Ontogeny of the digestive and vision system of wreckfish

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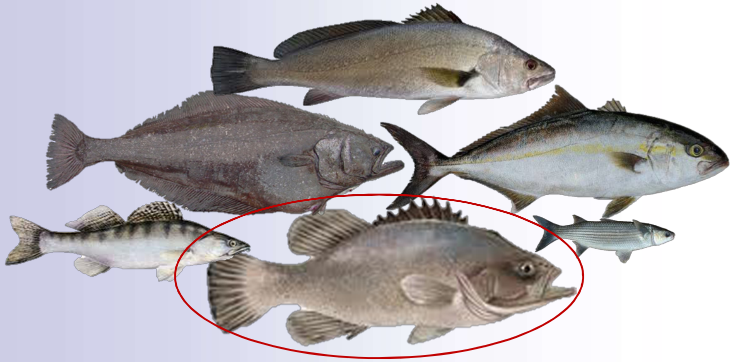


Ioannis Papadakis (HCMR)

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

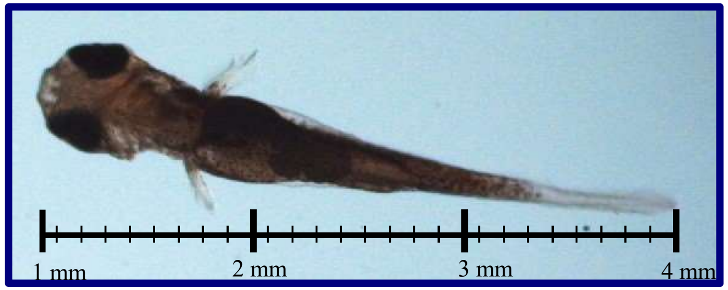
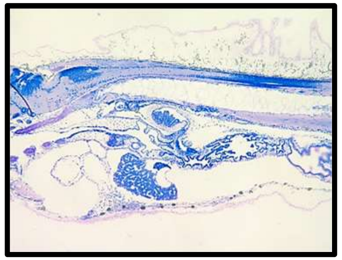
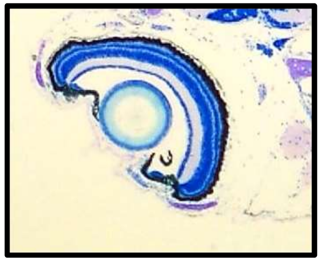
The aim of the study

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.

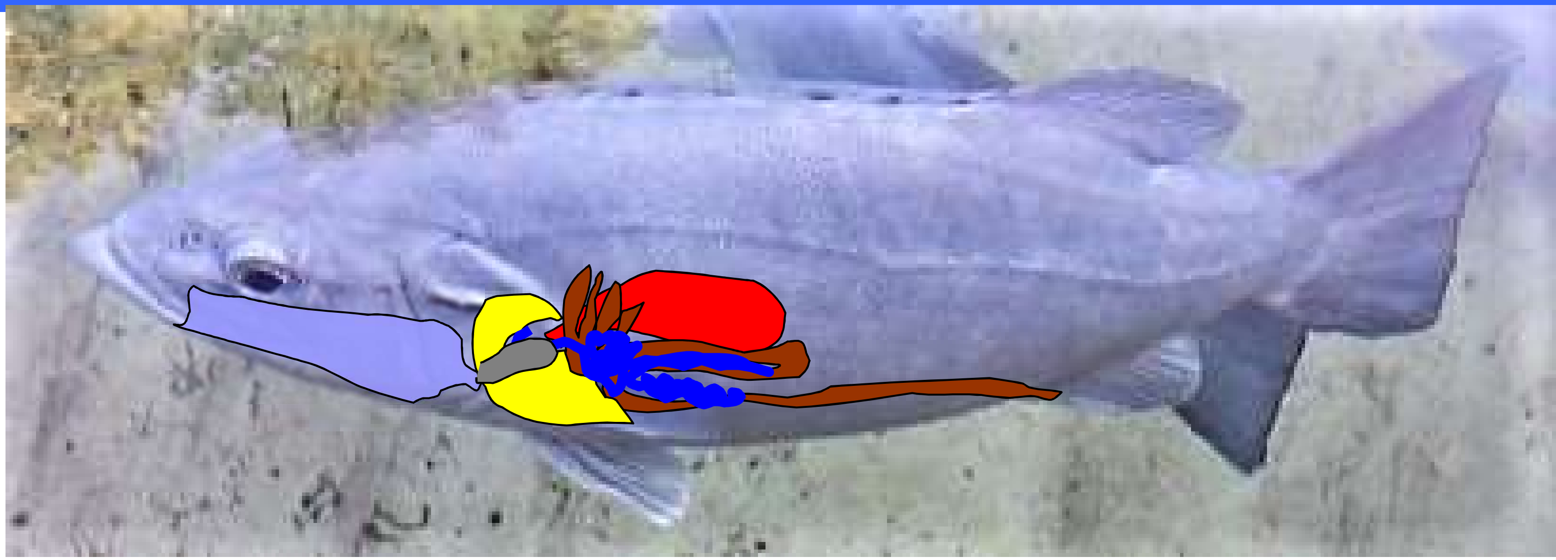
The aim of this study was the extraction of the related information, in order to develop - optimize the larval rearing protocol of wreckfish.

Materials & methods
Sampling - Methodology

Parameters	Methodology	
Growth performance		Fixed larvae (from MC2)
Ontogeny of digestive system and eye	 	Histology
Feeding preferences	Stomach contents of the larvae	Histology
Estimation of malnutrition periods	Lipid deposition in the liver and the length of intestinal villi	Histology, image analysis
Estimation of visual acuity (visual distance)	Diameter of lens, number of cone cells in the retina	Histology, image analysis

