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Comparative morphometrics, stereology and histology of sea bass *Dicentrarchus labrax* L. larvae reared in a conventional and axenic model system

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- Intensive aquaculture
- Antibiotic resistance
- Probiotic as alternative
- Host-microbe interactions
- Hosts tolerance to microflora, mucosal immunity, bacterial adhesion and translocation

axenic feed chain

Artemia nauplii - sea bass larvae

Quantitative and qualitative analysis of sea bass larvae reared in AXENIC and XENIC treatment

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1.Morphometrical, stereological and histological analysis

2. Challenge test

Materials and methods

Disinfection procedure of eggs:

- **Sea bass eggs: 15000 eggs/mL**
- **Glutaraldehyde: 200 mg/L for 3 minutes**
- **Incubation: in 10 mg/L rifampicin and 10 mg/L ampicillin**
  filtered (0.2µm), autoclaved seawater
  1500 eggs/L

Materials and methods

Set up for larvae culture:

- **Vials of 10 mL**
- **12 fish larvae/replicate**
- **Replicates are discarded after 1 counting**
- **Feeding *Artemia nauplii* from DAH 7 (30 *nauplii* /vial) each second day**

Materials and methods

Treatments

axenic

- rotating setup (AxRot)
- non-moving setup (AxS)

~~xenic~~

- ~~rotating setup (XeRot)~~
- ~~non-moving setup (XeS)~~

Rotation:
4 rpm, longitudinal axis

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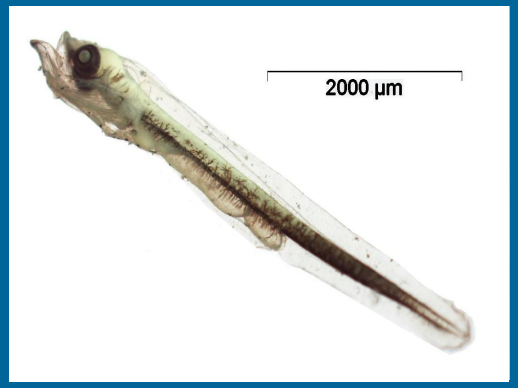
Materials and methods

