RESAMA: A Network for Monitoring Health and Husbandry Practices in Aquatic Research Facilities

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HEALTH MONITORING OF ZEBRAFISH
Overview

- Fishes and amphibians have gained a new role as model organisms in human biology and biomedical studies.
- Advances in husbandry and health management for these species have lagged well behind developments in science and technological innovation.
- Knowledge is sparse and desorganised: lot of theory but less of experimentation.
- Health of aquatic animals remains in most facilities mainly unmanaged, and few veterinarians are trained to deal with aquatic model species.
RESAMA (Réseau d’Etudes Sanitaires des Animaux Modèles Aquatiques: Network for the Study of Aquatic Model Animal Health)

- Increase the knowledge on pathogen and health issues in amphibian and fish facilities
- Improve husbandry and health management practices
- RESAMA focus on the commonly aquatic species in research: Danio rerio and Xenopus laevis.
- Indeed, other aquatic species less frequently encountered are also incorporated in the study: Xenopus tropicalis, Oryzias latipes, Astyanax mexicanus ...
The first step consisted in defining the current state of:

- Husbandry practice,
- Heath management practice,
- List of pathogen

The second step will be to link each other, when possible.
HOW IT WORKS?

- This work received support from the program ‘‘Investissement d’avenir’’ ‘‘TEFOR promoted by three founder research organisms (CNRS, INRA, INSERM) as well as the University of Auvergne.
- Network strong of 60 partners located mainly in France but some abroad
- Each partner facility is visited for free at least once during the time course of the project, which is financed until 2019.
- A brief report of the visit and the results are giving back,
- RESAMA aims to publish results of the study (facilities stay anonymous)
- The network is still recruiting partners.
Protocole of the visit

- Team: one veterinarian and one specialized animal caretaker
- A brief presentation of the aims of the network was made to the staff.
- The veterinarian did a historical review of the facility (results of previous health monitoring, existence of previous epizooty, mortality rate etc.) followed by the health monitoring and sampling visit of the facility.
- Meanwhile, a zootechnic assessment is organized to review the design of the facility (technical solutions chosen, water production system, day/night cycle, etc.) as well as Standard Operation Protocols (water parameter controls, feeding practices, strain management, etc.).
Sampling procedure

- Each water unit (animal and water)
- Animals presented clinical signs of illness were sample first
- If several animals presented the same clinical sign, the worst is choosen,
- If animals present differents clinical sign each set were selected
- If no animal presented clinical sign: randomly (sentinel first)
Materials and Methods
Necropsy

- euthanized using an overdose of benzocaine (250 mg/mL)
- External examination: detect any lesion.
- For fish:
  - 50% of the samples were necropsied, the rest were kept for histological analysis.
  - Cutaneous mucus, branchial arches, pectoral and caudal fins as well as any lesion were systematically observed under microscope. Each internal organ: macro and microscopy
- For Amphibians,
  - all the animals went through necropsy.
  - Skin scrap of the belly and the back were performed and each organ was submitted to a macroscopic observation. Skin, lung, gall bladder, urinary bladder, gut (first, middle and posterior parts) as well as its content were carefully examined under microscope.
Histology

- **Fixative:** Tissues and organs fixed in 10% buffered formalin

- **Organs:**
  - **Fish:** entire body
  - **Amphibian:** every organ (skin, lung, spleen; kidney; liver, gut, bladders...)

- **Stain:** HE, Fite Faraco, PAS, Giemsa, GRAM
Bacteriology

- Mostly when suspected not sure
- Organs:
  - Fish: heart, kidney
  - Amph: kidney, spleen, liver
- Medium: Anacker-Ordal and Tryptic Soy agar and incubated at 20°C+-2°C
- ID: API + Maldi-Tof (matrix assisted laser desorption/ionisation time-of-flight analyser)
PCR

- Use mainly for ID:
  - Mycobacterium spp. (multispecies)
  - Pseudoloma neurophilia
  - Batrachochytrium spp.
  - Ranavirus

- Mycobacterium species confirmed by sequencing for ID cobacterium spp
ZEBAFISH
RESULTS

HEALTH ANALYSIS
HUSBANDRY PRACTICE

FELASA 2016 congress, Brussel
## Sample size (not updated)

<table>
<thead>
<tr>
<th>Species</th>
<th>Visited Facilities</th>
<th>Sampled animals</th>
<th>Analysis</th>
<th>sex ratio</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>male</td>
<td>female</td>
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<tr>
<td>Danio rerio</td>
<td>17</td>
<td>612</td>
<td>931</td>
<td>284</td>
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<td>Oryzias latipes</td>
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<tr>
<td>Xenopus tropicalis</td>
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<td>Xenopus laevis</td>
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<td>Xenpous borealis</td>
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</table>
# Bacteriology

**Bacteria detected**

- Acinetobacter junii
- Aeromonas caviae
- A. veronii
- Citrobacter freundii
- Chryseobacterium indologenes
- Mycobacterium chelonae
- M. fortuitum
- M. gordonae
- M. marinum
- M. mucogenicum
- Mycobacterium (partial histology only)
- M. putrida
- Pseudomonas aeruginosa
- M. putrida
- Shewanella (partial characterisation only)
- Shewanella putrefaciens
- Stenotrophomonas maltophilia
- Vibrio (partial characterisation only)
- Vibrio alginolyticus

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**Facilities**

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<th>4</th>
<th>4</th>
<th>7</th>
<th>6</th>
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<td>11</td>
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<tr>
<td>M. fortuitum</td>
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<td>2</td>
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<tr>
<td>M. gordonae</td>
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<td>M. marinum</td>
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**Animals**

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Parasitology

<table>
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<th>Facilities</th>
<th>Animals</th>
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<td>Flamingolepis liguloides</td>
<td>1</td>
</tr>
<tr>
<td>Pseudoloma neurophilia</td>
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</tbody>
</table>

No other parasite
Histology

- Histological analysis allowed us to assess infectious diseases and underline numerous non-infectious diseases on zebrafish
- nephrocalcinosis,
- neoplasia,
- egg-binding,
- gut distension.
- Egg-binding, seminoma and steatosis were very common in all the zebrafish facilities we sampled.
- 45% of the female displaying egg-binding also had granulomatous infections associated with acid fast bacteria (most probably mycobacteriosis).
Conclusion
a brief view of health monitoring realised in 17 zebrafish facilities between the end of 2012 and September 2015.

Only 6 facilities had scheduled health monitoring program prior to our visit.

Non-infectious diseases were quite prevalent and diverse in all species.

Others, like nephrocalcinosis, liver neoplasia, egg-binding were more limited to some facilities.

A large number proportion of the pathogens found in research facilities are common to most of the aquatic animals held there.

These results comfort our approach to assess aquatic facilities as a whole.
Thank you for your attention