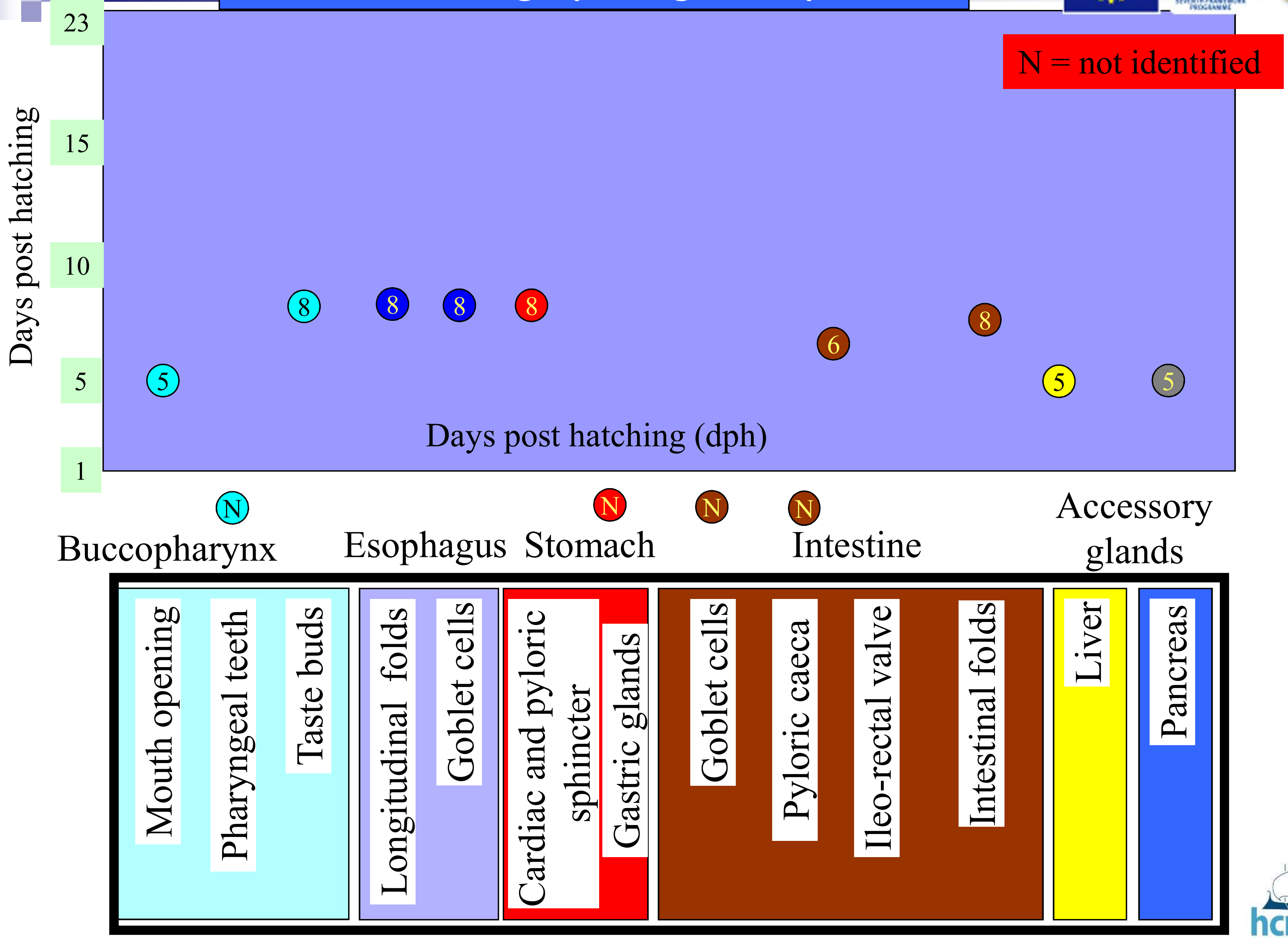
Results

Digestive system ontogeny

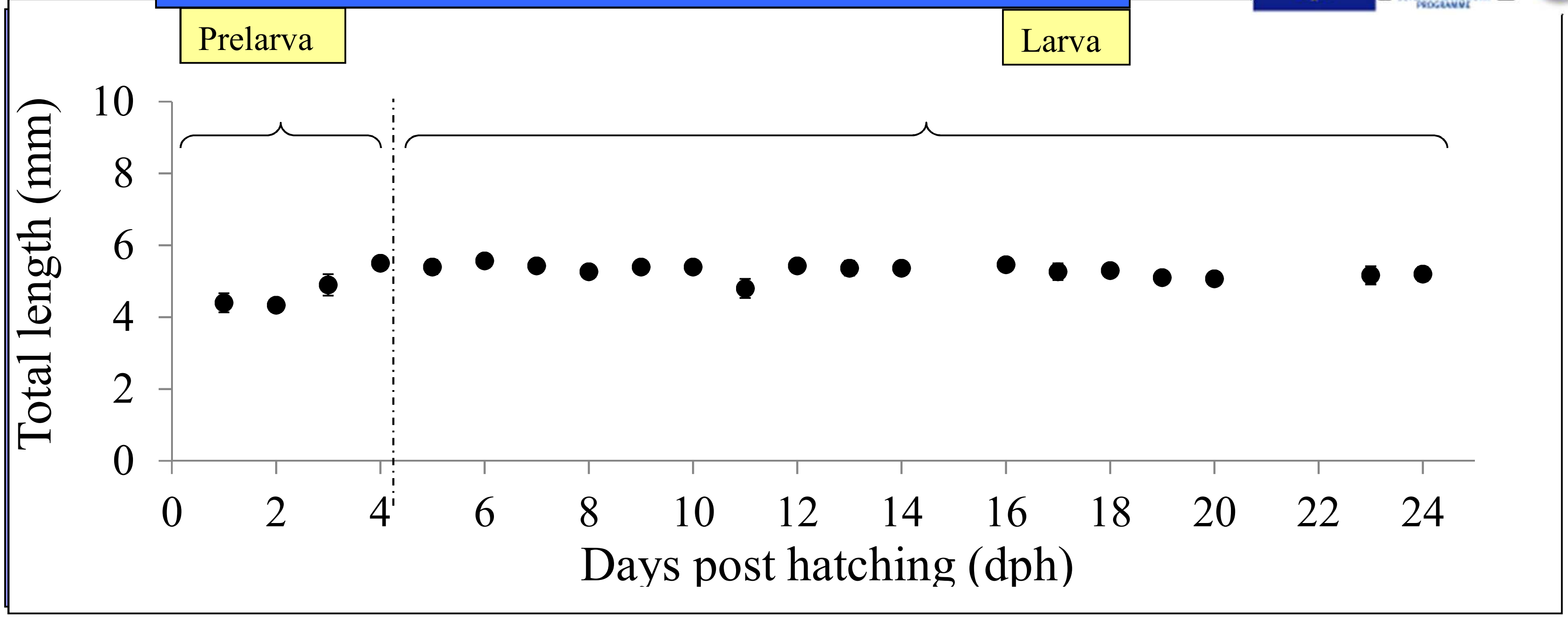


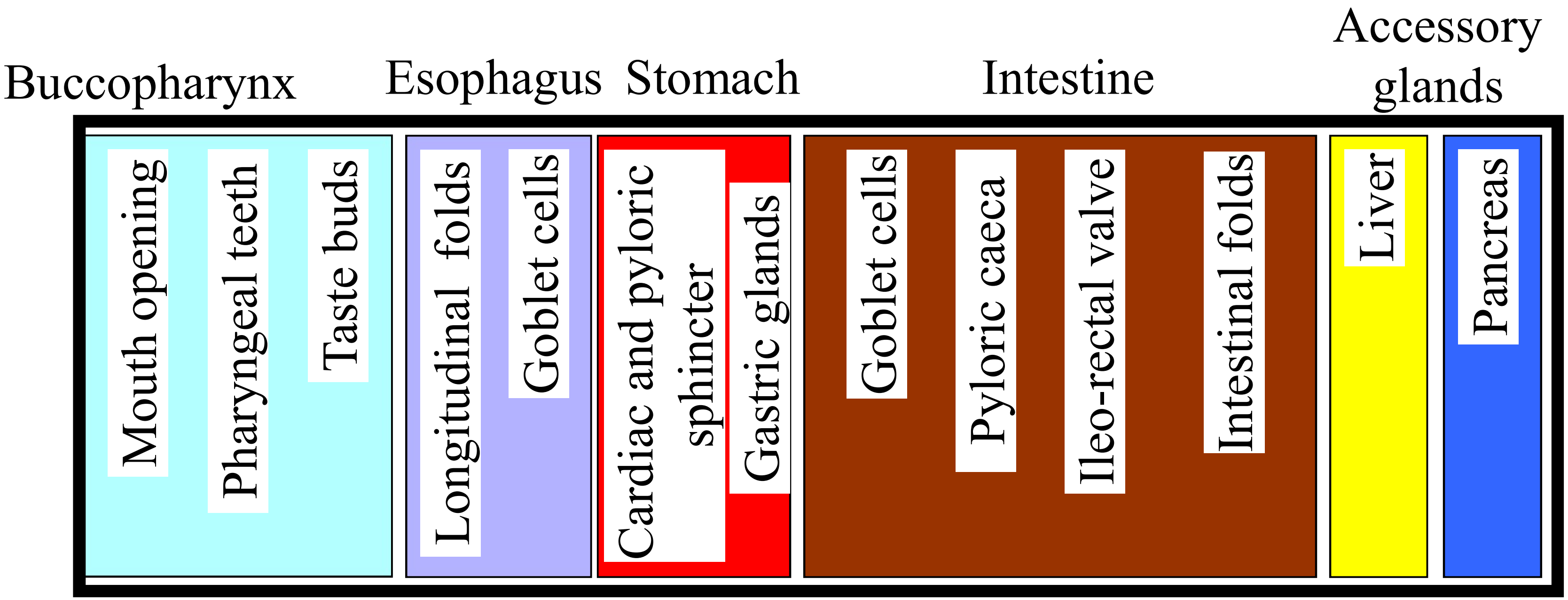
Buccopharynx	Esophagus	Stomach	Intestine	Accessory glands
Mouth opening	Longitudinal folds	Cardiac and pyloric sphincter	Goblet cells	Liver
Pharyngeal teeth	Goblet cells	Gastric glands	Pyloric caeca	Pancreas
Taste buds			Ileo-rectal valve	
			Intestinal folds	

Results – ontogeny of digestive system



Results – ontogeny of digestive system

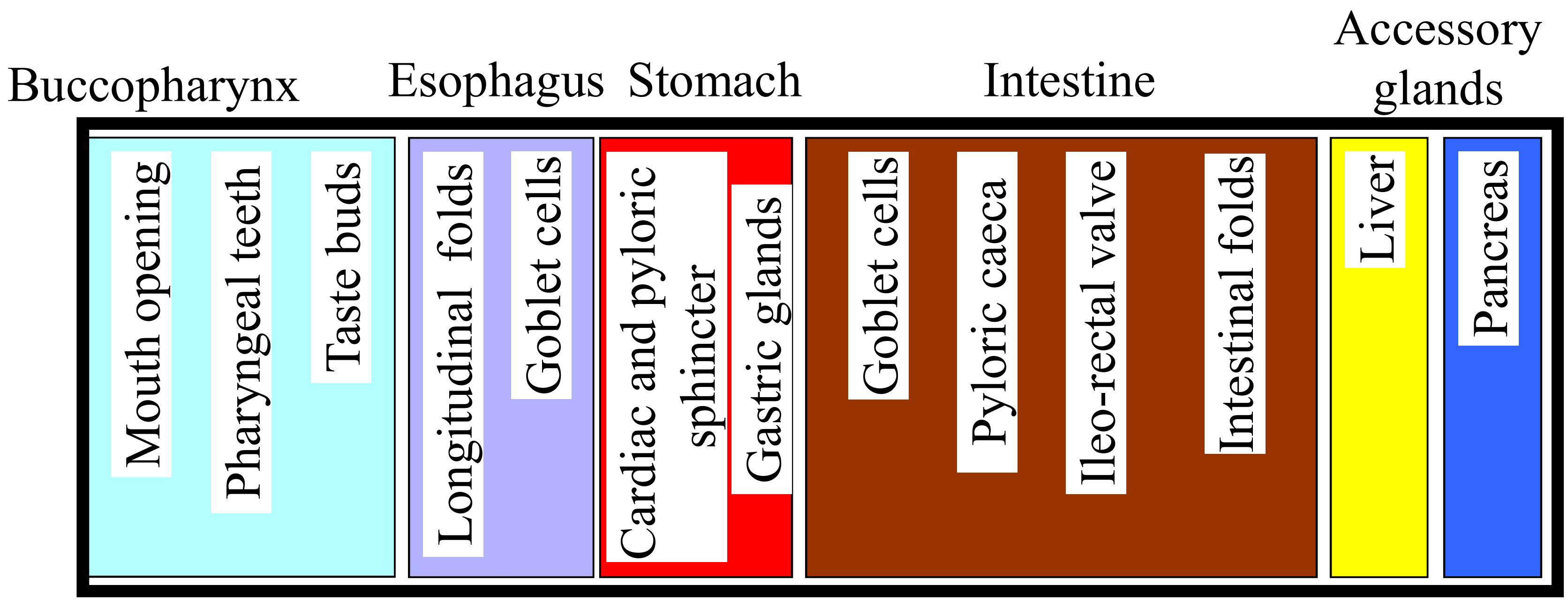




Results – ontogeny of digestive system

Days post hatching

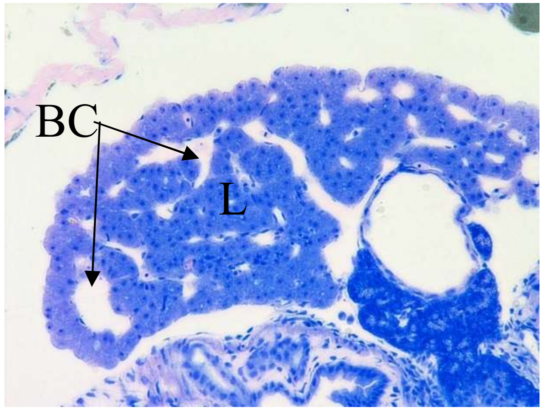
The development of the digestive system of wreckfish is a slow process (temperature related).
The ontogenetic status until 8 dph (5.8 mm) shows that the larvae were able to consume food items but they didn't.



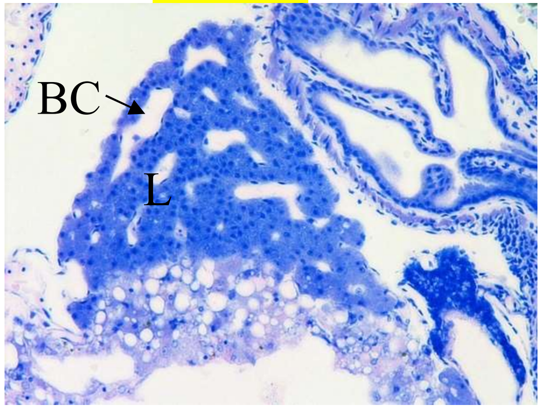
Results – Liver lipids and stomach contents

