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

Laurent Legendre[1], Brigitte Guillet[2], Emmanuel Leguay[3], Emmanuel Meunier[3], Sophie Labrut[4], Nicolas Keck[5], Massimiliano Bardotti[6], Lorraine Michelet[7], and Frédéric Sohm[1]

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

amagen@inaf.cnrs-gif.fr

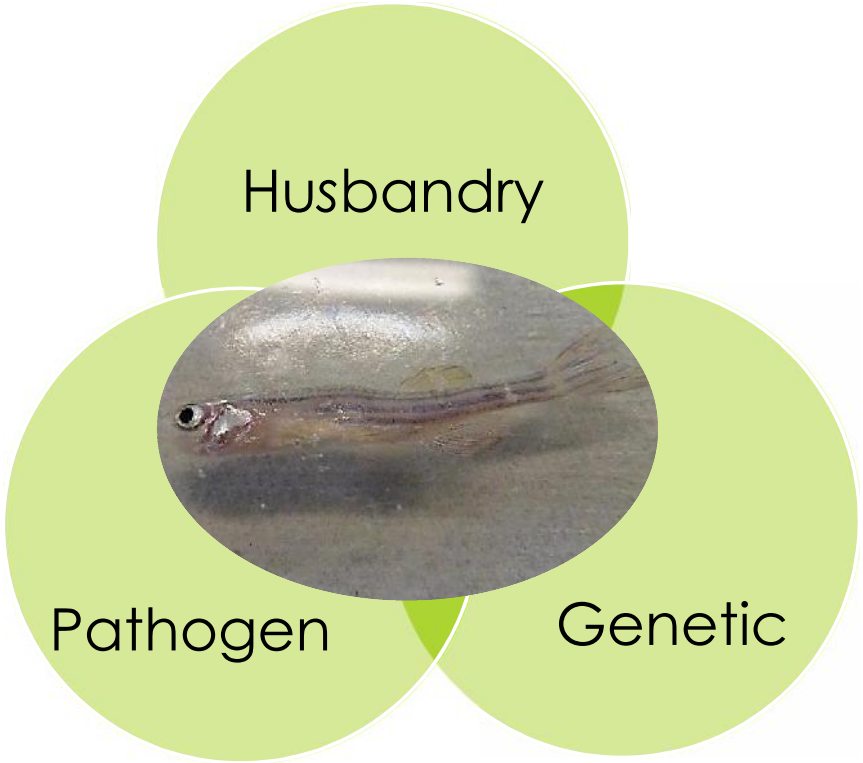
AIMS

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 …

Objectifs

- 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 do 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.
- The 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 singn, the worst is choosen,
- If animals present differents clinical sign each set were selected
- If no animal presented clinical sign: randomly (sentinelle first)

