

# ARRESTED DEVELOPMENT: The Molecular and Endocrine Basis of Flatfish Metamorphosis

## ARRDE

Shared-cost research project from FP5 (Q5RS-2002-01192)  
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

Total cost: 1.357.111 €; EC contribution: 1.277.610 €  
Commencement: 2002-10-01; completion: 2006-03-31

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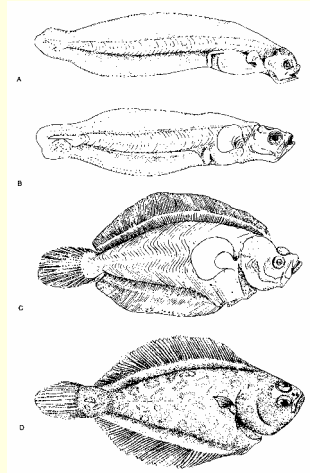
EU scientific officer: Mario Lopes dos Santos



## Halibut life history: pelagic to benthic



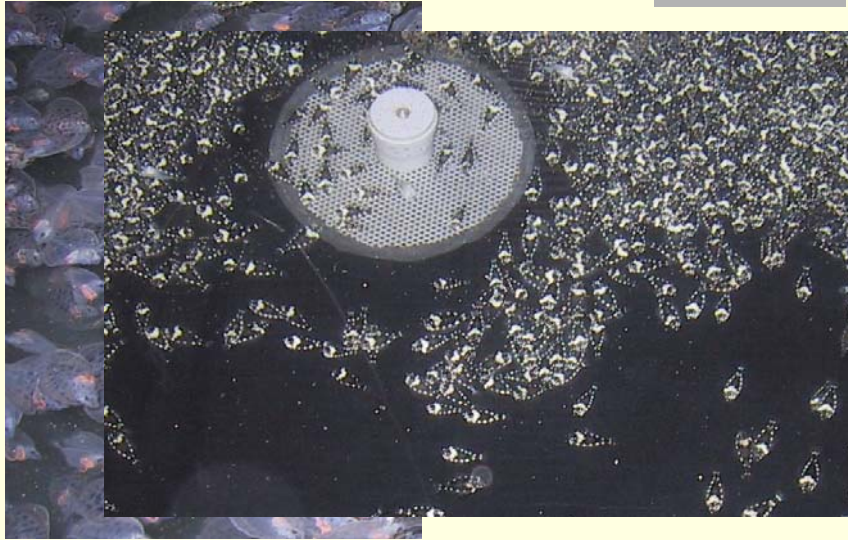
## Metamorphosis: a key life-history transition and a production bottleneck



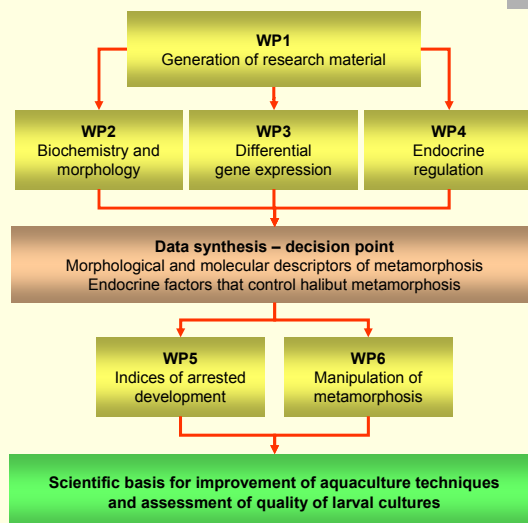
## Broodstock stripping



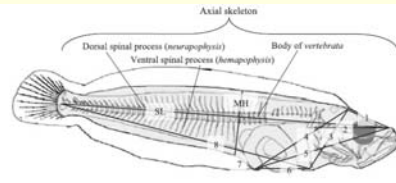
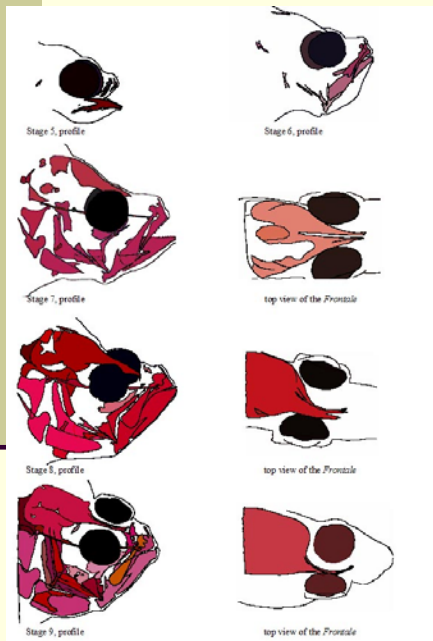
## Metamorphosis: key life-history transition and production bottleneck



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## Staging scheme



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Aquaculture 239 (2004) 445–465

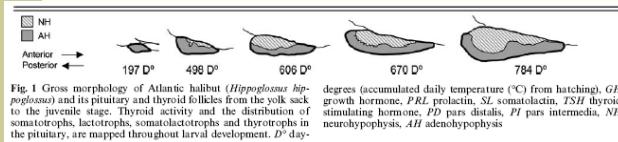
Aquaculture

[www.elsevier.com/locate/aqua-online](http://www.elsevier.com/locate/aqua-online)