BC = Blood capillaries L = Liver

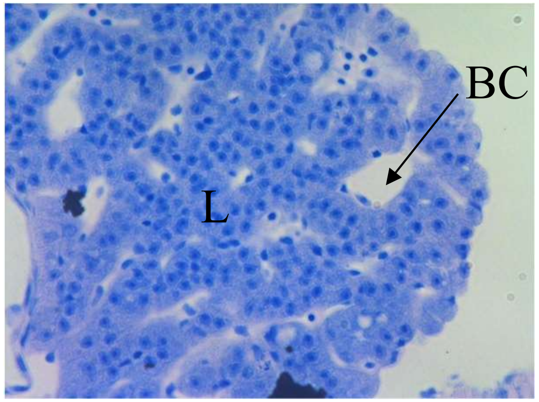
Liver



10 dph

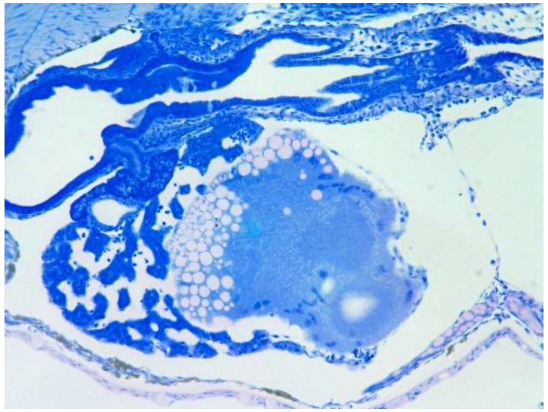


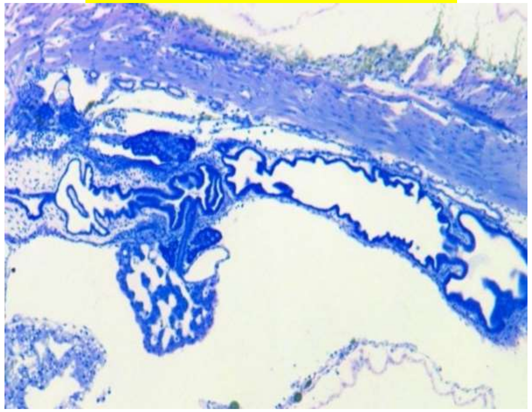
16 dph

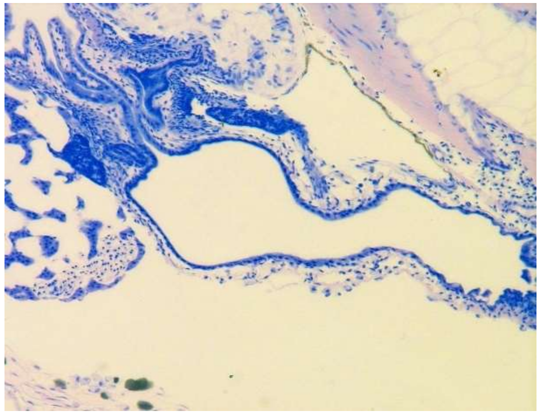


23 dph

Digestive canal



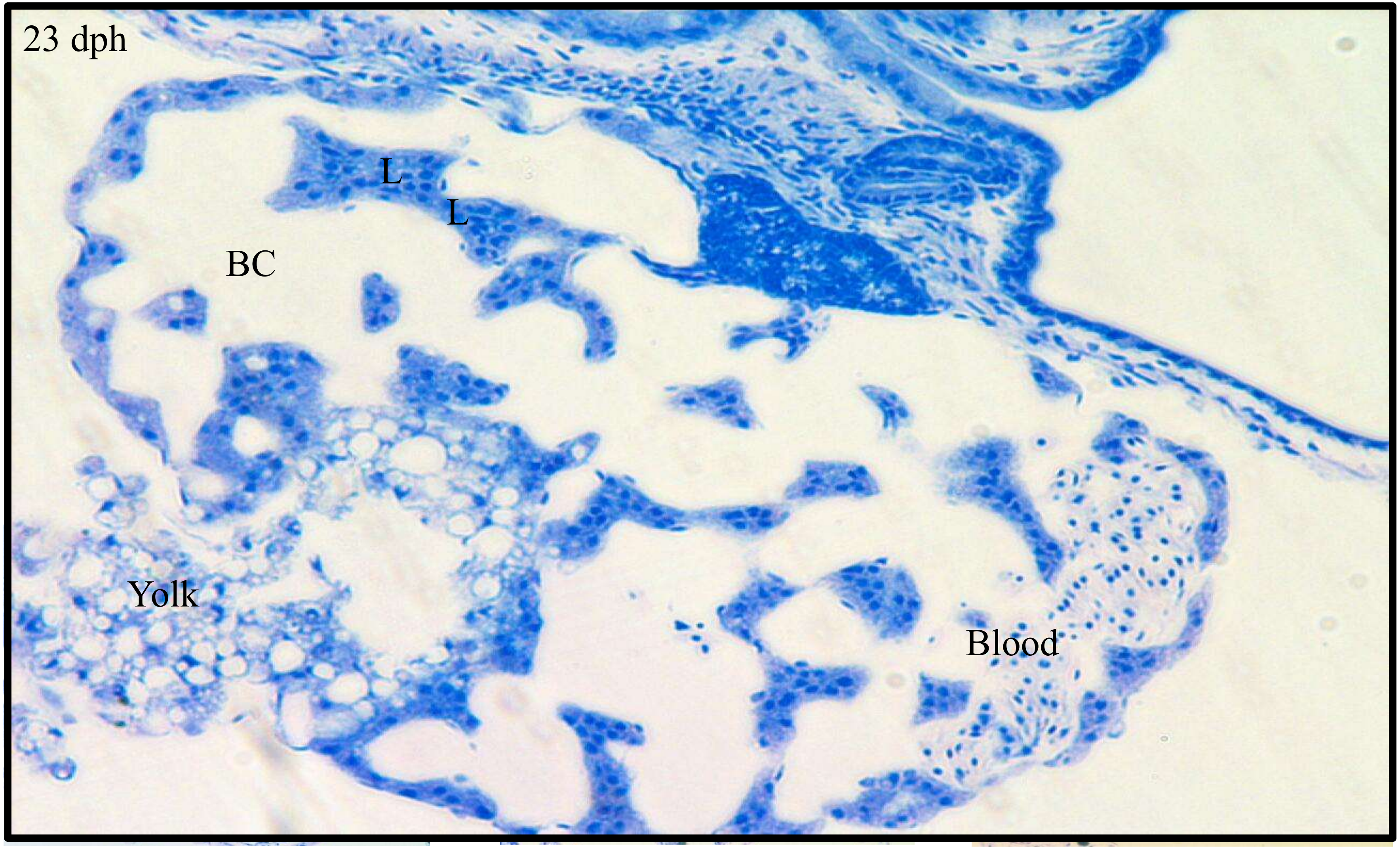




Area of the digestive canal

Results – Liver lipids and stomach contents

BC = Blood capillaries L = Liver



Area of the digestive canal

Results – Liver lipids and stomach contents

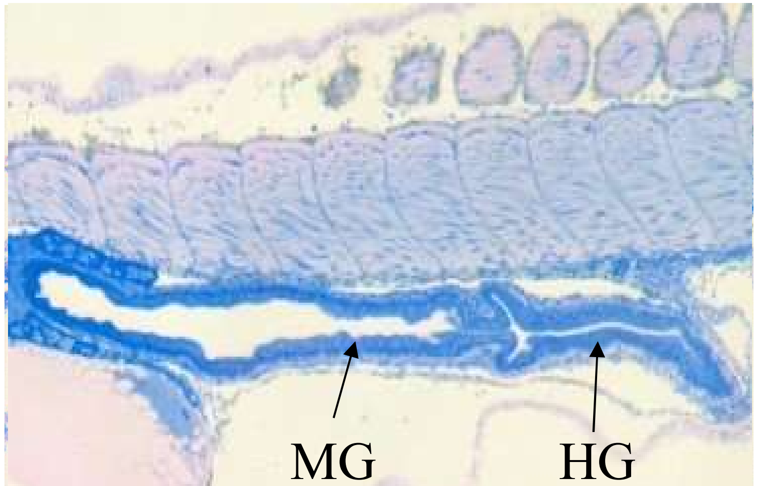
1. There were no lipids identified in the liver tissue during the rearing period (malnutrition period).

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

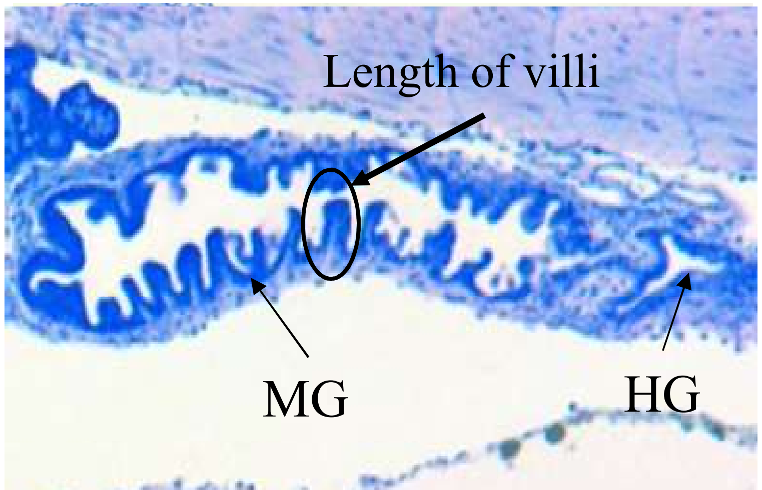
3. The diet of the larvae was exclusively autotrophic, based on the nutrients that were in the yolk sack.

Results – Length of intestinal villi

7 dph

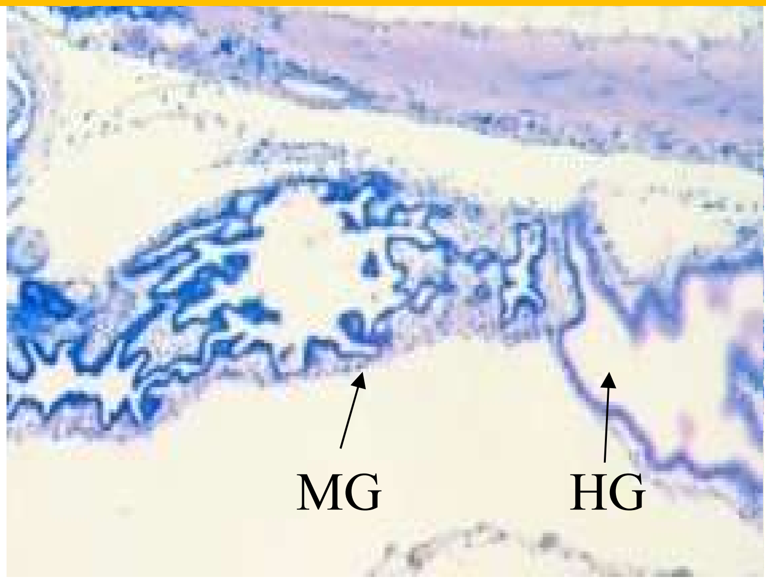


11 dph

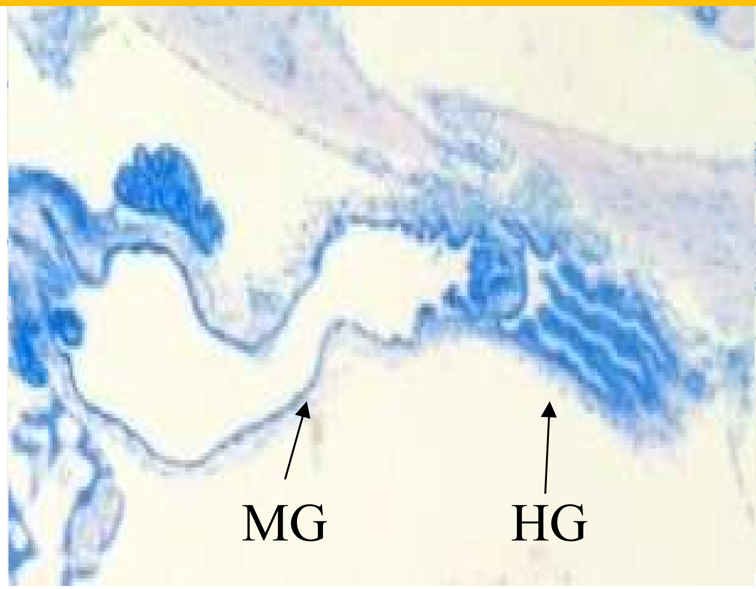


The size of intestinal villi is a histological index that is related with the nutritional status of the fish. (Hall and Bellwood 1995; McLeese and Moon 1989)

16 dph



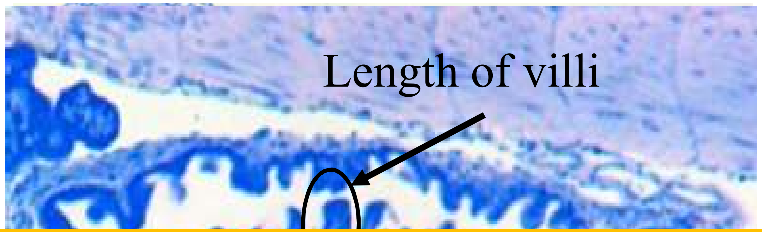
23 dph



Results – Length of intestinal villi

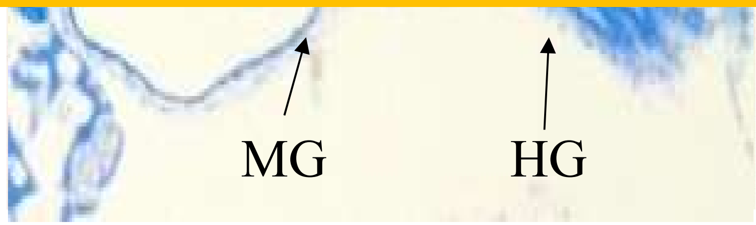
7 dph

11 dph



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)

