies in juveniles (Argüello-Guevara *et al.* submitted for publication). However, it is known that accelerating development and growth rates by temperature, precocious and incomplete ossification may occur [7]. This study was aimed to evaluate the possible association between temperature culture and fat-soluble nutrient content in normal and with evident cranial anomalies juveniles of *Seriolella violacea*.

Corresponding Author: Wilfrido Argüello-Guevara. Escuela Superior Politécnica del Litoral, Centro Nacional de Acuicultura e Investigaciones Marinas. Km 30.5 Vía Perimetral, P.O. Box: 09-01-5863, Guayaquil, Ecuador.

MATERIALS AND METHODS

Naturally spawning (176,000 eggs) of *S. violacea* was obtained from wild broodstock maintained at the Laboratory of Marine Fish of Universidad Católica del Norte (Coquimbo, Chile) in September 2013. A total of 140,000 larvae were hatched. Larvae were stocked in six 500-L tanks (40 larvae L⁻¹). Two temperatures 14°C (T14) and 18°C (T18) were evaluated by triplicate during 80 days post-hatch (DPH). Culture was carried out following UCN protocol for this species. Larvae were gradually fed with enriched rotifers (*Brachionus plicatilis*), *Artemia* (INVE®)-enriched nauplii and metanauplii and subsequently BIOMAR® artificial diet (48.5% Protein, 16.5% Lipids, 2% Fiber, 12% Ash, 10% Moisture). At the end of the culture period, samples of juveniles (10 g wet weight) were taken of all tanks, considering normal condition (NC) and with obvious cranial anomalies (deformed condition, DC; Fig. 1). Samples were rinsed with distilled water and frozen at -20°C until analysis. Fatty acid profile and vitamins A, E, D₃ contents in tissues of fish by GC/FID and HPLC/DAD respectively, was determined. Two-ways analysis of variance (ANOVA) was used to evaluate the differences among fat-soluble nutrient contents in normal juveniles and with cranial skeletal anomalies in T14 and T18. D₃ concentrations data were transformed using 1/x². When ANOVA's showed mean differences Post-hoc comparisons were analyzed using Tukey's test ($\alpha = 0.05$). SigmaStat 3.1® program was used.

RESULTS AND DISCUSSION

Fat-soluble nutrient contents in tissues of NC and DC juveniles are shown in Table 1. Interaction between temperature and NC or DC reported no significant

differences ($P > 0.05$) for any fat-soluble nutrient. In general, temperature had a significant effect on some fatty acid content, but not on vitamin content in examined juveniles, while condition (NC or DC) significantly influenced on vitamins A and E content, being higher ($P < 0.05$) in normal juveniles. ARA and DHA content were significantly higher in T14. Likewise, EPA: DHA and "SUM" n-3 and "SUM" n-6 ratios were significantly higher in T14 than T18. In our study, both treatments, larvae were fed with the same enriched live-prey and subsequently juveniles were fed *ad libitum* with the same artificial diet. However, the period of feeding was different between treatments due to temperature and larval development (T14 were added live preys by 68 days and T18 by 53 days, whilst artificial diet in T14 was added by 29 days and T18 by 40 days). This could be the reason for the differences in fatty acids profile in tissue of juveniles, being in many cases significantly higher in T14 (longer fed with live prey enriched [8]), as concluded Guillou *et al.* [9], fatty acids last a long time to disappear from tissues, because the "feeding history" has a long lasting effect and generally fatty acid patterns of predator reflects prey's nutrient contents [8,10].

Abnormalities in larvae of *Latris lineata* have been associated with higher requirements of DHA: EPA ratios, greater than 2:1 [11] especially around flexion of the notochord [12]. In our study (Table 1) the highest DHA: EPA ratio was found in T14 (1.34±0.17), according with registered in larval tissues of milkfish (*Chanos chanos*) fed with DHA-enriched prey (1.54±0.09) where a reduction of opercular abnormalities was noted [10]. However, in this treatment (T14), was recorded a frequency of cranial skeletal anomalies of 20.4±9.8 %, although was not statistically different to T18 (7.3±2.9 %). On the other hand, the condition NC and DC resulted in no statistical differences in any fatty acid measured in tissues.

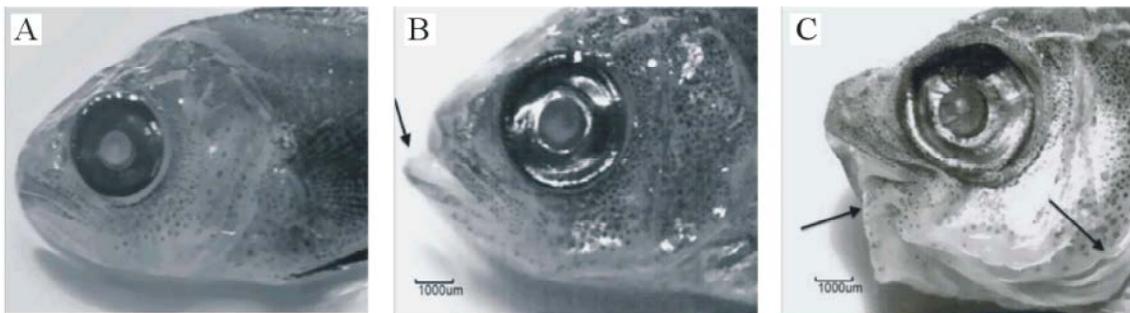


Fig. 1: Juveniles (80 DPH) of *Serirolella violacea*. Normal (A) and with evident cranial skeletal anomalies juveniles (B and C). Arrows indicate the affected element.

