7-12 febrero 2021 X Firma 2021 online X Foro Iberoamericano de los Recursos Marinos y la Acuicultura
Sinergia entre ciencia e industria para el desarrollo y la sostenibilidad

Tissue-expression pattern of *elovl4* genes in *Sparus aurata* and *Solea senegalensis*: from larvae to adult.

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Introduction

Very long-chain (>C_{24}) fatty acids (VLC-FA) play critical roles during early development of vertebrates. However, studies on VLC-FA in fish are scarce. The biosynthesis of VLC-FA is mediated by Elovl4 proteins. Such ability is itself dependent on the complement of *elovl4* genes and the functions of their corresponding encoded enzymes. For a better understanding of the metabolism and the potential tissue-specific requirements of VLC-FA in marine teleosts, the present study aimed to determine the tissue-expression pattern of genes that coding for both Elovl4 isoforms, *elovl4a* and *elovl4b*, in different windows of development (larvae and adults) of *S. aurata* and *S. senegalensis*.

Materials and methods

-Tissue expression of *elovl4* genes in 24 hours post-hatching (hph) larvae.
Whole-mount *in situ* hybridization (WISH).
-Tissue expression of *elovl4* genes in adult fish.
RT-PCR (screening) and qPCR (selected tissues).

Results and Discussion

***S. aurata* larvae:** in agree with the observed in *Danio rerio* larvae [1], *elovl4a* was widely distributed in the head region (**Fig. 1B**). Moreover, *elovl4b* was specifically expressed in the eyes (**Fig. 2C**) showing a strong signal in the retinal epithelium (**Fig. 2D**). No signal was detected for sense control probes of *elovl4a* (**Fig. 1A**) and *elovl4b* (**Fig. 2A, B**) genes.

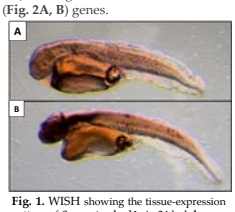

Fig. 1. WISH showing the tissue-expression pattern of ***S. aurata elovl4a*** in 24 hph **larvae**. Larvae were hybridized with either sense (A) or antisense (B) probes

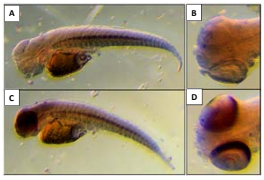

Fig. 2. WISH showing the tissue-expression pattern of ***S. aurata elovl4b*** in 24 hph larvae. Larvae were hybridized with either sense (A, B) or antisense (C, D) probes.

***S. senegalensis* larvae:** curiously, and in contrast with the observed in *D. rerio* larvae [1], *elovl4a* expression signal was located in the eyes (**Fig. 3C**, **D**). Oppositely to the tissue-expression pattern shown by *S. aurata* larvae, *elovl4b* expression signal was widely distributed in the cephalic region (**Fig. 4B**). As expected, no signal was detected for sense control probes of *elovl4a* (**Fig. 3A, B**) and *elovl4b* (**Fig. 4A**) genes.

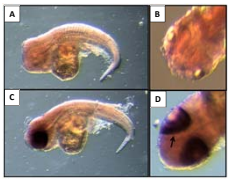

Fig. 3. WISH showing the tissue-expression pattern of *S. senegalensis elovl4a* in 24 hph larvae. Larvae were hybridized with either sense (A, B) or antisense (C, D) probes. Black arrow denote a strong expression signal in retinal epithelium.

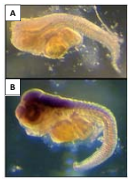

Fig. 4. WISH showing the tissue-expression pattern of *S. senegalensis elovl4b* in 24 hph larvae. Larvae were hybridized with either sense (A) or antisense (B) probes.

In **adults from both fish**, rtPCR results denoted a differential *elovl4a* and *elovl4b* tissue-specific expression pattern (**Fig. 5A, B**). As expected, qPCR results confirmed a similar *elovl4* expression pattern between *S. aurata* larvae and adults, with *elovl4a* being mostly expressed in brain (**Fig. 5C**), and *elovl4b* in eye (**Fig. 5E**). Curiously, for *S. senegalensis*, an opposite *elovl4* tissue-expression pattern was observed between the pre- and post-metamorphic stages. These differences could be connected with the important neural tissues remodeling carried out during metamorphosis process, after which, the cognitive system and feeding habits of *S. senegalensis* are consequently adapted to the strong nocturnal activity developed in the post-metamorphic stage. However, this assumption requires further exploration. Independently to the species-specific expression differences observed, this results suggest a role of Elovl4a/b enzymes in the local biosynthesis and incorporation of VLC-FA in fish neural tissues [2,3].

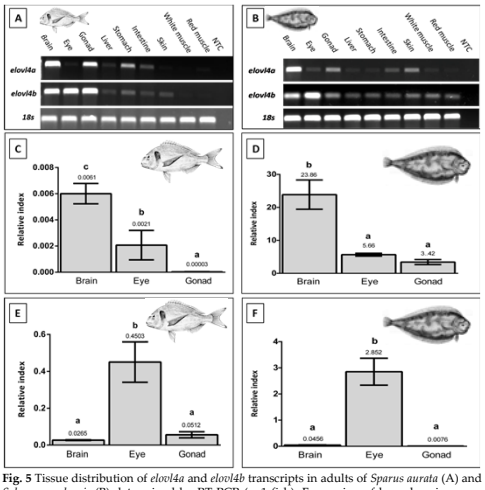

Fig. 5 Tissue distribution of *elovl4a* and *elovl4b* transcripts in adults of *Sparus aurata* (A) and *Solea senegalensis* (B) determined by RT-PCR (n=1 fish). Expression of housekeeping gene 18s is also shown. Expression in selected tissues of *Sa elovl4a* (C) *Ss elovl4a* (D), *Sa elovl4b* (E) and *Ss elovl4b* (F) transcripts was also determined by qPCR. The results, shown as relative index, are β-actin normalized values (gene copy number/*β-actin* copy number). Bars represent means and standard deviations (n=3 fish). Different letters (a, b, c) denote significant differences (ANOVA and Tukey HSD test, $P \leq 0.05$) among tissues.

Conclusions

Neural tissues are the major site of ***elovl4* expression**.

In contrast to found for *S. auarata*, the ***elovl4a*** and ***elovl4b*** **tissue-expression pattern** seem to be **stage-specific** in ***S. senegalensis***. These results suggest that the investigation of *elovl4* genes, and consequently of their encoded Elovl4 proteins in teleosts, requires a **species-specific approach**.

References

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Acknowledgments: AGL2013-40986-R,AGL2011-23502 (MINECO) and PROMETEO II / 2014/085 (*G.V.*).