USING DIFFERENT NORMALIZATION STRATEGIES AND REFERENCE GENES WHEN EXAMINING THE EXPRESSION OF PEPT1 IN THE DEVELOPING DIGESTIVE TRACT OF LARVAL ATLANTIC COD (*Gadus morhua*).

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BACKGROUND

Housekeeping genes, or so called reference genes for Q-RT-PCR, may vary in their expression with the experimental conditions. When studying ontogeny of genes that are influenced by both diet and hormonal stimuli, it is important to evaluate the use of housekeeping genes for normalisation. The growth rate of the developing larvae also challenges the use of housekeeping genes as cells divide at a high rate. Our studies aim to describe the ontogeny of the digestive system in developing marine fish larvae. This information is vital in order to formulate proper feeds in aquaculture. Similar to most other marine fish larvae, Atlantic cod has a very simple digestive system at the onset of exogenous feeding and may therefore have a limited capacity to completely digest complex proteins into free amino acids (1). Absorption of peptides via the oligopeptide transporter (PepT1) might therefore be very important to utilise dietary proteins (1,2). We have shown for the first time that PepT1 mRNA was expressed in the gut of Atlantic cod larvae at hatching by *in situ* hybridization, but no expression was detected in the oesophagus nor the posterior part of the hindgut (3).

RESULTS

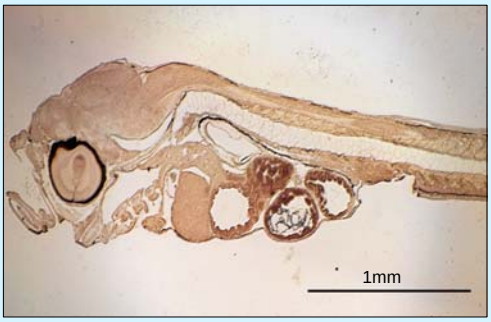

Fig 1 . Specific PepT1 mRNA staining shown in the digestive tract of Atlantic cod larvae.

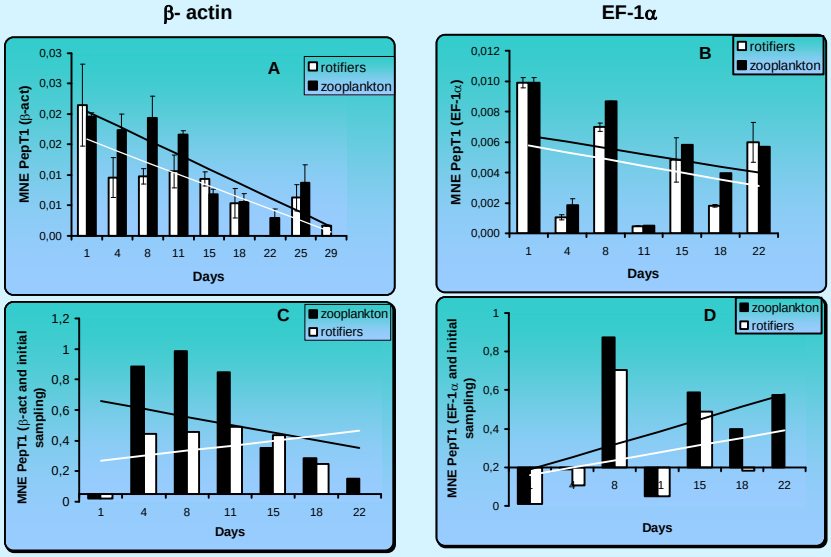

Fig. 2. Relative expression of A. cod PepT1 normalised to β- actin (Fig. 2A) or EF-1α (Fig. 2B) and to the given reference gene and to the initial sampling (Fig. 2 C and D.)

- Specific PepT1 mRNA staining in the midgut and hindgut in zooplankton fed Atlantic cod larvae was observed (Fig.1).
- There was a decreasing trend in relative expression of PepT1 in both zooplankton and rotifier fed larvae when normalised to either β- actin (Fig. 2A) or EF-1α (Fig. 2B).
- There was a decreasing trend in relative expression of PepT1 in zooplankton fed larvae, but an increasing trend was shown in rotifier fed larvae when normalised to β- actin and the initial sampling (Fig. 2C).
- There was an increasing trend in relative expression of PepT1 in both zooplankton fed larvae and rotifer fed larvae when normalised to EF-1α and the initial sampling (Fig. 2D).

DISCUSSION

• When using reference genes for Q-RT-PCR, one should evaluate several candidates. Based on calculations on this dataset performed using the *geNorm* VBA applet, EF-1 α was ranked as the most stable gene of those tested (results not shown). Together this suggest EF-1 α as a promising reference gene candidate in ontogeny studies of developing marine fish larvae. At present work is being done to further evaluate several promising gene candidates; ribosomal protein s9 and ubiquitin. Together these evaluated reference genes may be used to create a gene index. This constitutes the goal of our present studies, as this is believed to enable studies of several target genes which describe the ontogeny of the digestive system in developing marine fish larvae. However, the alternative of using absolute quantification may also be considered as several clones are available.

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