Staging of Atlantic halibut (*Hippoglossus hippoglossus* L.) from first feeding through metamorphosis, including cranial ossification independent of eye migration

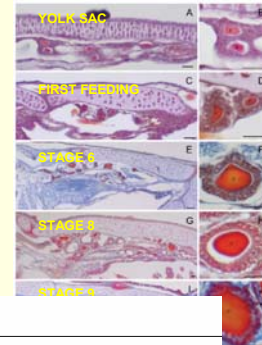
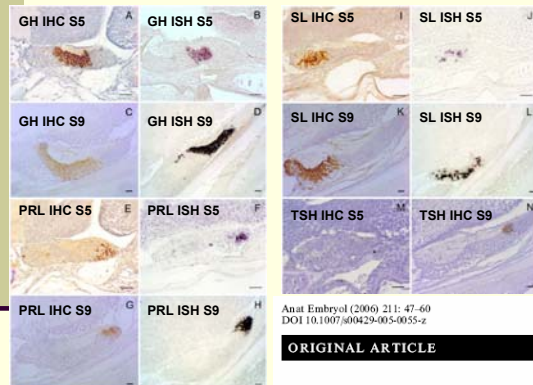
Ø. Sæle<sup>a,\*</sup>, J.S. Solbakken<sup>b</sup>, K. Watanabe<sup>a</sup>, K. Hamre<sup>c</sup>,  
D. Power<sup>d</sup>, K. Pittman<sup>a</sup>

## Pituitary development



## Endocrine development

### Thyroid development



Anat Embryol (2006) 211: 47–60  
DOI 10.1007/s00429-005-0055-z  
ORIGINAL ARTICLE

Ingbjörg Eir Einarsdóttir · Nadra Silva  
Deborah M. Power · Heiddis Smáradóttir  
Björn Thrandur Björnsson

Thyroid and pituitary gland development from hatching through metamorphosis of a teleost flatfish, the Atlantic halibut

## Molecular approaches

- Protein isolation; RIA
- Candidate gene cloning; PCR and ISH
- Microarray establishment

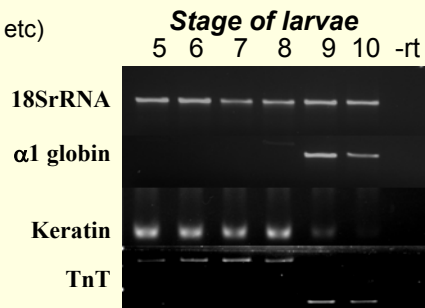
## Candidate gene cloning

gene/protein	Application			
	IHC	PCR	ISH	Microarray
GHR 1				
GHR 2		√	√	
GHR trunc			√	
IGF1-R1				
IGF1-R2				
PRLR			√	
PRL	√	√	√	
GH	√	√	√	
SL	√	√		
Transthyretin	√	√		
TR $\alpha$ A		√	√	√
TR $\alpha$ B		√	√	√
TR $\beta$ A		√	√	√
TB $\beta$ B		√	√	√
GR	√			
Deiodinases (types I-III)		√	√	√
Digestive enzymes (trypsin, amylase etc)		√	√	√
Muscle markers (myosin, troponin etc)		√	√	√
Globins		√	√	√
Immunoglobulins			√	
Epidermal keratins		√	√	√

## Markers of metamorphosis

- Skin (keratins)
- The haematopoietic system (globins)
- The GI tract (amylases, proteases etc)
- Muscle (myosins, troponins etc)
- The immune system (IgM)

The molecular studies have been supported by morphological analyses of skin, blood etc

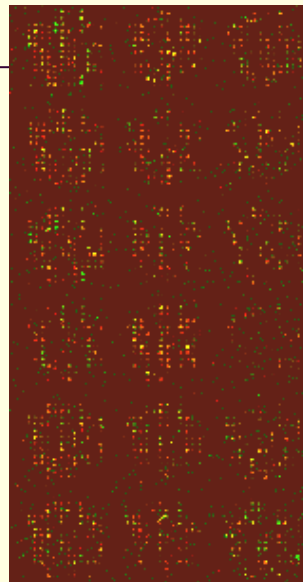


**Stage 9 appears to involve dramatic changes in marker expression**

## Microarray analysis

A microarray has been constructed consisting of:

- 288 clones from each of 4 subtractive libraries (1152 in total).
- 480 clones selected randomly from a larval cDNA library.
- A few candidate genes (TR, deiodinases, PitX2 etc).
- Approx. 1,000 of the arrayed clones have been subjected to single-pass sequencing.
- Screening is underway to identify changes in gene expression during normal metamorphosis, differences between normal and abnormal larvae and changes in gene expression induced by hormone treatment.





## ARRDE milestones

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- Staging scheme to characterise progression and quantify success of metamorphosis
- Generation of microarray of genes induced or repressed during halibut metamorphosis
- Morphological, endocrine and molecular description of metamorphosis in halibut
- Integration of data to determine the underlying molecular, biochemical and hormonal changes which explain metamorphic arrest

STRANGE BREW / John Deering



Thank you!

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