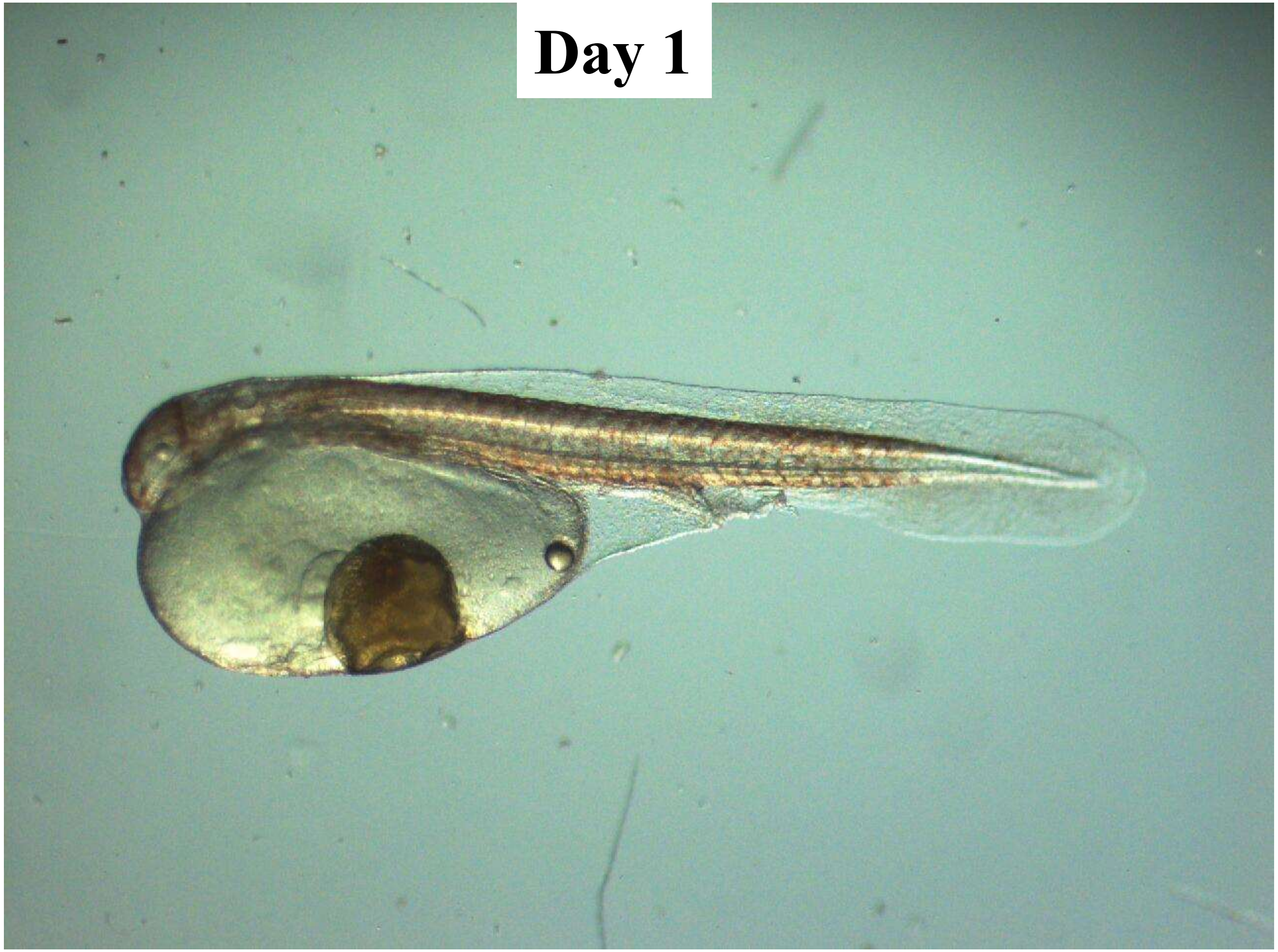
- **Wreckfish larvae were affected by a malnutrition - starvation period (16-23 dph) that resulted in the atrophy of the intestinal structures, which appeared as a reduction of villi size.**
- **The autotrophic condition that existed during this period was not adequate to cover the nutritional requirements of wreckfish larvae.**



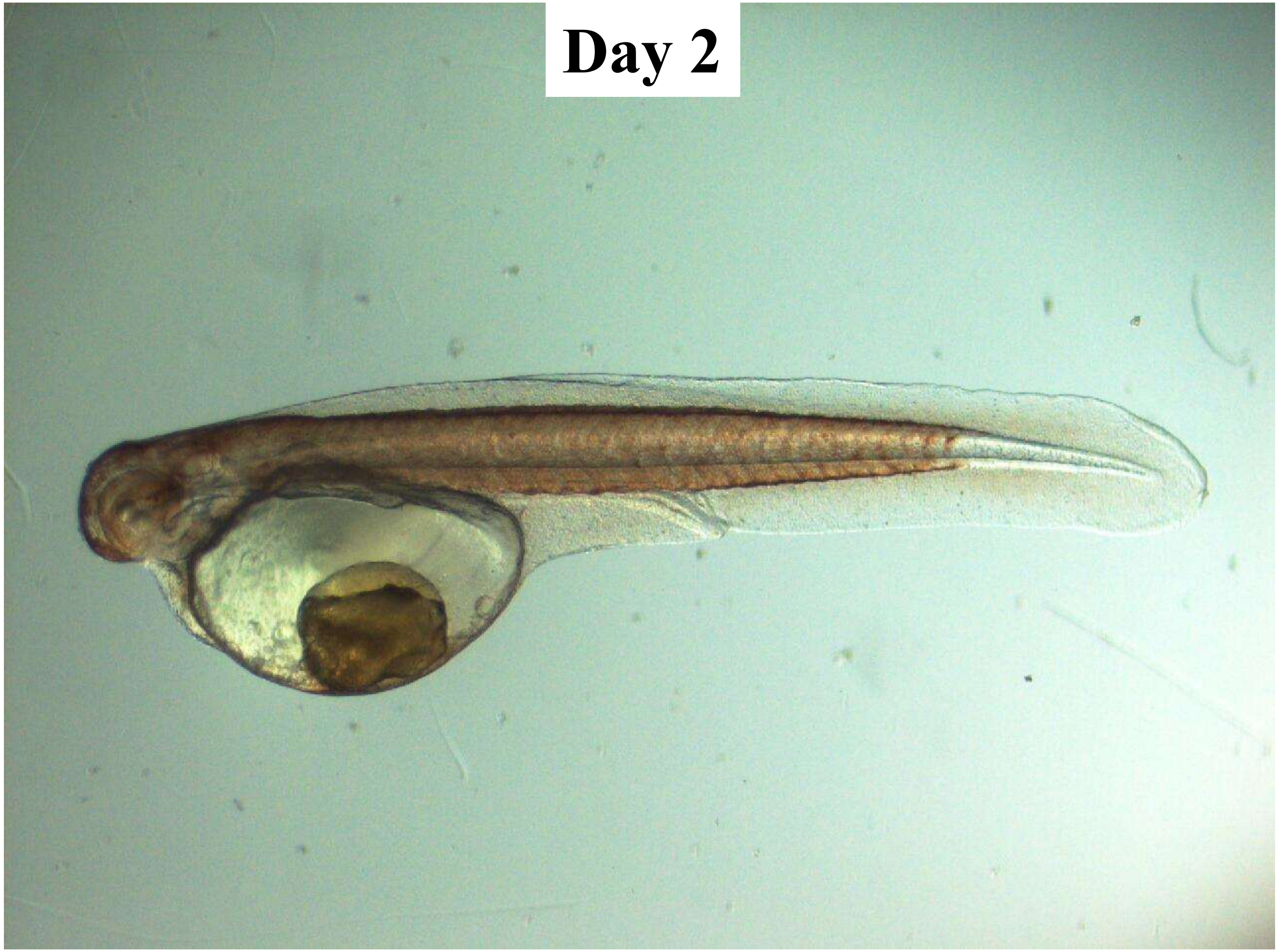
Results

- 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.