Samples ➡ DAH1,

DAH6,

DAH9,

DAH14,



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Materials and methods

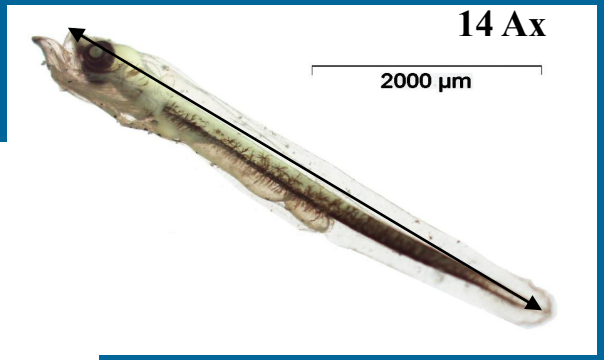
stereomicroscopy

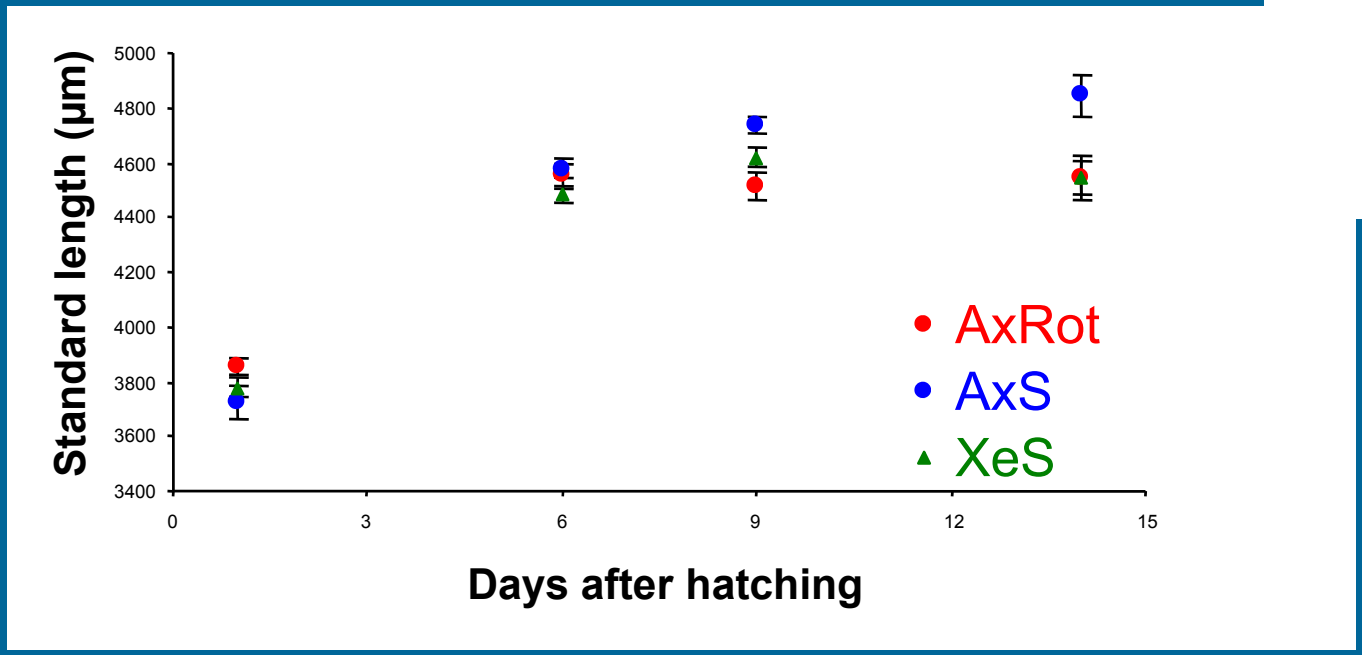
light microscopy

morphometry

qualitative analysis
stereology

1. Morphometrical analysis





From DAH6 onwards till the end of the experiment, AxS larvae exhibited the best growth amongst the three treatments

Standard length (mean ± S.E.M.) of larvae on DAH1, DAH6, DAH9 and DAH14 reared in axenic rotor, axenic static and xenic static treatment (n=8-20).

2. Stereology based on Cavalieri method

Ratio between the volume of the digestive tract and total body volume based on stereology using the Cavalieri method.

Vd/Vt

	AxRot (n=2)	AxS(n=2)	XeS(n=2)
DAH1	1.89%	1.89%	1.76%
DAH6	8.05%	7.59%	7.68%
DAH9	7.63%	8.28%	7.64%
DAH14	7.89%	10.14%	8.29%

V: volume in mm3, CE: coefficient of error, VD: volume of the digestive tract mucosa; VT: volume of the total body; VD / VT: ratio between the volume of the digestive tract and total body volume.

The AxS larva revealed the most developed digestive tract with a Vd/Vt value of 10.14 %

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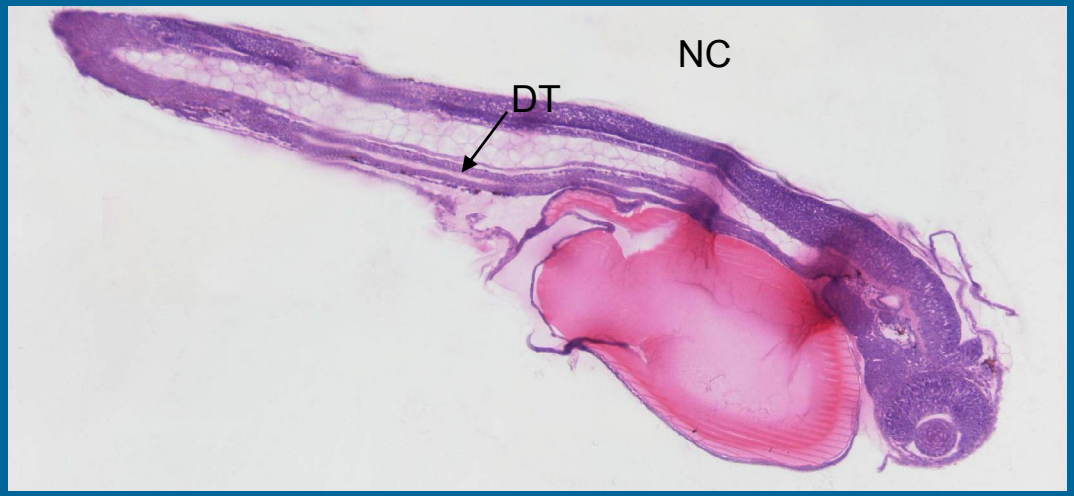
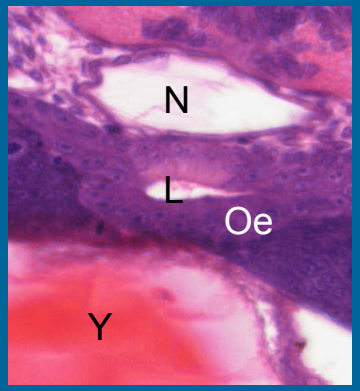
3. Qualitative histology (1)

