

# Follow-up meeting GENBAS

*Location: RBINS*

August 22, 2014

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22/08/2014

**Present:** Jos Snoeks, Maarten Van Steenberge, Loic Kéver, Jeroen Van Houdt, Erik Verheyen, Sofie Derycke

**Excused:** Eric Parmentier, Pascal Poncin

Participants arrived between 11.30 – 12.30h, informal lunch from 12h-13h

Start meeting: 13.30h

1/ Overview project => all people have been hired:

Erik Verheyen: Sofie Derycke  
Jos Snoeks: Maarten Van Steenberge  
Erik Parmentier & Pascal Poncin: Loic Kéver  
Jeroen Van Houdt: Koen Herten

2/ Overview of the work already performed, problems encountered and actions to be taken for each WP

## WP1: Behavioural and acoustic experiments (presented by Loic)

*Task 1.1: Installation of the tanks, experimental set up.*

*Task 1.2: Recording and characterisation of the behaviour during con- and heterospecific encounters.*

*Task 1.3: Recording and characterisation of acoustic communication during con- and heterospecific encounters.*

*Task 1.4: Data analysis behaviour and acoustics.*

### Overview:

4 aquaria of 300 liter have been installed;  
five fish (2 males and 3 females) of *O. ventralis* have been kept in two aquaria for three months  
Three kinds of sounds were recorded (1 during feeding, 1 when males chase females, 1 not yet linked to particular behavior, but sounds like quivering  
When two males are present in the tank, they fight a lot, but no sound was recorded  
A bigger tank can be used as well

### Problems:

1. no reproduction has been observed, why?
  - a. 300 litres too small? In aquaria of 1000 liters reproduction was good (*Erik*)
  - b. *Jos* will phone Wilfried to inform about spawning conditions with hobbyists; females may not be ready to spawn since their belly is not swollen
  - c. Ratio male/female not optimal (*Maarten*); generally 1 male for three females
  - d. create hiding spaces for females, PVC tubes (*Erik*); movies show that females are swimming at the top of the water which indicates they are stressed (*Jos*)

- e. what are you feeding them? Flakes + Artemia optimal food source (*Jos and Erik*)
  - f. fish quickly know when you are there (even when using a 'wall', they feel the vibrations in the water); they may court when you are not looking => make records during 24 hours; only enter the room to feed them (*Erik*)
  - g. pay attention to the water quality (pH)
  - h. Never mix lots!
2. standard error on sound profiles are very high, which forms a problem if we want to investigate significant differences between species
- a. sounds do not need to be different between species; it is possible that they only produce a sound when they see a conspecific, and keep quite in the absence of conspecifics (*Erik*)

Actions to be taken:

1. Keep the five fish in the smaller tank; put new specimens in the bigger tank (*Loic*)
2. Genotype them to verify their origin => take ca 5mm of the dorsal or anal fin (definitely not the pelvic fins!) (*Loic and Maarten*)
3. Purchase 10 wild caught fish of each species (3 males and 7 females) (*Maarten*)

**WP2 : Specimen and tissue collection (presented by Maarten)**

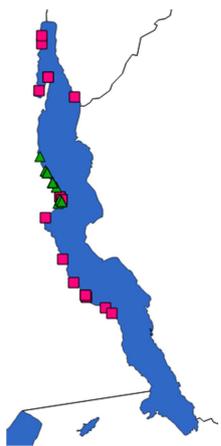
*Task 2.1: Purchase, import and determination of live fishes.*

*Task 2.1: DNA extraction samples genotyping by sequencing.*

*Task 2.2: Brain dissection and RNA extraction samples.*

Overview:

Specimens can be bought at the Aquatref meeting in Düsseldorf to reduce the costs of transport; Wildcaught € 30 specimen, Aquarium bred: €10-25, transport €55 box; specimens for each species are available from different localities



(Kigoma).