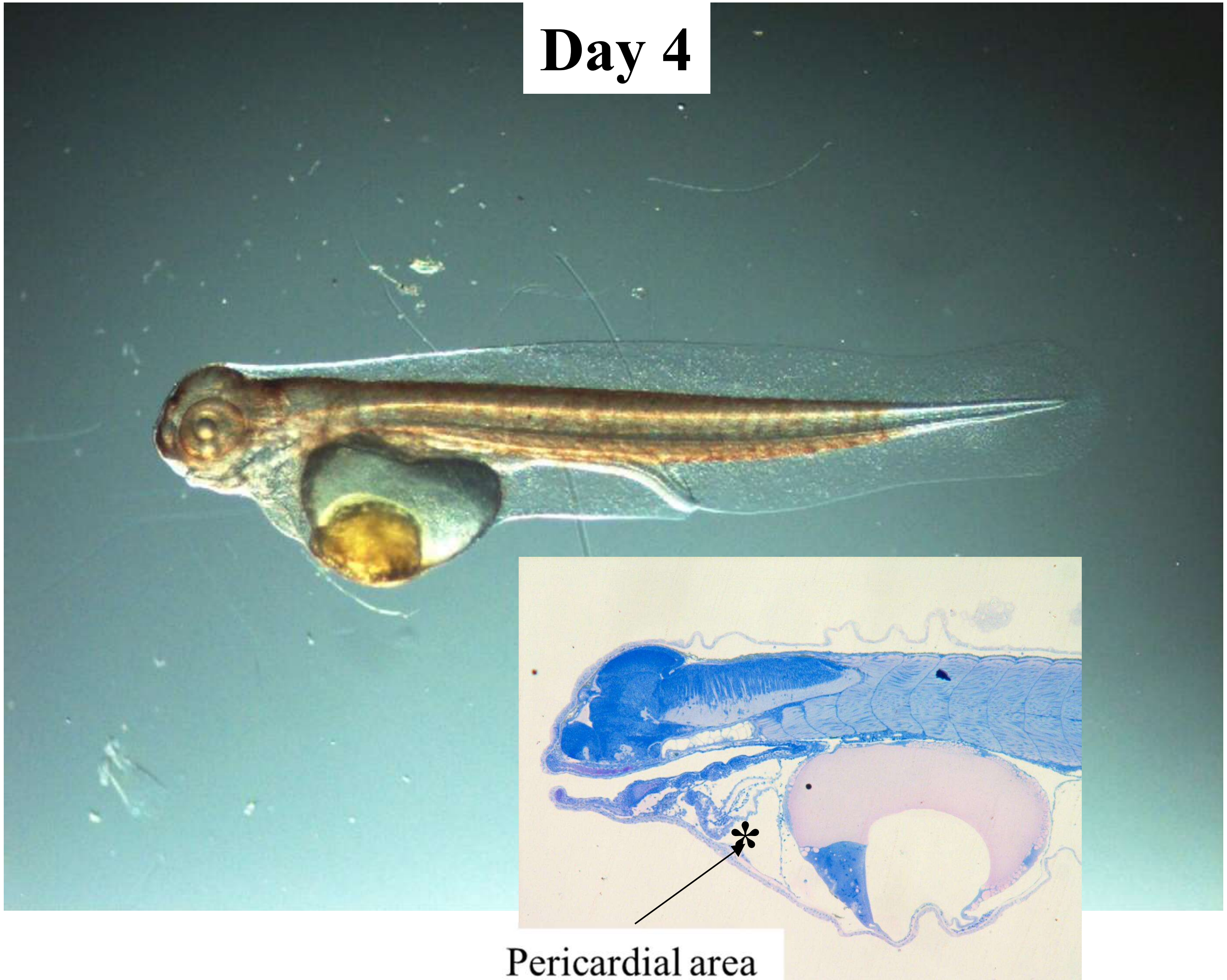
Results

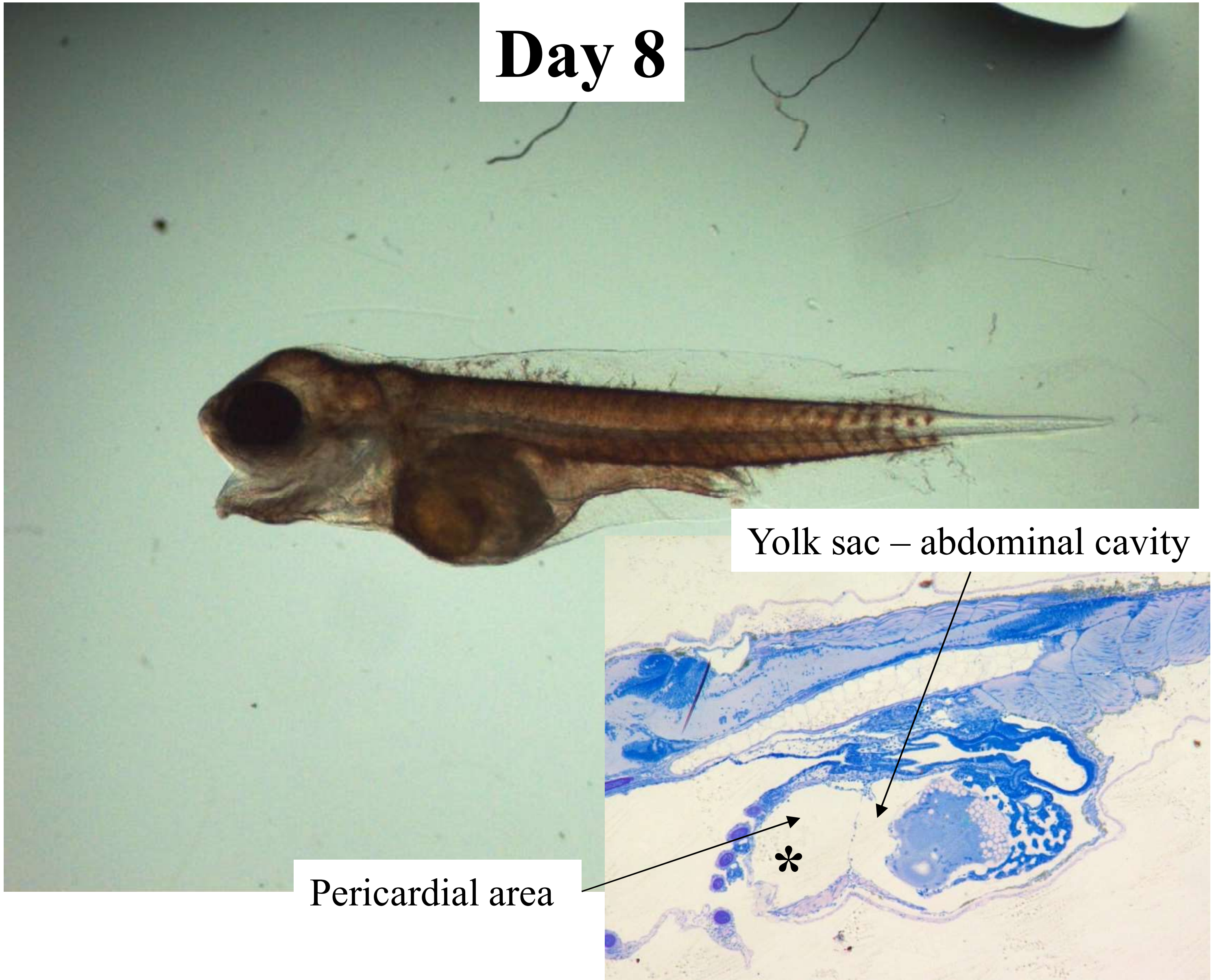


Results



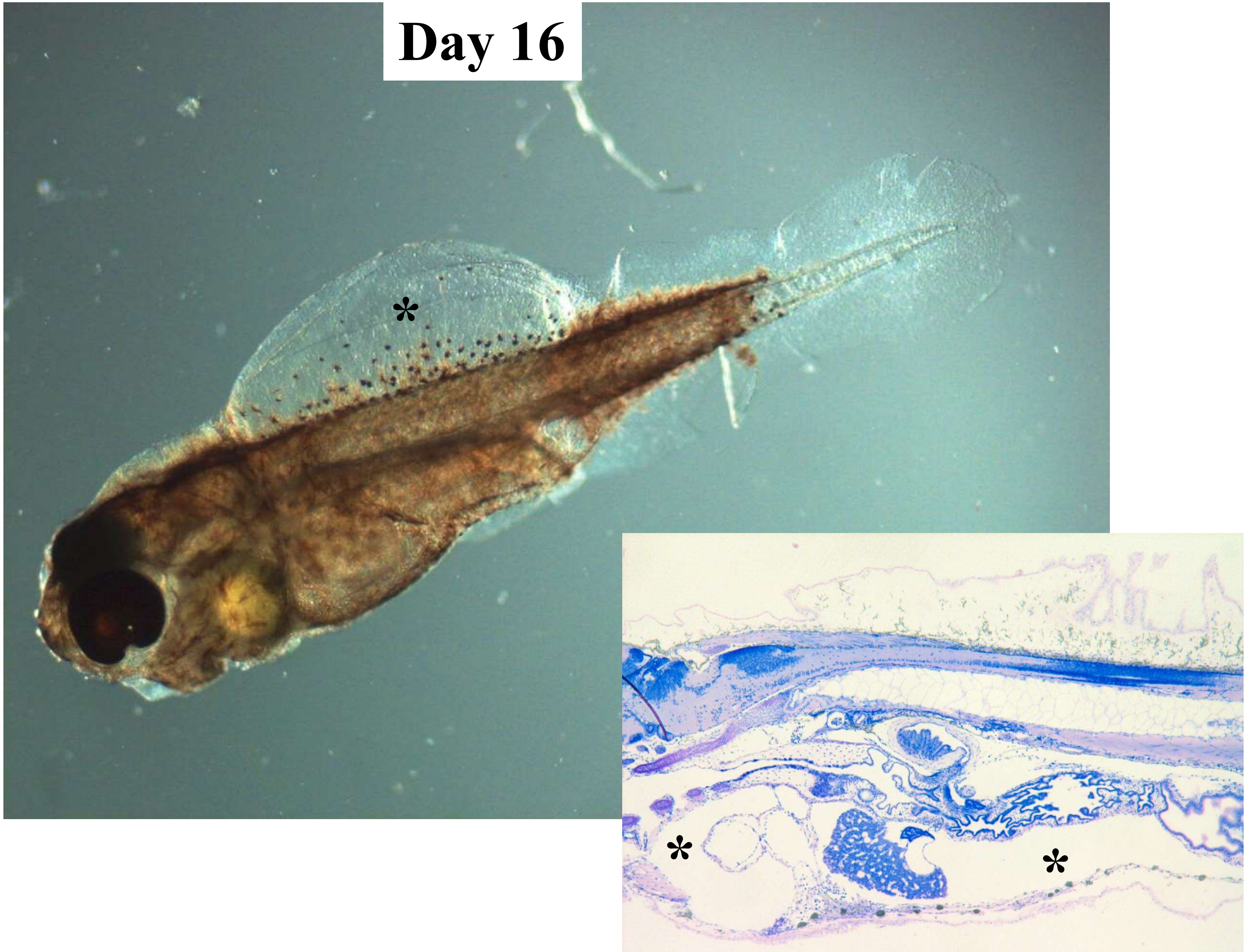
Results – edema (*)




Day 8
Yolk sac – abdominal cavity
*
Pericardial area

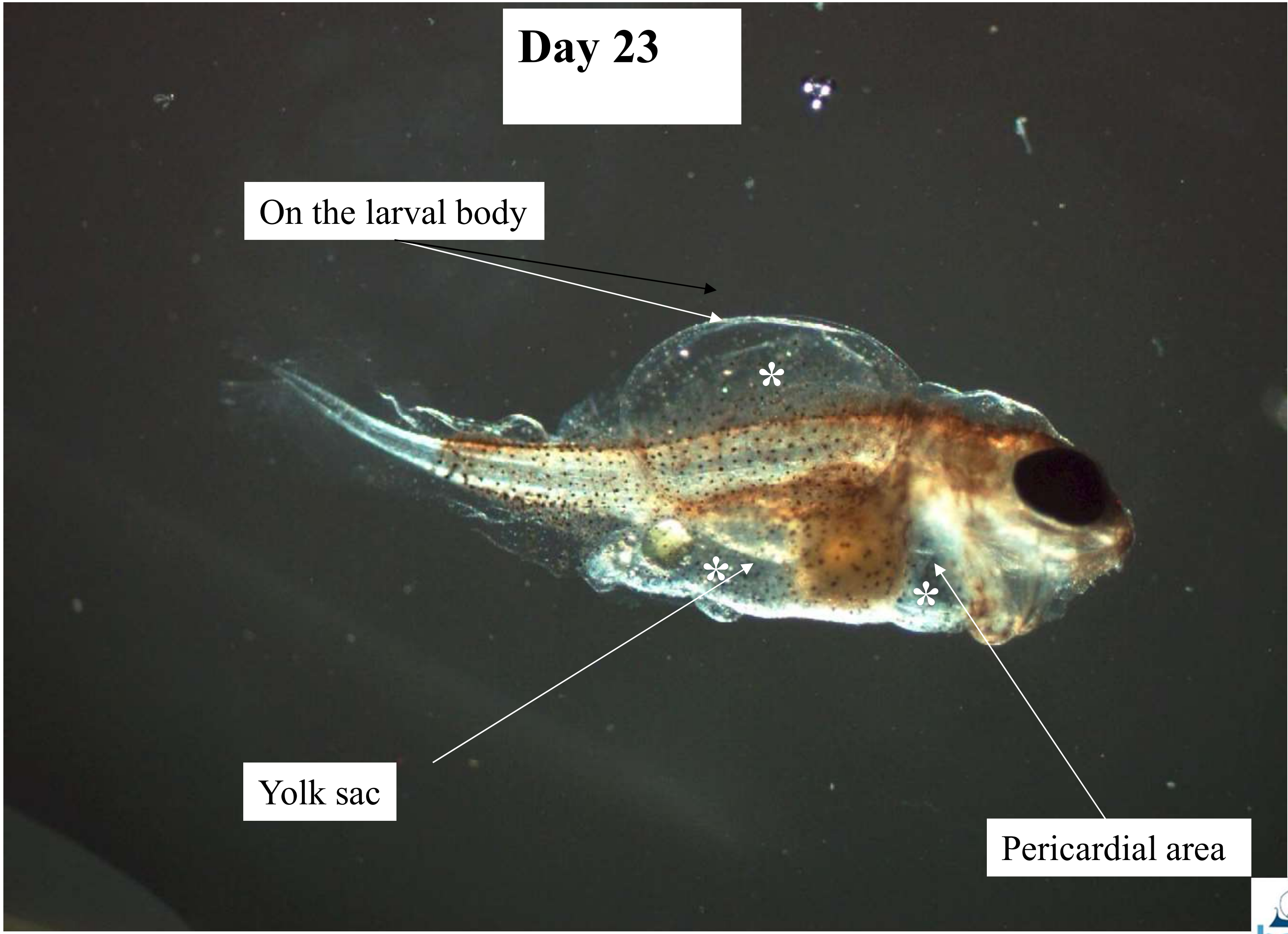
Results – edema (*)

Results – edema (*)



Results – edema (*)

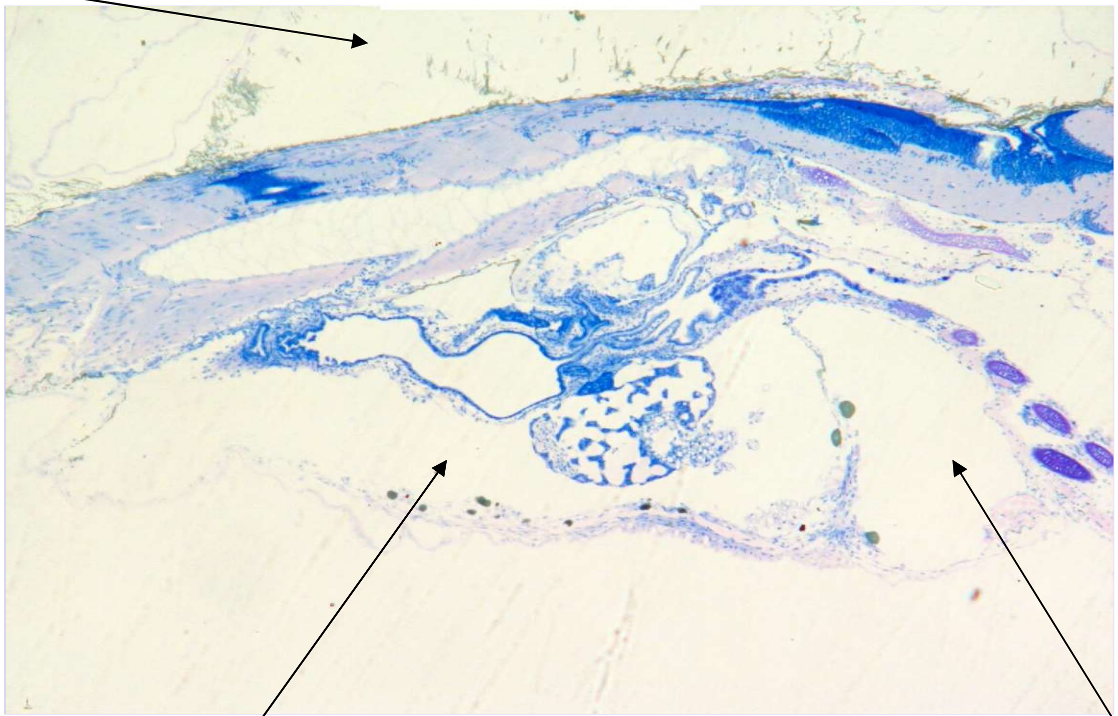
Results – edema (*)



Results – edema (*)

Day 23

On the larval body



Yolk sac

Pericardial area

Results – edema (bibliography)

Aquatic Toxicology 177 (2016) 515–525

Contents lists available at ScienceDirect

Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

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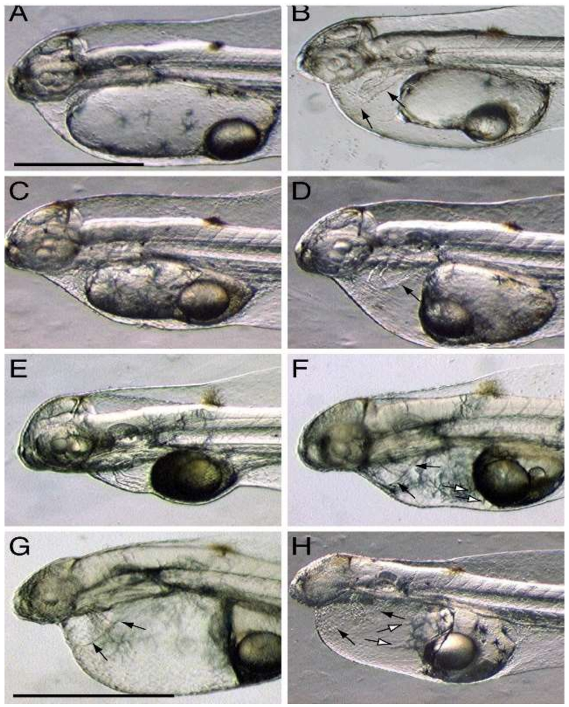
AQUATIC TOXICOLOGY

The influence of heart developmental anatomy on cardiotoxicity-based adverse outcome pathways in fish

CrossMark

John P. Incardona*, Nathaniel L. Scholz

Environmental and Fisheries Sciences Division, Northwest Fisheries Science Center, National Marine Fisheries Service, NOAA Fisheries, 2725 Montlake Blvd. E., Seattle, WA 98112 USA



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.

