Ontogeny of the digestive and vision system of wreckfish

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Larval rearing success is the main bottleneck for the industrial-scale farming of any species.

The development of the digestive and visual systems is essential for the survival of larvae and is directly related to the employed rearing protocol.
The digestive system and the visual system constitute two of the most important systems for the survival of fish.

- With the digestive system, the organism is provided with the necessary nutrients that allow it to survive and subsequently to grow and to reproduce.

Feeding protocols
(qualitative – quantitative composition)

- With the vision system (eye) fish detect food for capture.

Light conditions
The study aimed at:

a) the ontogeny of digestive system and eye,
b) the identification of critical phases during larval rearing (malnutrition periods),
c) the estimation of feeding preferences in accordance to the feeding protocol,
d) the visual ability in the different developmental stages.

The aim of this study was the extraction of the related information, in order to develop - optimize the larval rearing protocol of wreckfish.
Materials & methods

Sampling - Methodology

Parameters

- Growth performance
- Ontogeny of digestive system and eye
- Feeding preferences
- Estimation of malnutrition periods
- Estimation of visual acuity (visual distance)

Methodology

- Fixed larvae (from MC2)
- Stomach contents of the larvae
- Lipid deposition in the liver and the length of intestinal villi
- Diameter of lens, number of cone cells in the retina

Histology

Histology, image analysis
Results

Digestive system ontogeny

Buccopharynx  Esophagus  Stomach  Intestine  Accessory glands

Mouth opening  Pharyngeal teeth  Taste buds  Longitudinal folds  Goblet cells  Goblet cells  Goblet cells  Pyloric caeca  Ileo-rectal valve  Intestinal folds  Liver  Pancreas

Cardiac and pyloric sphincter  Gastric glands
Results – ontogeny of digestive system

Days post hatching (dph)

1
5
10
15
23

Buccopharynx
Esophagus
Stomach
Intestine
Accessory glands

Mouth opening
Pharyngeal teeth
Taste buds
Longitudinal folds
Goblet cells
Cardiac and pyloric sphincter
Gastric glands
Goblet cells
Pyloric caeca
Ileo-rectal valve
Intestinal folds
Liver
Pancreas

N = not identified
Results – ontogeny of digestive system

Days post hatching (dph)

Total length (mm)

Buccopharynx
Esophagus
Stomach
Intestine
Accessory glands

Mouth opening
Pharyngeal teeth
Taste buds
Goblet cells
Cardiac and pyloric sphincter
Gastric glands
Goblet cells
Pyloric caeca
Ileo-rectal valve
Intestinal folds
Liver
Pancreas
The development of the digestive system of wreckfish is a slow process (temperature related). The ontogenetic status until 8 dph (5.8 mm) shows that the larvae were able to consume food items but they didn't.
Results – Liver lipids and stomach contents

BC = Blood capillaries  L = Liver

10 dph  16 dph  23 dph

Liver

Digestive canal

Area of the digestive canal
Results – Liver lipids and stomach contents

BC = Blood capillaries  L = Liver

Area of the digestive canal
1. There were no lipids identified in the liver tissue during the rearing period (malnutrition period).

2. There were no food items identified in the stomach contents.

3. The diet of the larvae was exclusively autotrophic, based on the nutrients that were in the yolk sack.
The size of intestinal villi is a histological index that is related with the nutritional status of the fish. (Hall and Bellwood 1995; McLeese and Moon 1989)
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- Wreckfish larvae were affected by a malnutrition - starvation period (16-23 dph) that resulted in the atrophy of the intestinal structures, which appeared as a reduction of villi size.

- The autotrophic condition that existed during this period was not adequate to cover the nutritional requirements of wreckfish larvae.
The presence of the large yolk sac and the large oil droplet, indicates the presence of a long autotrophic larval stage.

The large oil droplet increase the buoyancy of the wreckfish larvae.
Day 1
Day 2
Results – edema (*)

Day 4

Pericardial area
Day 8

Results – edema (*)

Pericardial area

Yolk sac – abdominal cavity

*
Results – edema (*)

Day 12
Results – edema (*)

Day 16
Day 19

Results – edema (*)
Day 23

Results – edema (*)

- Pericardial area
- On the larval body
- Yolk sac
Day 23

- On the larval body
- Yolk sac
- Pericardial area

Results – edema (*)
The edema has been connected with disruptions of cardiac function during the process of heart morphogenesis.