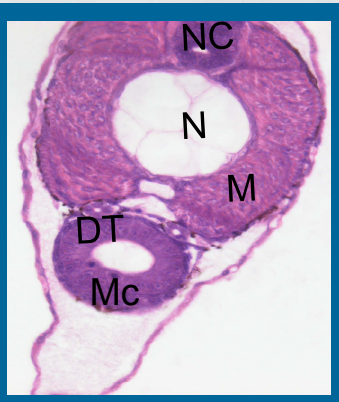
DAH 1

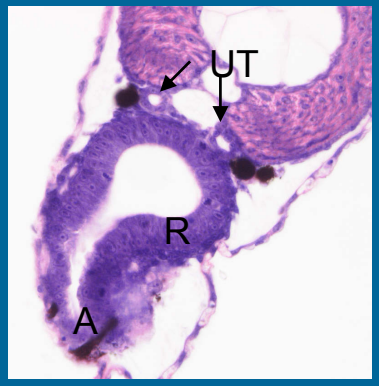
- Undifferentiated straight duct
- Closed mouth and anus
- Intestinal epithelium varying in size from cubic to columnar
- Anal region (pseudo)stratified epithelium
- Mitotic cells
- No mucus production

No difference between AxRot-AxS-XeS larvae







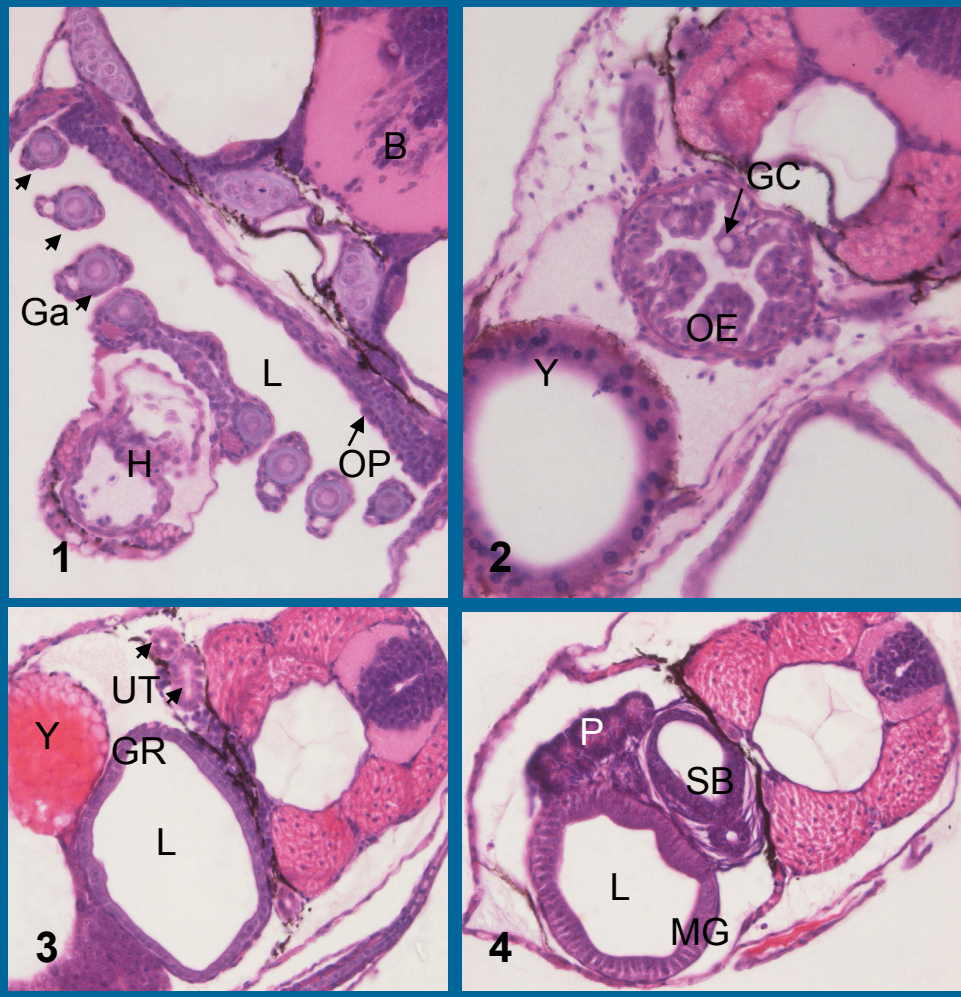


3. Qualitative histology (2)

DAH 6

- Differentiation of the digestive tract into:
  1. Oropharynx - simple squamous epithelium
     - stratified epithelium
  2. Oesophagus - (pseudo)stratified epithelium with goblet cells
  3. Gastric region - simple cubic to columnar epithelium
  4. Midgut and hindgut - high columnar epithelium