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Results – edema (bibliography)

Aquatic Toxicology 177 (2016) 515–525

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Aquatic Toxicology

journal homepage: www.elsevier.com/locate/aquatox

ELSEVIER

The influence of heart developmental anatomy on cardiotoxicity-based adverse outcome pathways in fish

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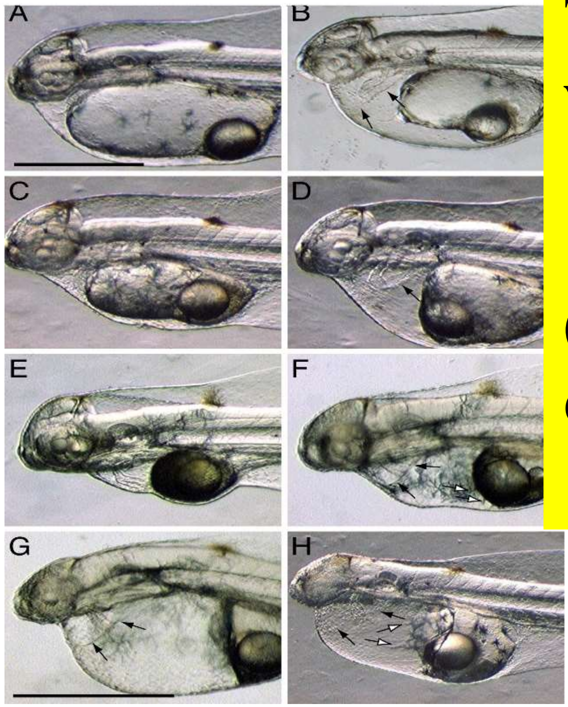
Environmental and Fisheries Sciences Division, Northwest Fisheries Science Center, National Marine Fisheries Service, NOAA Fisheries, 2725 Montlake Blvd. E., Seattle, WA 98112 USA



The accumulation of edema is related with:

- **General anatomy of yolk-sac**
- **The species**

(marine vs freshwater or/vs pelagic vs demersal)

- **Toxic factors**

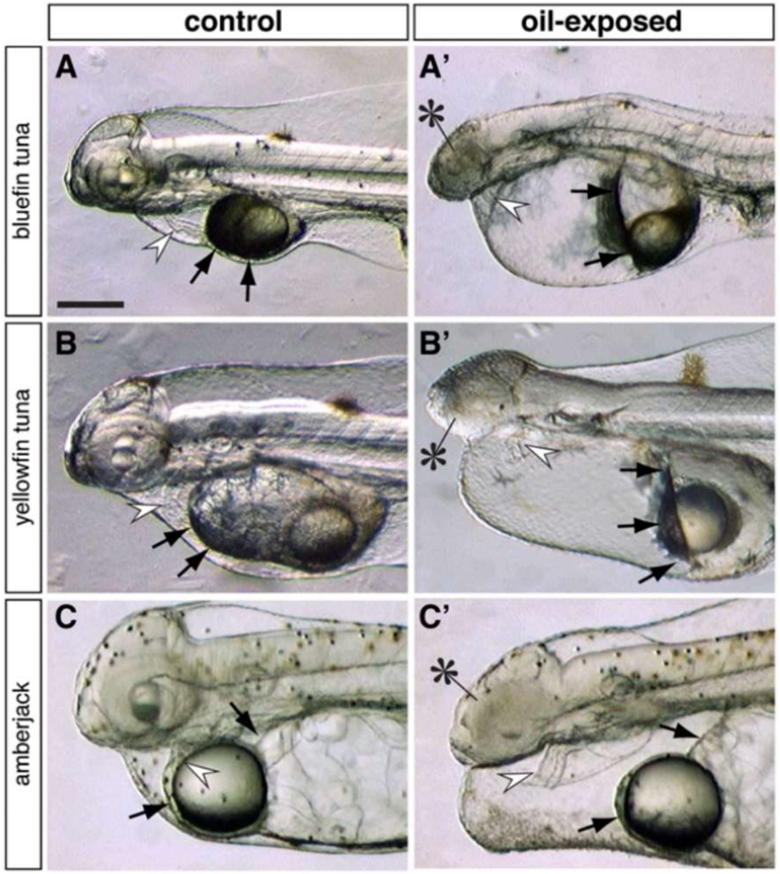
Results – edema (bibliography)

Deepwater Horizon crude oil impacts the developing hearts of large predatory pelagic fish

John P. Incardona[a,1], Luke D. Gardner[b], Tiffany L. Linbo[a], Tanya L. Brown[a], Andrew J. Esbaugh[c], Edward M. Mager[c], John D. Stieglitz[c], Barbara L. French[a], Jana S. Labenia[a], Cathy A. Laetz[a], Mark Tagal[a], Catherine A. Sloan[a], Abigail Elizur[d], Daniel D. Benetti[c], Martin Grosell[c], Barbara A. Block[b], and Nathaniel L. Scholz[a]

E1510–E1518 | PNAS | Published online March 24, 2014

➢ **Toxic factors**



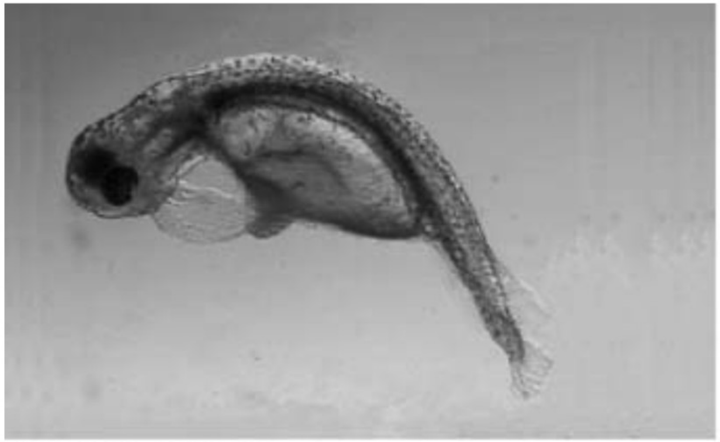
Results – edema (bibliography)

Fish Physiol Biochem (2009) 35:625–640
DOI 10.1007/s10695-008-9284-4

➢ **Toxic factors**

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

Barbara Jezierska · Katarzyna Ługowska ·
Małgorzata Witeska



Cardiac edema

Results – edema (bibliography)

➢ **Toxic factors**

Theranostics 2013, Vol. 3, Issue 4 258

IVYSPRING INTERNATIONAL PUBLISHER

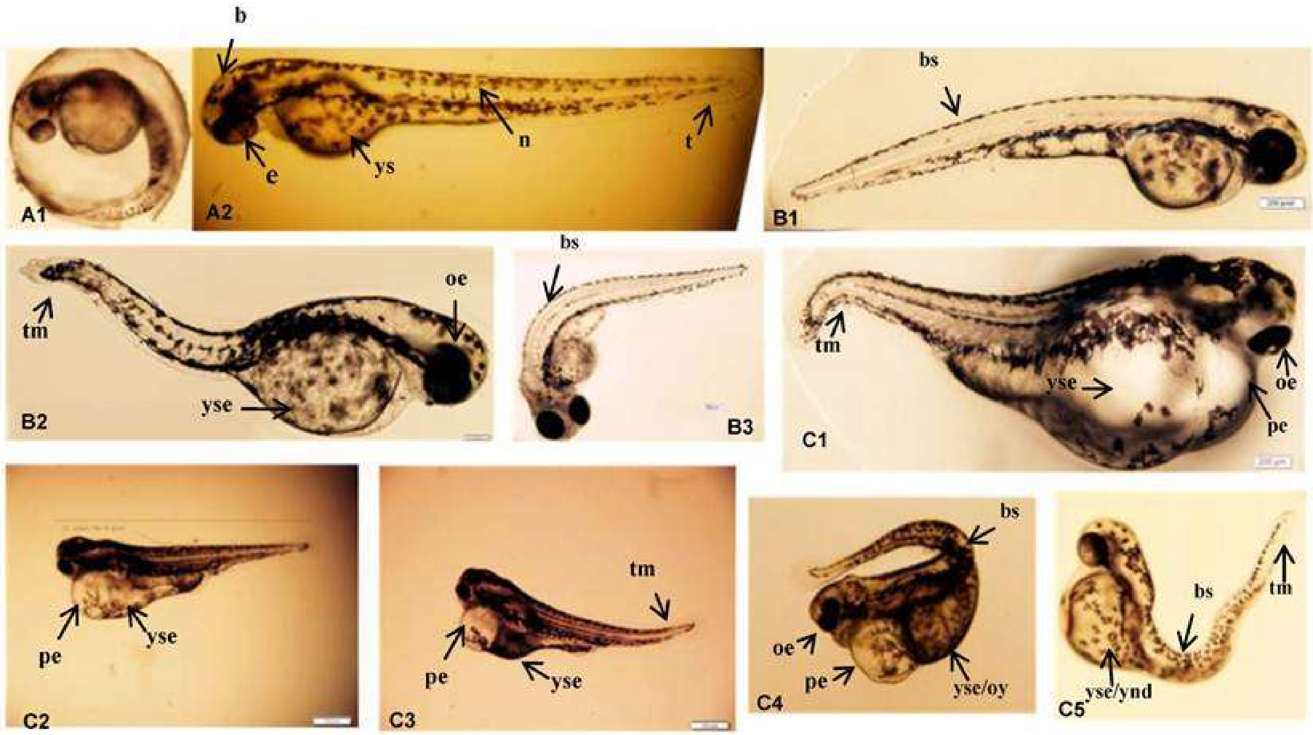
Theranostics

2013; 3(4):258-266. doi: 10.7150/thno.5701

Research Paper

Toxicity Assessments of Near-infrared Upconversion Luminescent LaF_3:Yb,Er in Early Development of Zebrafish Embryos

Kan Wang[1], Jiebing Ma[1], Meng He[1], Guo Gao[1], Hao Xu[1], Jie Sang[2], Yuxia Wang[2], Baoquan Zhao[2] and Daxiang Cui[1]✉



Results – edema (bibliography)

➤ **Toxic factors**

OXFORD

SOT Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 145(1), 2015, 177–195

doi: 10.1093/toxsci/kfv044
Advance Access Publication Date: February 23, 2015

Advanced Morphological — Behavioral Test Platform Reveals Neurodevelopmental Defects in Embryonic Zebrafish Exposed to Comprehensive Suite of Halogenated and Organophosphate Flame Retardants

Pamela D. Noyes, Derik E. Haggard, Greg D. Gonnerman, and Robert L. Tanguay[1]

Department of Environmental & Molecular Toxicology, Environmental Health Sciences Center, and the Sinnhuber Aquatic Research Laboratory, Oregon State University, Corvallis, Oregon 97331

[1]To whom correspondence should be addressed. Fax: 541-737-6074. E-mail: robert.tanguay@oregonstate.edu.