Table 1: Mean values (\pm standard deviation) of fat-soluble nutrient in tissues of *Serirolella violacea* juveniles reared at 14°C and 18°C. Significant effects of temperature ($T^{\circ}C$), condition (*Cond.*) normal (NC) or with cranial anomalies (deformed condition, DC) and interaction (*I*) are represented with ** ($P < 0.05$) for each nutrient.

Fatty acids (FA)					2-way ANOVA			
	% = g AG / 100 g fat	T14-NC	T14-DC	T18-NC	T18-DC	$T^{\circ}C$	<i>Cond.</i>	<i>I</i>
C14:0		2.42 \pm 0.45	2.62 \pm 0.37	4.37 \pm 0.34	3.53 \pm 0.44	**		
C16:0		23.03 \pm 1.39	22.29 \pm 2.37	23.43 \pm 2.44	21.95 \pm 2.25			
C18:0		9.22 \pm 0.06	9.06 \pm 0.43	6.64 \pm 0.78	6.98 \pm 0.38	**		
C18:1 <i>n</i> -9		15.40 \pm 0.78	14.95 \pm 1.54	18.01 \pm 1.76	19.20 \pm 2.32	**		
C18:2 <i>n</i> -6		4.98 \pm 0.21	5.99 \pm 1.18	7.32 \pm 0.70	9.20 \pm 3.49	**		
C18:3 <i>n</i> -3		2.22 \pm 0.21	1.96 \pm 0.49	1.18 \pm 0.14	0.80 \pm 0.40	**		
C20:4 <i>n</i> -6		2.22 \pm 0.55	2.15 \pm 0.67	0.70 \pm 0.25	0.77 \pm 0.09	**		
C20:5 <i>n</i> -3		8.53 \pm 1.46	9.08 \pm 1.70	10.20 \pm 1.89	9.16 \pm 0.82			
C22:6 <i>n</i> -3		10.59 \pm 0.70	13.00 \pm 3.35	8.34 \pm 2.84	9.13 \pm 0.30	**		
FA saturated		34.66 \pm 1.82	33.97 \pm 2.31	34.54 \pm 3.25	32.60 \pm 2.28			
<i>n</i> - 3		21.34 \pm 1.95	24.04 \pm 5.30	19.73 \pm 4.78	19.09 \pm 0.75			
<i>n</i> - 6		7.20 \pm 0.60	8.14 \pm 1.66	8.02 \pm 0.91	9.96 \pm 3.46			
C22:6 <i>n</i> -3 / C20:5 <i>n</i> -3		1.26 \pm 0.19	1.42 \pm 0.13	0.80 \pm 0.13	1.00 \pm 0.06	**		
<i>S n</i> - 3 / <i>S n</i> - 6		2.97 \pm 0.12	2.95 \pm 0.10	2.44 \pm 0.38	2.07 \pm 0.67	**		
Vitamins								
μ g / 100 g								
Vitamin A		19.3 \pm 2.8	13.0 \pm 3.6	20.0 \pm 0.9	17.5 \pm 3.1		**	
Vitamin D3		130.3 \pm 26.4	113.3 \pm 6.4	132.0 \pm 4.2	134.7 \pm 4.7			
Vitamin E		146.7 \pm 33.7	102.7 \pm 30.7	201.2 \pm 24.6	140.2 \pm 59.1		**	

Probably, because flour and fish oil represents main ingredients in fish feed (as enrichment and artificial diet used in this study). Therefore, larval requirements of highly unsaturated fatty acids (*n*-3 HUFA) are easily covered [13]. High values of palmitic acid (C16:0) registered in tissues of juveniles in both treatments should be noticed, probably indicate the preference of this fatty acids by *S. violacea*.

A higher percentage of vitamin A (VA) and E (VE) were found in normal juveniles in both treatments and were significantly different from deformed juveniles in this study, while temperature did not exert significant effect on the concentration of these vitamins. Furthermore, vitamin D₃ content was not different because temperature or condition in juveniles (Table 1). Although other studies have shown the influence of dietary VA and VE on the occurrence of skeletal abnormalities, in parallel to our study, in *S. violacea* was recorded that the earliest occurrence of abnormalities in the lower jaw was to 5 DPH, during endogenous feeding [6]. Outcomes of Bohórquez-Cruz [6] and our results indicate that different levels of VA and VE found in tissues of juveniles were not the causative factor to produce the occurrence of cranial anomalies observed in *S. violacea*, due to the presence of

cranial anomalies still in early stages of development. However, proper management of levels of these nutrients in diet [14], could mean a reduction in skeletal abnormalities in later stages of development. Therefore, cannot rule out the effect of a possible mechanical stress during collection and transport of eggs [15], broodstock nutrition and endogenous reserves of yolk sac [16] as possible causative factors of cranial skeletal abnormalities in early stages of development.

In conclusion, fat-soluble nutrient content in tissues of *S. violacea* juveniles reflect the composition of enrichment and artificial diet used, associated with the acceleration of the growth rate and feed intake by indirect effect of temperature, but are not the cause of occurrence or frequency of cranial skeletal abnormalities in this species.

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