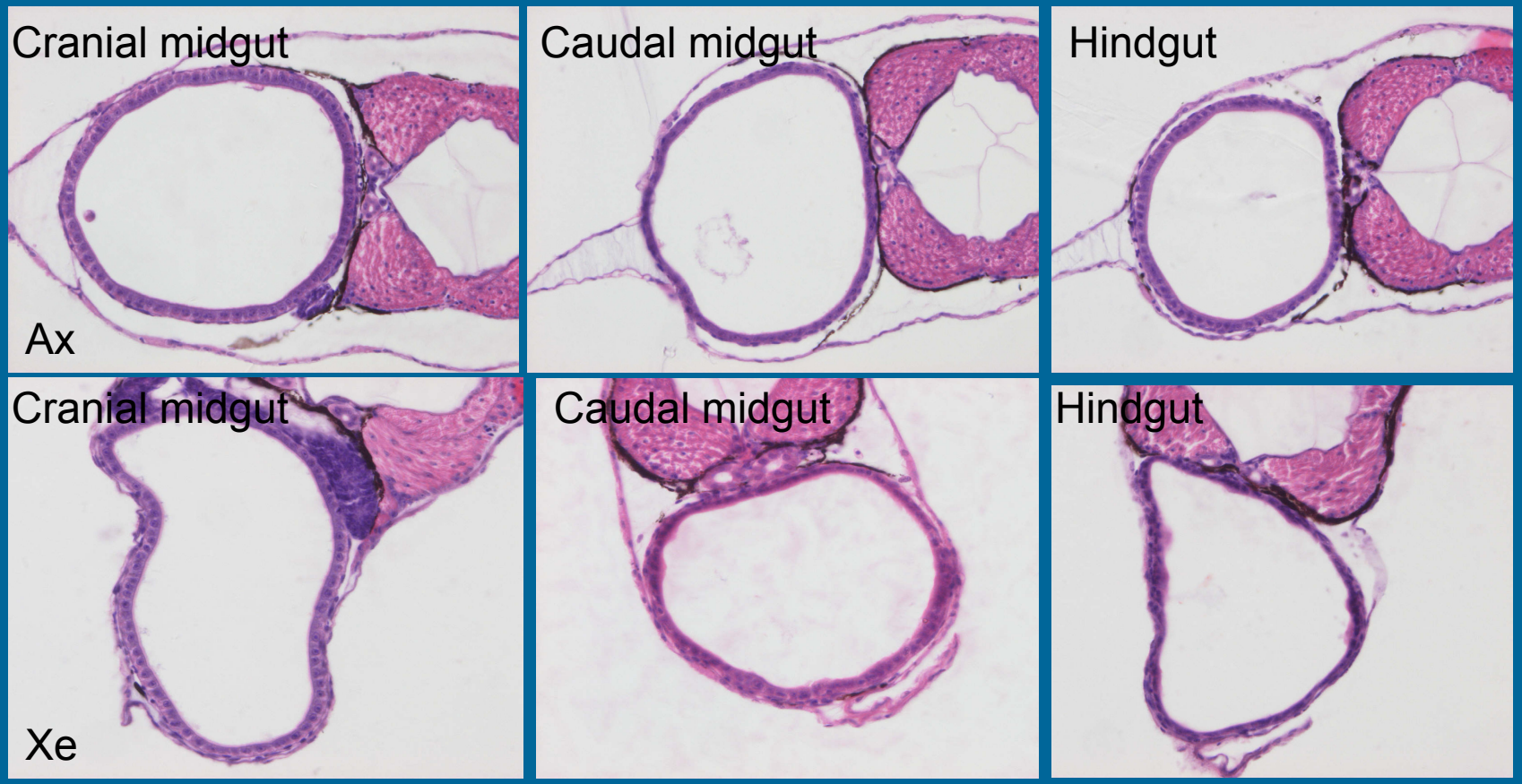
According to preliminary results no differences between AxRot-AxS-XeS larvae