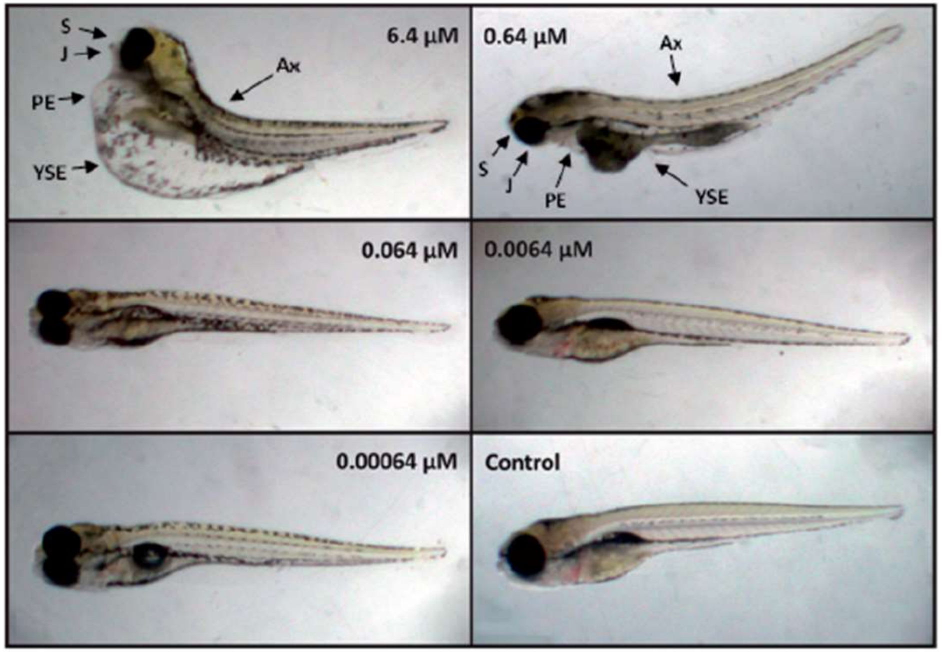


FIG 2. Morphological deformities observed in zebrafish larvae exposed to mITP flame retardant at 0, 0.00064, 0.0064, 0.064, 0.64, and 6.4 μM for 5 days. Deformities denoted as: Ax, axis; S, snout; J, jaw; PE, pericardial edema; YSE, yolk sac edema.

Results – edema (bibliography)

Toxicology Reports 4 (2017) 614–624

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

ELSEVIER

toxicology reports

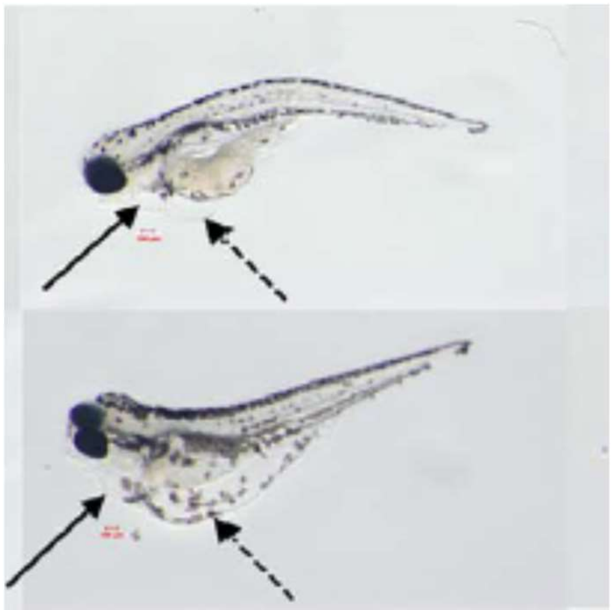
Full length article

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

Check for updates

Jie Yang[a], Hui Zhao[b], King Ming Chan[a,*]

[a] School of Life Sciences, The Chinese University of Hong Kong, Sha Tin, N.T., Hong Kong Special Administrative Region, China
[b] School of Biomedical Sciences, The Chinese University of Hong Kong, Sha Tin, N.T., Hong Kong Special Administrative Region, China



- **Toxic factors**

ELSEVIER

Aquaculture 169 (1998) 69–85

Aquaculture

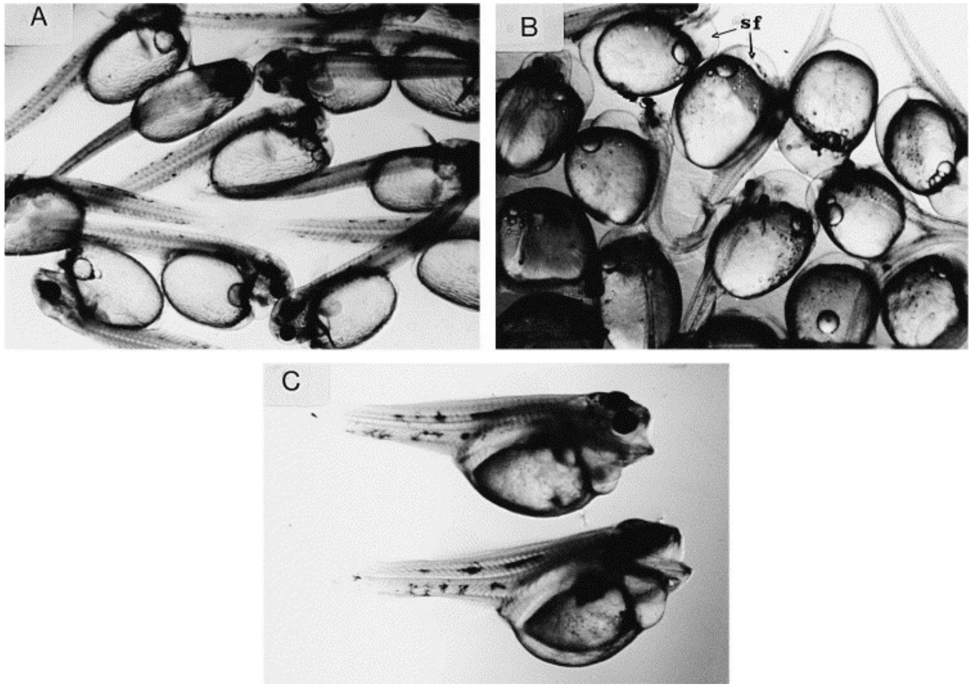
Characterisation of 'swollen yolk-sac syndrome' in the Australian freshwater fish Murray cod, *Maccullochella peelii peelii*, and associated nutritional implications for large scale aquaculture

Rasanthi M. Gunasekera [a,*], Geoff J. Gooley [b], Sena S. De Silva [a]

[a] *School of Ecology and Environment, Deakin University, P.O. Box 423, Warrnambool, Victoria, 3280 Australia*

[b] *Marine and Freshwater Resources Institute, Snob's Creek, Private Bag 20, Alexandra, Victoria, 3714 Australia*

Accepted 27 July 1998



- **Nutritional factors**
- **Poor nutrition of the broodstock**

Results – edema (bibliography)

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).

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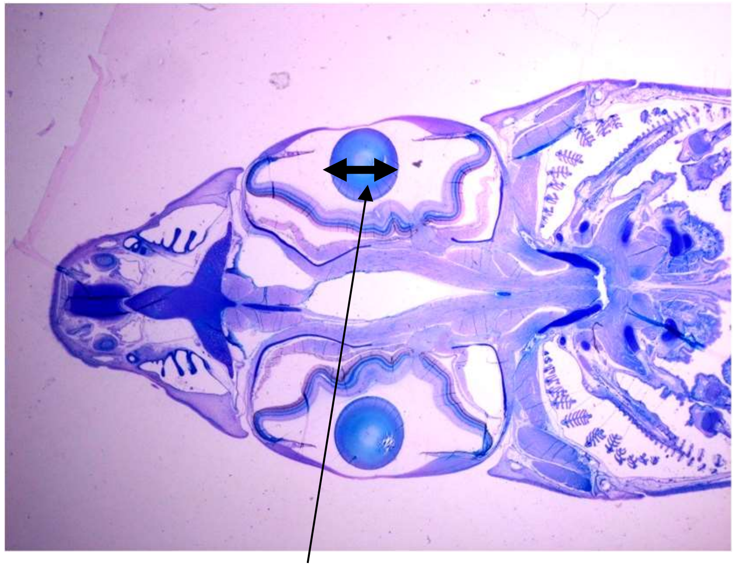
Eye-Vision Measurements

Retina

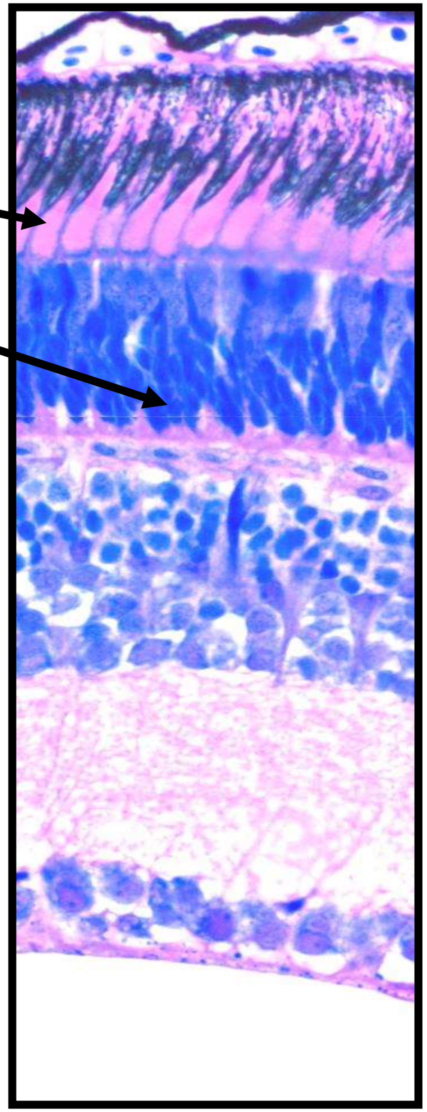


Lens diameter

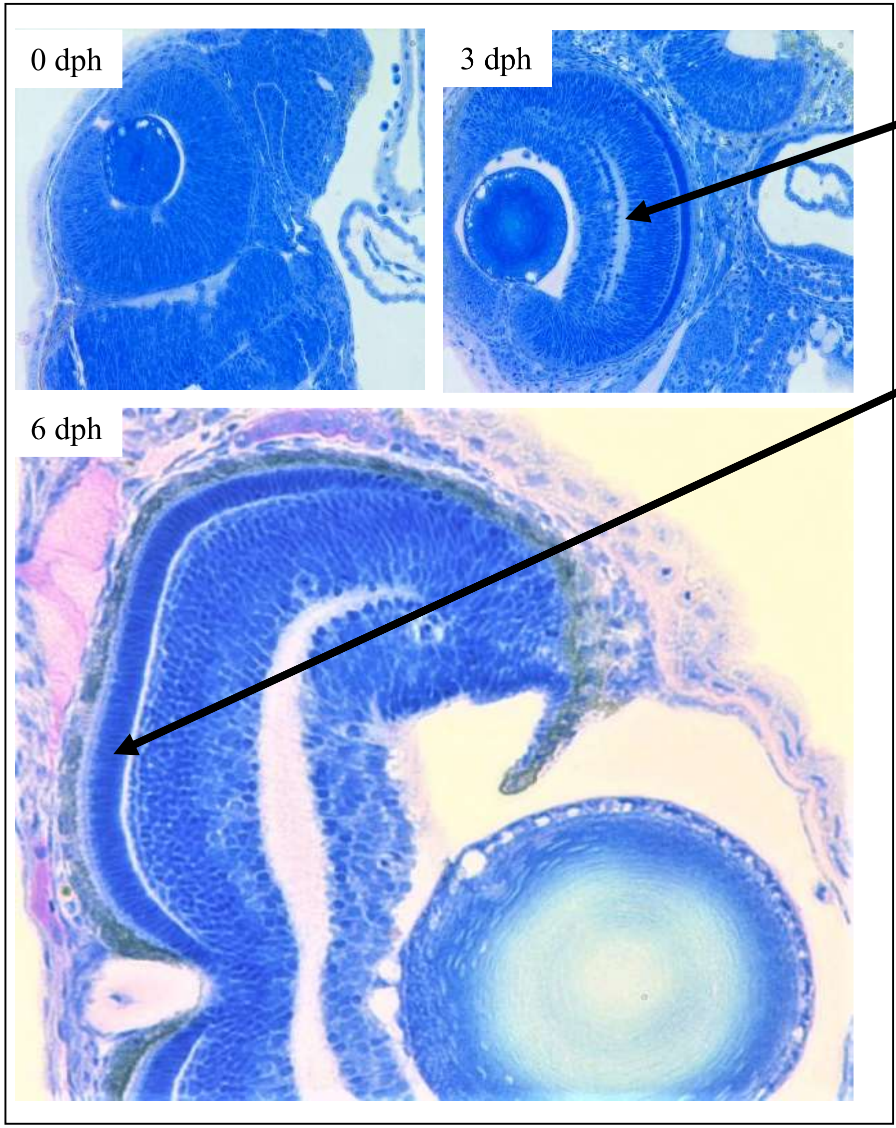
Number of:

Cone cells

Rod cells



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).

