MOLECULAR ONTOGENY OF DIGESTIVE ENZYMES IN PERSIAN STURGEON (*Acipenser persicus*)

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Introduction

The Persian sturgeon (*Acipenser persicus*) is a species of primary commercial interest which inhabits the Caspian and Black seas. This species is overfished mainly because of the high value of its roe and, in consequence, is listed as a critically endangered species. Sea ranching and production in hatchery is envisioned as a way to solve the problem. It is therefore necessary to advance the knowledge of the biology of larvae and juveniles to improve the rearing techniques. During the early development of the digestive system, digestive capacity is provided by the pancreatic enzymes (proteases, lipases and glucosidases) in conjunction with alkaline proteolytic enzymes secreted by the intestine, prior to acid digestion. While the pattern of digestive activities during the ontogeny has been profusely studied in many species, the expression patterns of the mRNA transcripts encoding the digestive enzyme precursors are comparatively scarce (Rønnestad et al., 2013). In this study, cDNAs coding for trypsinogen (*try*), two isoforms of pepsinogen (*pga*), proton pump (*atp4a*), bile salt activated lipase (*cel*) and β-actin (*actb*) from Persian sturgeon were partially cloned and their gene expression were analyzed using real time PCR from hatching to 34 days post hatch (dph).

Materials and methods

Sturgeon larvae were obtained from artificial fertilization of wild captured fish from the Southern Caspian Sea. Larvae were reared in standard circular Vniro tanks connected to a filtered flow-through freshwater system and fed with *Artemia* nauplii, then co-fed with *Daphnia* sp., and finally with *Daphnia* sp. Larvae were cultured under the natural light with average water temperature of 18.4±0.7 °C along the trial. Between 0 and 34 dph, 14 sampling points were chosen and 5-8 larvae were processed per sampling point. Larvae fixed in RNAlater® were weighted prior to total RNA extraction. Gene cloning and sequencing was carried out according to the methodology explained in Pujante et al. (2015). For real time PCR, specific primers were designed. The quantification was performed using SYBR Green and $\Delta\Delta C_T$ method. *actb* was chosen as internal reference control and a mix of equal cDNA amounts for all the samples was used as calibrator.

Results

Persian sturgeon larvae grew from 16 mg at hatching to 45 mg at 34 dph (see Fig. 1). *try* and *cel* expression increased progressively from first feeding, being maximum from 18 dph. The expression of both *pga* was detected from 8 dph and increased quickly reaching the maximum levels also at 18 dph. Although when studied the average value of the cycle threshold (Ct) of the calibrator sample used in all the plates, pga1 had 2.36 times more expression than *pga*2. Interestingly, the expression of *atp4a* picked at 10-12 dph and then declined to reach high values again at the end of the studied period (Fig. 2). Data is shown as mean ± standard error of the mean.

Discussion and conclusions

Although a progressive increase in the expression of all the digestive enzymes occurred from first feeding, the maximum potential seems to be attained at 18 dph, age at which the growth notably increased.

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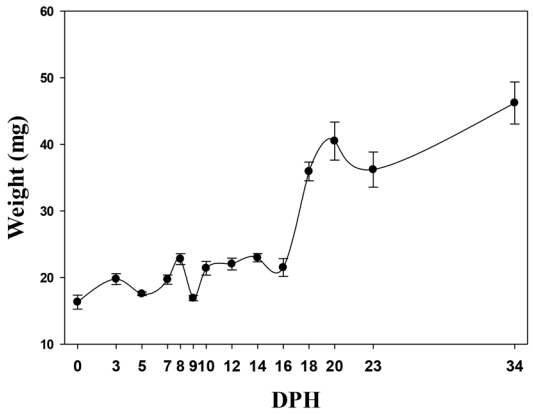

Fig. 1. Changes in the weight of Persian sturgeon larvae during development.

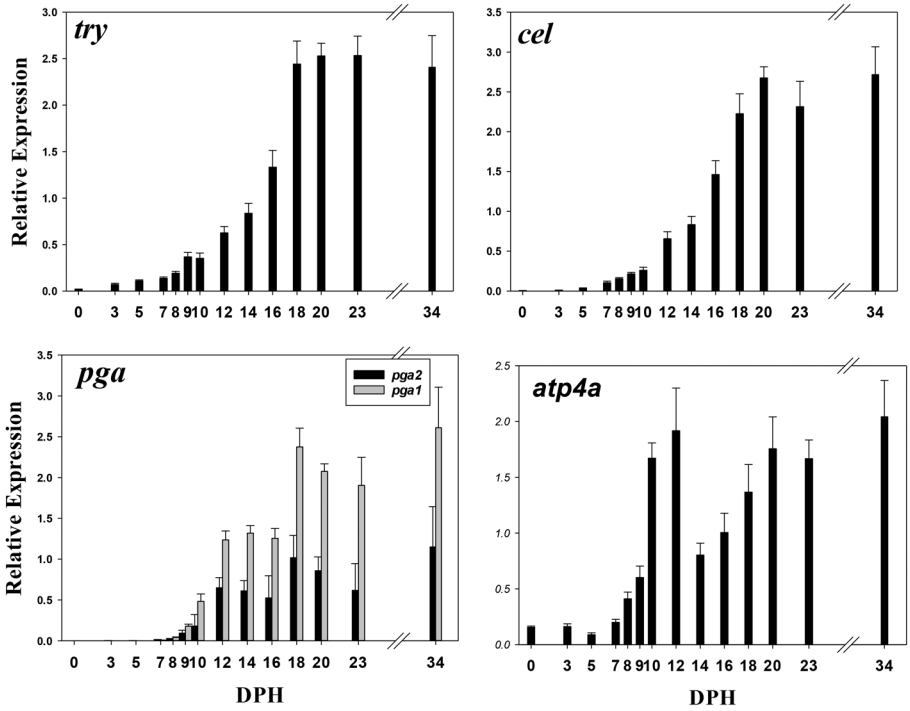

Fig. 2. Transcriptional changes of *try*, *cel*, *pga*1 and *pga*2, and *atp4a* of whole-body Persian sturgeon larvae during development.

References

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Acknowledgments

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