ARRESTED DEVELOPMENT: The Molecular and Endocrine Basis of Flatfish Metamorphosis (**ARRDE**, Q5RS-2002-01192)

Shared-cost research project from FP5
Quality of Life and Management of Living Resources
Key Action 5: Sustainable Agriculture, Fisheries and Forestry, and Integrated Development of Rural Areas Including Mountain Areas

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Objectives:

Flatfish species such as halibut, turbot and sole form a major focus of the diversification of European aquaculture industry. However, production has been severely hampered by biological problems in larval rearing. Despite juvenile quality being determined during larval metamorphosis, knowledge of flatfish metamorphosis is still rudimentary, especially in terms of **the molecular and endocrine basis**. As normal metamorphosis is a prerequisite for post-larval development, growth and survival of the fish, it is literally vital, in nature as in hatcheries, for this transition to proceed correctly.
The objective of this proposal is to seek the biological basis for arrested metamorphosis, and thus ways to alleviate it, in order to gain knowledge-based control over the resulting juvenile quality and production quantity, using the Atlantic halibut as a model species.
In order to reach the over-all project objective, the following goals need to be reached:

- Elucidating the morphological and molecular transformations that constitute metamorphosis.
- Elucidating the complex interaction between age, growth rate, size and metamorphic stage.
- Determining the endocrine mechanisms which regulate metamorphosis.
- Determining the underlying cause(s) of arrested development during metamorphosis.
- Recommending rearing practices that will alleviate the problem.

Workplan/workpackages:

WP1: Staging and Sampling
WP2: Differential gene expression during metamorphosis using microarrays
WP3: Biochemical and morphological markers for metamorphosis
WP4: Endocrine regulation of metamorphosis
WP5: Biochemical, molecular and endocrine indices of arrested metamorphosis
WP6: Experimental manipulation of metamorphosis

Molecular/genomic approach:

1. Candidate genes:

Numerous genes, thought to be of key interest for elucidating the regulation of the metamorphic process as well as give insights into the process itself were chosen for cloning and subsequent molecular studies. In certain instances, this has been complemented by immunohistohemistry studies of the proteins (see Table):

gene/protein	Application			
	IHC	PCR	ISH	Microarray
GHR 1				
GHR 2		√	√	
GHR trunc			√	
IGF1-R1				
IGF1-R2				
PRLR			√	
PRL	√	√	√	
GH	√	√	√	
SL	√	√		
Transthyretin	√	√		
TRαA		√	√	√
TRαB		√	√	√
TRβA		√	√	√
TBβB		√	√	√
GR	√			
Deiodinases (types I-III)		√	√	√
Digestive enzymes (trypsin, amylase etc)		√	√	√
Muscle markers (myosin, troponin etc)		√	√	√
Globins		√	√	√
Immunoglobulins			√	
Epidermal keratins		√	√	√

2. Microarray analysis:

A targeted microarray has been constructed for use in assessing global changes in gene expression during metamorphosis. This consists of approximately 1,500 clones spotted in triplicate. Five hundred of the clones used were randomly selected from a larval cDNA library, with the remainder being obtained from subtractive hybridisations designed to enrich for genes that are either up-regulated or down-regulated at metamorphosis. Approximately 1,000 of the selected clones have been subject to single-pass sequencing, allowing for the identification of several hundred previously undescribed halibut genes. Screening of the microarray is now underway to identify changes in gene expression during normal and abnormal metamorphosis and between untreated and hormone treated larvae.

Comprehensive project data analysis is currently been carried out to be presented in the ARRDE FINAL REPORT