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Publication date:
2013

Document Version
Publisher's PDF, also known as Version of record

Biochemical, histological and molecular study of digestive tract development in European eel larvae (*Anguilla anguilla*) prior to exogenous feeding

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INTRODUCTION

European eel natural stocks have declined dramatically these last decades mainly due to intensive fisheries. In order to reduce the fishing pressure, a sustainable aquaculture industry needs to be developed and to be self-sustaining with fry.

Controlled reproduction and larval development of eel constitute two major bottlenecks for the aquaculture development of this species. In the PRO-EEL project, the capability to produce viable eggs and larvae of European eel, *Anguilla anguilla*, has increased. However, survival rate reduces to zero during the first feeding stage, due to difficulties to feed the larvae. Hence identifying development of their digestive system and adequate feed is a main focus.

OBJECTIVES

This study aimed at:

- examining the developmental pattern of the digestive tract in eel larvae
- comparing results to what is known for other marine fish species in order to define appropriate feeding strategies for *A. anguilla*

MATERIALS & METHODS

Experiments were performed at the DTU eel facility, DK, as part of PRO-EEL, using standard procedures [1,2].

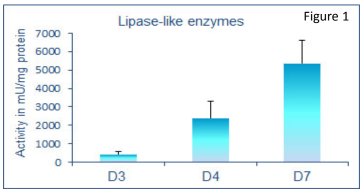
- Wild female broodstock and farmed male broodstock
- Gamete production through hormonal induction
- Incubation of embryos at 20°C in 36ppt seawater
- Prior to hatch (45 HPF) transfer to 20 l containers and
- addition of 40ppm penicillin and 65ppm streptomycin

Sampling: 100-150 larvae sampled from 3 to 14 DPH and preserved:

- -80°C (enzyme studies)
- RNAlater (gene expression studies)
- Glutaraldehyde/formalin fixative (for histology)

Specific primers were designed using data from EEL GENOME project: http://www.zfgenomics.org/sub/eel

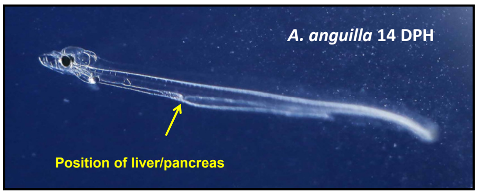
RESULTS & DISCUSSION

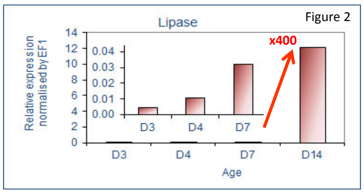


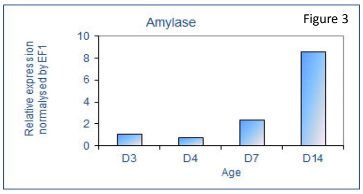
Enzymatic study 1

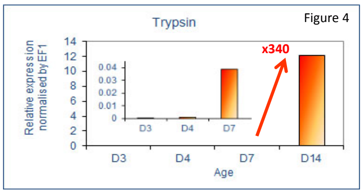
Very high activities in lipase-like enzymes (Fig. 1) were detected immediately after hatching, and the activities continued to increase in the developing larvae.

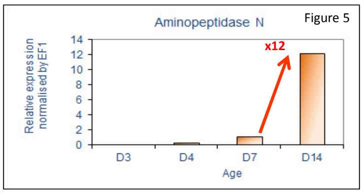
Such enzymatic pattern is hardly detected at early developmental stages in other marine fish species [3] suggesting a specific and important nutritional need in neutral lipids for eel larvae.











Enzymatic study 2

The activity of the studied enzymes paralleled the increase in expression of their corresponding coding gene (Fig. 2, 3, 4, 5 for lipase, amylase, trypsin and aminopeptidase N respectively). This result suggests that eel larvae have functional capabilities for digestive enzyme synthesis as early as the first days of their life. In addition, we have noted that the gene expression of all the enzymes considered in this study highly increased from day 7 to day 14, suggesting that this date is close to the settlement of the eel digestive functions, just after mouth opening and beginning of the exogenous feeding.

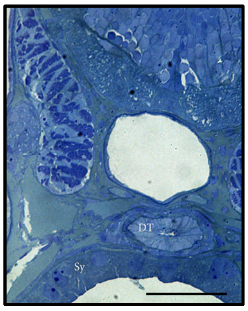


Figure 6. D6 – anterior digestive tract (DT) and yolk syncytium (Sy). Scale bar 50µm.

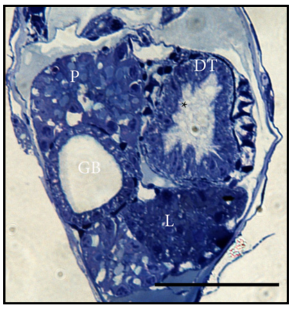


Figure 7. D12 – liver (L), pancreas (P), gall bladder (GB), anterior digestive tract (DT). Scale bar 50µm.

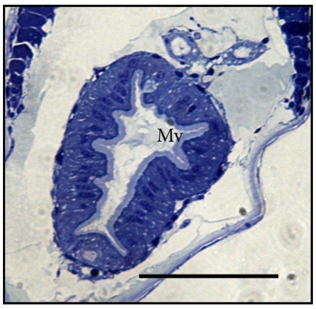


Figure 8. D12 – posterior digestive tract with microvilli (Mv). Scale bar 50µm.

Histological study

One day after hatching, the digestive tract was poorly developed and was only observed in the anterior part of the larvae, close to the yolk oil droplet. At D6 (Fig.6), the digestive tract (DT) appeared as a straight tube with a tight lumen. A solid yolk syncytium layer was present (Sy).

At D12 (Fig. 7 and 8), immature liver (L), pancreas (P) tissue, and the gall bladder (GB) were observed. Numerous cilia in the intestinal tract were observed anterior to the liver (*), and villi structures with a dense microvilli layer (Mv) was observed in the digestive tract posterior to the liver.

Scan to view video of active larvae in feeding stage

CONCLUSION

This study indicates that the developmental pattern of digestive enzymes in eel larvae differs from that of other marine fish larvae. In particular, it evidences an important enzymatic potential for lipid hydrolysis. Data suggest that eel larvae have an elevated nutritional requirement for lipids, an information that opens the way to develop new strategies for feeding eel larvae.

ACKNOWLEDGMENTS

This research was part of the PRO-EEL project: "Reproduction of European Eel: Towards a self-sustained Aquaculture (Grant no. 245257)" funded by the European Commission through the FP7 Programme. P. Lauesen, C. Graver and M. Krüger-Johnsen participated in the reproduction experiments providing larvae for the study.

References: [*1*] Ohta et al. (1997) *Fish Physiology and Biochemistry* ; [*2*] Tomkiewicz (2012) *DTU Aqua Report No 249 ;* [*3*] Zambonino Infante J.L. and C.L. Cahu. 2001. *Comp Biochem Physiol C* .