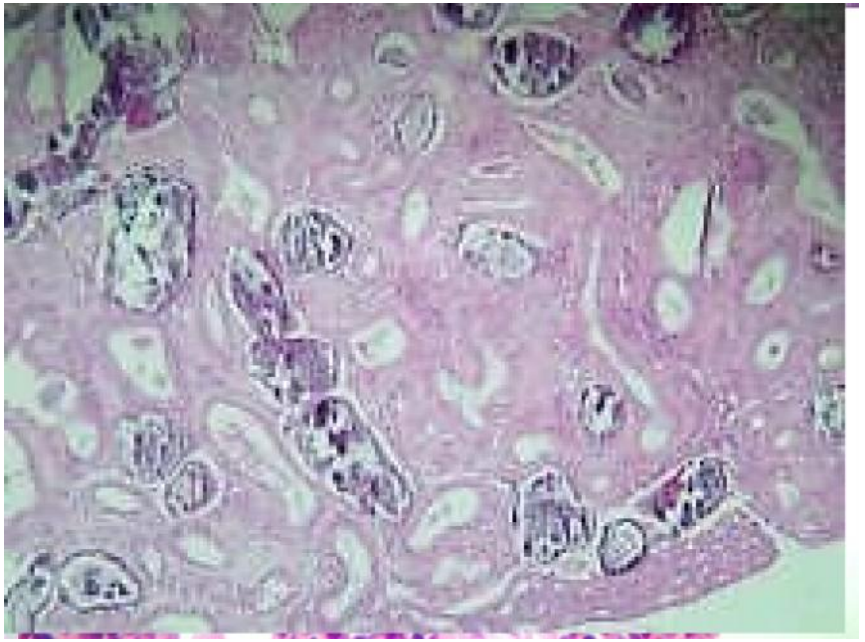
Materials and Methods

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



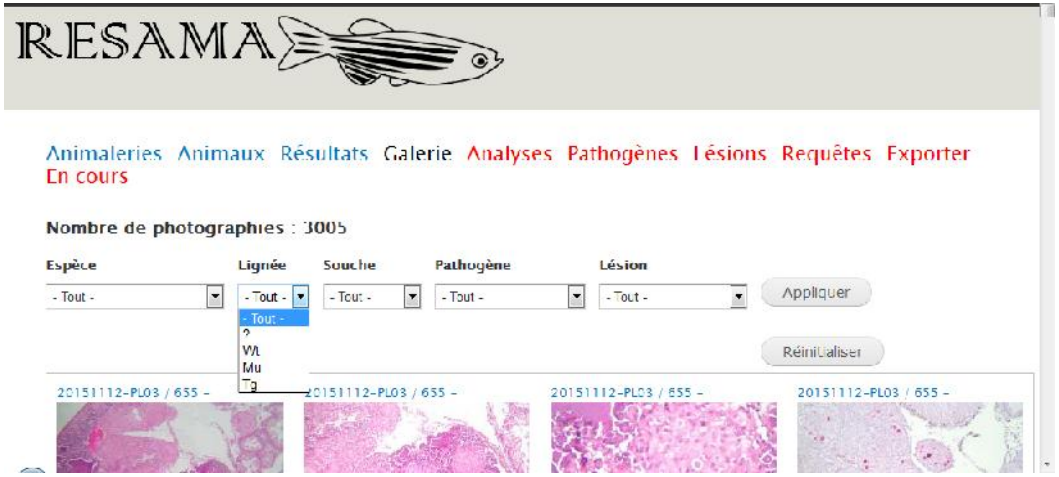
Bacteriology

- Mostly when suspected st not
- Organs:
  - Fish: heart, kidney
  - Amph: kidney, spleen, liver
- Medium: Anacker-Ordal and Tryptic Soy agar and incubated at 20°+-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

DATABASE


RESAMA
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En cours
Nombre de photographies : 3005
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Lignée
Souche
Pathogène
Lésion
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Appliquer
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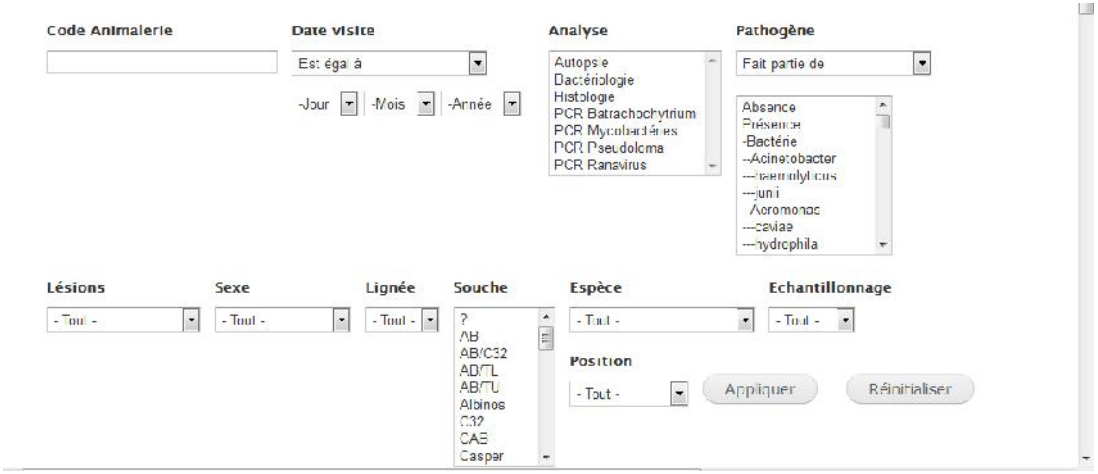
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Date visite
Est égal à
-Jour
-Mois
-Année
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Autopsie
Bactériologie
Histologie
PCR Batrachochytrium
PCR Mycobactéries
PCR Pseudoloma
PCR Ranavirus
Pathogène
Fait partie de
Absence
Présence
-Bactérie
--Acinetobacter
---haemolyticus
---jun i
Aeromonas
---caviae
---hydrophila
Lésions
Sexe
Lignée
Souche
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ZEBRAFISH RESULTS