New samples (ca 100 specimens from 7 localities) have been collected during the 2014 expedition (indicated in green); an additional set of ca 30 specimens from 13 localities were collected in 2010 (indicated in red)

Problems:

1. Do we use specimens from the same region, or from different regions for the behavioural experiments?
  - a. Same area, since specimens are confronted with each other and genomic signals may be more clear (*Erik*)
2. Taxonomic delineation of *O. heterodonta* is problematic, disagreement between authors (Hanssens & Snoeks 1997; Konings 2014); all authors do agree that *O. heterodonta* occurs in the northern region

- a. In view of the taxonomic problems with *O. heterodonta*, we restrict ourselves for the moment to *O. ventralis*, *O. boops* and *O. nasuta*
3. Wildcaught or aquarium bred specimens for RNA seq analyses? Difficult question, both have pros and cons; check literature to see what others have used (*Sofie*); aquarium bred will probably reduce variation among individuals and may thus be more suited for RNA seq analyses (*Jeroen*); topic to discuss with follow-up committee.
4. Dissection of brains: killing has to be done as quick as possible so that the expression is related to courtship, not to killing

Actions to be taken:

1. Buy specimens: 30 (9 females-21 males) aquarium bred for preliminary experiments in RBINS and 10 (3+7) wildcaught for Liege (*Maarten*)
2. Setup 6 aquaria in RBINS by 13/09/2014 (*Maarten*)
3. Analyse all new samples (ca 130 specimens) for the D-loop fragment to further explore introgression patterns observed by Nevado et al. 2011 (DNA extraction and PCR D-loop planned for September (2010 samples) and october (2014 samples) (*Maarten*)
4. Check literature to get information on dissection of the brains; contact follow-up committee for advice, possibly invite stickleback expert for next meeting (*Sofie*)

**WP3: NGS**

*Task 3.1: Genotyping by sequencing*

*Task 3.2: RNA sequencing*

Overview:

Nothing to report at this point

**WP4: Genomics data analysis**

*Task 4.1: Preparatory data analysis for genotyping by sequencing.*

*Task 4.2: Data analysis genotyping by sequencing.*

*Task 4.3: Data analysis RNA sequences.*

Overview:

See sampling Maarten under WP 2

We aim at genotyping 300 specimens with GBS

Actions to be taken:

1. Preparatory phase GBS (*Sofie and Jeroen*):
  - a. investigate cichlid genomes in silico to determine restriction enzyme (Burtoni is closest related available genome)
  - b. test couple of old and new samples to ensure integrity of the samples: two from each of the three species

- c. comparing old/new samples and different species will allow to investigate whether the restriction enzyme is not cutting too much
2. Compile a database containing all old and new samples and life specimens (*Maarten*)

### WP5: data integration

*Task 5.1: Integration of the behaviour and acoustics with the gene expression.*

*Task 5.2: Integration of behaviour and acoustics with introgression patterns.*

*Task 5.3: Integration of the gene expression analysis with the genotyping by sequencing.*

#### Overview:

Nothing to report at this point

### WP6: Project coordination

*Task 6.1: Coordination of the project.*

*Task 6.2: Reporting and administration.*

*Task 6.3 Communication with the follow up committee.*

*Task 6.4 Dissemination of the results. (All)*

#### Overview

A technical bulletin explaining the project is required in Dutch, French and English (deadline several months ago)

A year report has to be submitted by the end of November => next meeting first week of November, in Liege

#### Actions to be taken:

1. An English draft of the technical bulletin will be sent to all partners; after approval of all partners, a Dutch version (*Sofie*) and French version (*Loic, Pascal or Eric*) will be created
2. A text for the RMCA website needs to be prepared (*Maarten => maybe you can use the technical fiche for this?*)
3. A dropbox folder will be created containing presentations and reports of meetings, as well as relevant literature that partners want to distribute (*Sofie*); the GENBAS folder contains:
  - a. A folder named 'Administration' with all administrative documents (contracts, reports required by Belspo, the original project application)
  - b. A folder named 'Reports\_Presentations\_meetings' with all reports and presentations of meetings by the partners
  - c. A folder named 'Interesting\_References' to which pdfs of literature can be added that are relevant for all partners.

LINK DROPBOX :

<https://www.dropbox.com/sh/0dlpvh63orhp37a/AADRqHIWcH8V9ISW-e8SAKGDa?dl=0>

4. A doodle for the next meeting in Liege will be distributed (*Sofie*)

#### 3/ Other remarks?

Loic mentions that "Ethical committee approval" is required before starting any tests

=> Maarten will receive the certificate very soon  
End of meeting at 16.45h