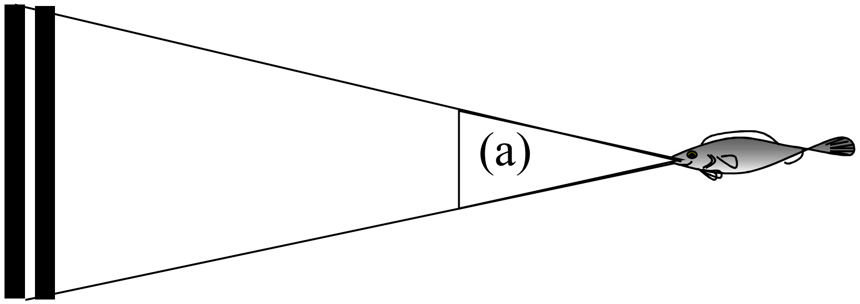
➢ **The fish weren't able to identify the food items in rearing environments with low light intensities.**

Results - Estimation of the histological visual acuity

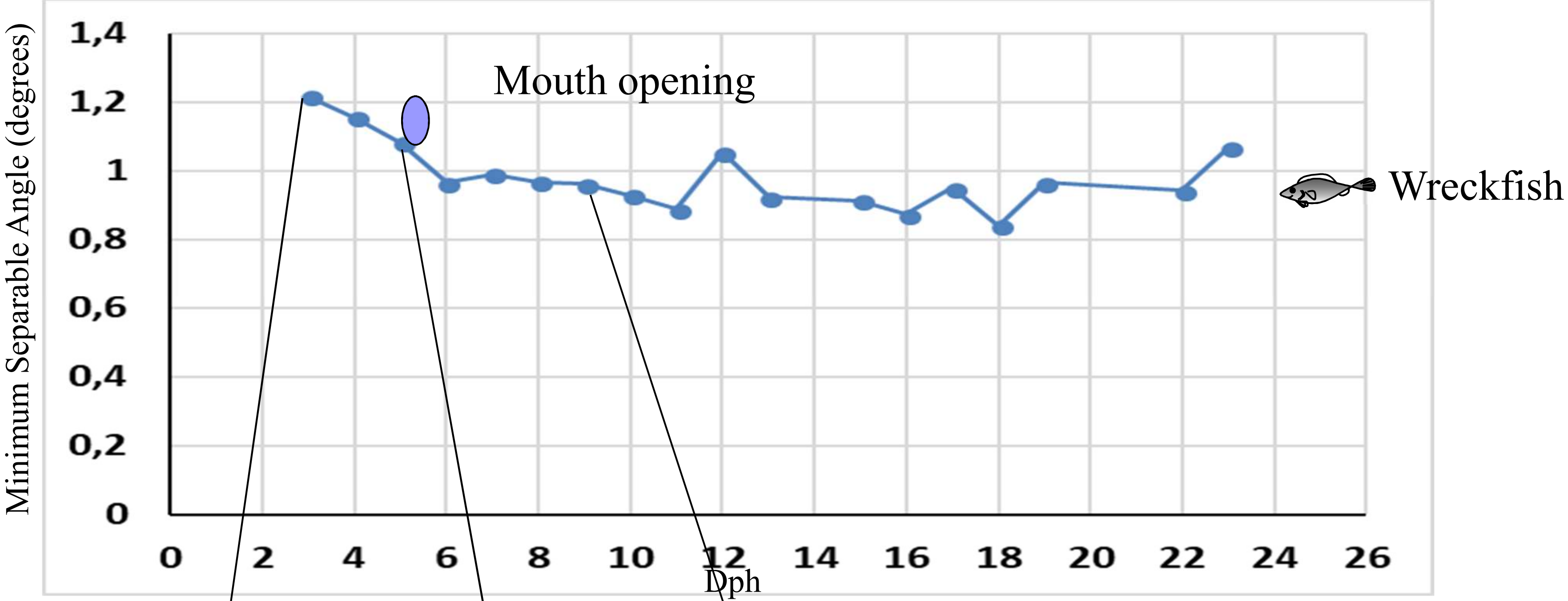
Visual acuity is defined as the minimum angle, at which two parallel objects can project at the eye and still be resolved as separate.

Histological visual acuity was expressed as the

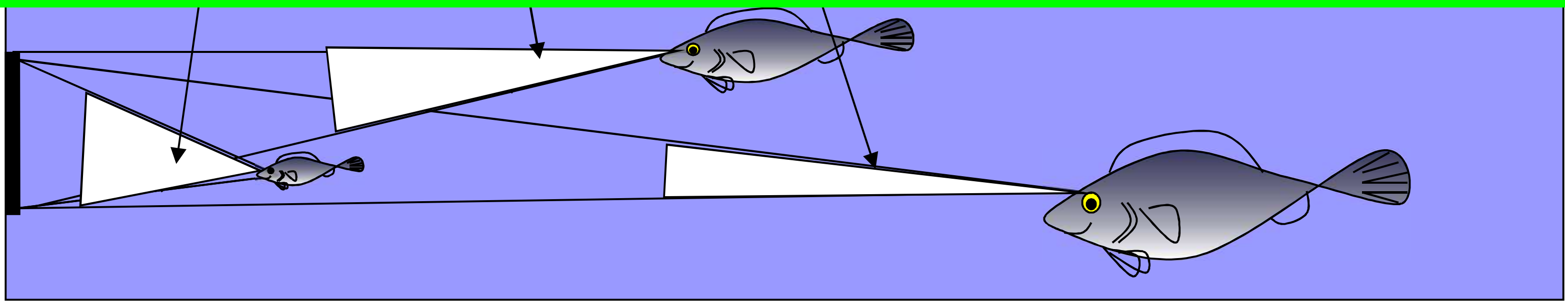
Minimum Separable Angle (MSA) = a,
which was calculated as:



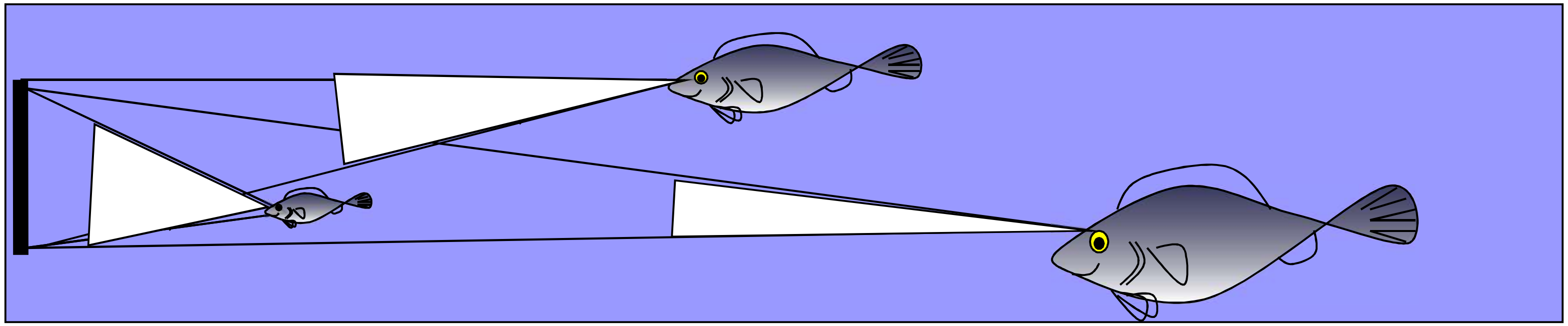
Results - Histological visual acuity



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)

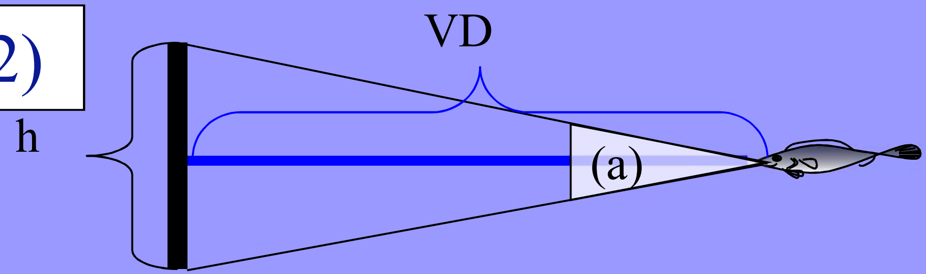


Estimation of the Visual Distance

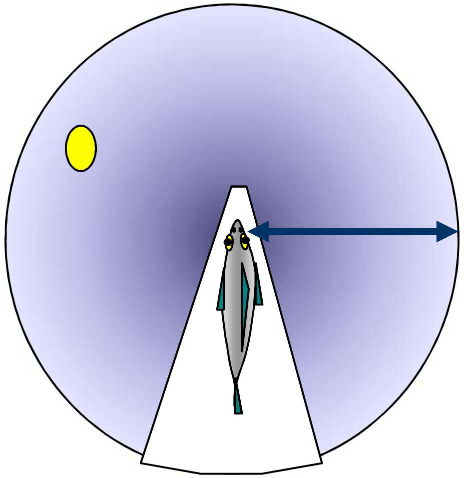
$$VD = 0.5*h/\tan(a/2)$$

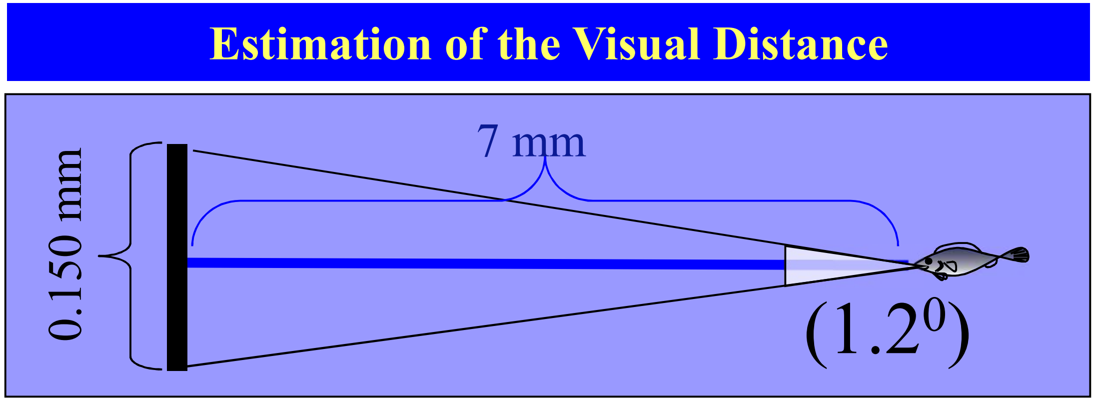
Visual Distance (VD)

h = object width (mm)



Results (Visual distance)





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.

Volume 1.4 ml
radius 7 mm

Rotifer

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.

Conclusions – size of larvae until to 5.8 mm

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.

Conclusions – size of larvae until to 5.8 mm

- **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) <u>Density of preys</u>
2) <u>Types of preys</u>
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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