HEALTH ANALYSIS

HUSBANDRY PRACTICE

Sample size (not updated)

Species	Visited Facilities	Sampled animals	Analysis	sex ratio		
				male	female	ND
Danio rerio	17	612	931	284	250	78
Oryzias latipes	4	85	115	46	29	10
Astyanax mexicanus	1	23	32	4	14	5
Xenopus tropicalis	4	39	75	13	24	2
Xenopus laevis	7	87	218	20	50	17
Xenpous borealis	1	1	3	1	0	0

BACTERIOLOGY

Bacteria detected	Acinetobacter junii	Aeromonas caviae	A. hydrophila	salmonicida	A. veronii	A. veronii biovar sobria	Chryseobacterium indologenes	Citrobacter freundii	Mycobacterium (partial histology only)	Mycobacterium chelonae	M. fortuitum	M. gordonae	M. marinum	M. mucogenicum	Pseudomonas aeruginosa	fluorescens	M. putida	Shewanella (partial characterisation only)	Shewanella putrefaciens	Stenotrophomonas maltophilia	Vibrio (partial characterisation only)	Vibrio alginolyticus
Facilities	1	4	4		7	6	1	2	14	11	2	5	1	1	1		1	2	2	2	2	1	
Animals	1	4	4		16	13	1	3	43	23	4	11	1	2	1		1	3	2	2	3	2	

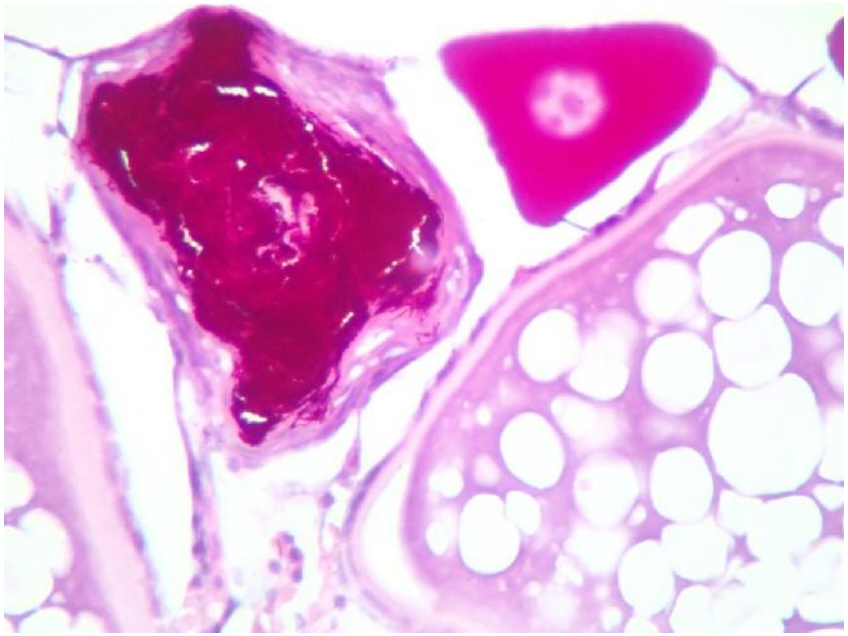
Parasitology

	Facilities	Animals
Flamingolepis liguloides	1	4
Pseudoloma neurophilia	14	116

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

amagen@inaf.cnrs-gif.fr