The circulation during the early larval stages plays a role in osmoregulation, yolk metabolism, and other developmental processes dependent on hydrostatic pressure.
The edema has been connected with disruptions of cardiac function during the process of heart morphogenesis. The circulation during the early larval stages plays a role in osmoregulation, yolk metabolism, and other developmental processes dependent on hydrostatic pressure. The accumulation of edema is related with:

- General anatomy of yolk-sac
- The species (marine vs freshwater or vs pelagic vs demersal)
- Toxic factors
Deepwater Horizon crude oil impacts the developing hearts of large predatory pelagic fish


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Toxic factors
The effects of heavy metals on embryonic development of fish (a review)

Barbara Jezierska · Katarzyna Ługowska · Małgorzata Witeska

Cardiac edema
Toxicity Assessments of Near-infrared Upconversion Luminescent LaF$_3$:Yb,Er in Early Development of Zebrafish Embryos

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Results – edema (bibliography)
Advanced Morphological — Behavioral Test Platform Reveals Neurodevelopmental Defects in Embryonic Zebrafish Exposed to Comprehensive Suite of Halogenated and Organophosphate Flame Retardants

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FIG 2. Morphological deformities observed in zebrafish larvae exposed to mFTP flame retardant at 0, 0.00064, 0.0064, 0.064, 0.64, and 6.4 μM for 5 days. Deformities denoted as: Ax, axial; S, snout; J, jaw; PE, pericardial edema; YSE, yolk sac edema.
Full length article

Toxic effects of polybrominated diphenyl ethers (BDE 47 and 99) and localization of BDE-99–induced cyp1a mRNA in zebrafish larvae

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Toxic factors
Characterisation of ‘swollen yolk-sac syndrome’ in the Australian freshwater fish Murray cod, Maccullochella peeli peeli, and associated nutritional implications for large scale aquaculture

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➢ Nutritional factors
➢ Poor nutrition of the broodstock
The edema affects the physiology (feeding) of wreckfish larvae and maybe is one of the main factors that is connected with wreckfish larval mortalities.

It can be related to:

a) Nutritional factors (broodstock nutrition)
b) Water quality (toxic factors in the water)
c) Diseases (under investigation).
d) General management of rearing factors, biotic and abiotic, during the larval rearing period (under investigation).
Eye-Vision Measurements

Retina

Number of:
- Cone cells
- Rod cells

Lens diameter
Results - Eye ontogeny

- Differentiation of retina layers started at 3 dph
- At 6 dph:
  a) Cone cells (photopic vision – day vision)
  b) Retina organization was completed
  c) The lens transparency increased.

- The fish were able to identify the food items in photic rearing environments.

- Rod cells didn’t appear during the first 23 dph.
  (scotopic vision – night or under low light intensities vision).

- The fish weren't able to identify the food items in rearing environments with low light intensities.
Visual acuity is defined as the minimum angle, at which two parallel objects can project at the eye and still be resolved as separate.

Histological visual acuity was expressed as the **Minimum Separable Angle (MSA) = \( \alpha \)**, which was calculated as: 

\[ \text{(a)} \]
Results - Histological visual acuity

Visual acuity (visual distance) increases as the total length of fish increases (the lens size in the eye also increases)
Results (Visual acuity – Visual distance)

Estimation of the Visual Distance

\[ VD = 0.5 \times h / \tan \left( \frac{a}{2} \right) \]

Visual Distance (VD)

\[ h = \text{object width (mm)} \]
At mouth opening, the histological visual acuity for wreckfish was estimated around $1.2^0$ (degrees). The distance or the radius of the visual field was calculated around $7$ mm for the identification of an object size of $0.15$ mm (rotifer). This corresponds to a volume of sphere of around $1.4$ ml. So the minimum concentration of rotifers has to be at least 1 rotifer per $1.4$ ml.
From the ontogenetical point of view the digestive system and the vision system of wreckfish larvae were developed to such an extent that larvae were able to identify, capture and assimilate zooplanktonic organisms.
Digestive system (feeding protocol)

- The presence of the large yolk sac and the large oil droplet, indicates the presence of a long autotrophic larval stage.

- The larvae are able to consume food from the time that the mouth opens, although no exogenous feeding items were identified in the stomach contents.

- The coexistence of autotrophic and heterotrophic stage for a long period during the first larval rearing period has to be taken in consideration for the development of new enrichment protocols for zooplanktonic preys that are used during the first feeding period.
From a hydrodynamic point of view, the big size of larvae, the large yolk sac and the large oil droplet increase the buoyancy of the wreckfish larvae.

The high buoyancy may negatively affect the movements and the swimming speed of the larvae for capturing food. For this reason:
1) **Density of preys**
2) **Types of preys**
   have to be tested.

Future studies have to be focused on the rearing conditions (temperature!!!!) during larval rearing stages of wreckfish.
Visual system (light conditions)

- The light conditions of the rearing environment have to be harmonized with the visual abilities of wreckfish larvae.

- The wreckfish larvae presented only photopic ability of vision. The lights above the rearing tanks have to be switched on when the pigmentation of the eye is completed (one day after mouth opening).

- The high visual capacity of wreckfish larvae indicates that they are able to identify food items very quickly and from far away. (High variability of prey size selection)

- The presence of phytoplankton will offer the larvae the ability to select the photic environment that they prefer to inhabit in the tank.
Thank you

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