3. Qualitative histology (3)

DAH 9

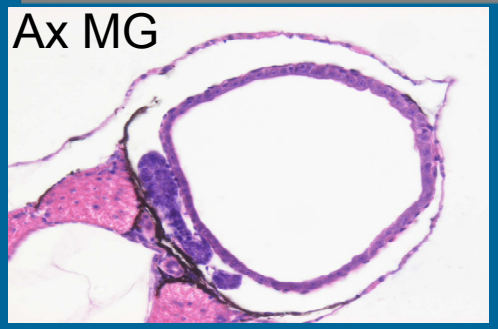
- variations in morphology of epithelial cell types among different treatments and within groups
- intestinal brush border better developed in axenic animals



3. Qualitative histology (4)

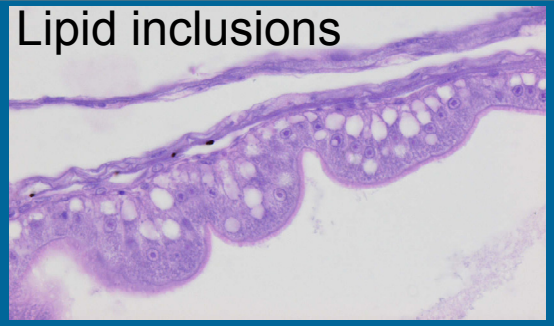
DAH 14

Ax MG

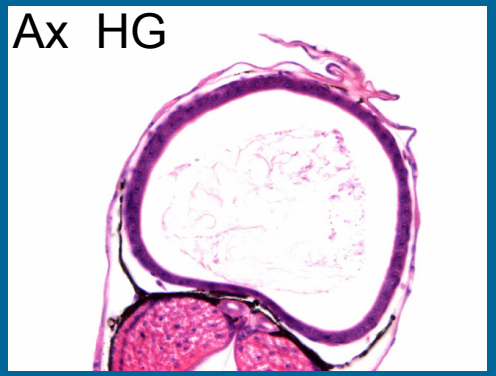


Xe MG

Lipid inclusions

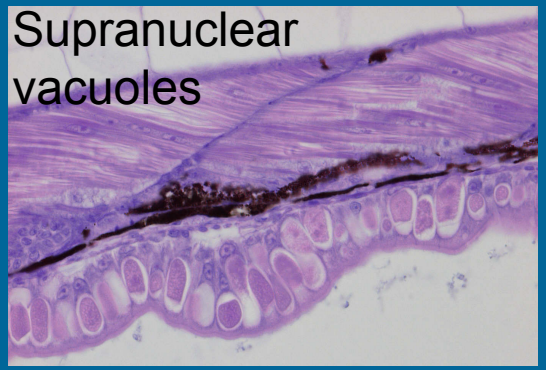


Ax HG



Xe HG

Supranuclear vacuoles



Conclusions

1. Morphometry - Enhanced growth in axenic static larvae

2. Stereology - Better developed digestive tract in axenic static larvae

3. Histology
   - lower epithelium height in axenic larvae
   - variation in regional morphology between and within treatment groups

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2. Challenge test

Experimental set up:

Vials of 10 mL
12 fish larvae/replicate
Replicates are discarded after 1 counting
Rotation: 4 rpm, longitudinal axis

Challenge:

Listonella anguillarum HI 610 (Serovar O2a)

Addition to the water at DAH 3 (10^5 CFU/mL) and through *Artemia* (10^3 CFU/Art) on DAH 7

Measurements:

Survival on DAH 5, DAH 7 and DAH 11

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Results

Survival was significantly lower from DAH 7 onwards

Survival of axenic versus challenged sea bass larvae

	Axenic	Challenge
DAH 5	**91.1 ± 2.6^{a}**	**100.0 ± 0.0^{a}**
DAH 7	**88.6 ± 5.5^{a}**	**58.6 ± 6.0^{b}**
DAH 11	**97.8 ± 1.5^{a}**	**29.1 ± 12.3^{c}**

Conclusions of the challenge test

1. *Listonella anguillarum* HI 610 (serovar O2a) is pathogenic to *Dicentrarchus labrax* larvae

2. Axenic sea bass larvae can be cultured in a rotating set up with high survival till DAH 11

Future research

- Mitosis and apoptosis in the digestive tract of the sea bass larvae

- Mucin histochemistry in the digestive tract in response to pathogens and probiotics

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