STSM CA15219 DNAqua-net, Call 2: Scientific Report

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STSM Host: Dr. Bernd Hänfling Evolutionary Biology Group, School of Biological, Biomedical and Environmental Sciences, University of Hull University of Hull, UK
Date of visit: 11th June 2017 – 31st July 2017
Title: Fine tuning eDNA metabarcoding pipeline for assessment of WFD fish biodiversity in aquatic ecosystems**.**

Stockholm, August 29th, 2017

1. BACKGROUND

Environmental DNA (eDNA) is an emerging tool for environmental aquatic monitoring has been shown to be extremely promising for detecting target species and monitoring fish communities (Valentini et al. 2015; Civade et al. 2016; Evans et al. 2017; Hänfling et al. 2016). All previous studies have shown that eDNA based metabarcoding has a better species detection rate than conventional fishing methods (Hänfling et al. 2016). Actual biomass of fish in the community makes it challenging to accurately predict fish community composition on the basis of eDNA samples (Brys et al. 2017). It is generally expected that primer specificity is an important driver in creating such species-specific efficiency in detection and quantification. For this STSM we aimed to systematically screen and test different primers on European freshwater fish species in order to reduce error rates, improve cost efficiencies and sensitivity and ultimately make this already powerful tool more streamlined and reliable.

For this STSM between AquaBiota Solutions /Stockholm University, Sweden (Dr. Micaela Hellström) and University of Kingston upon Hull (Dr. Bernd Hänfling) in collaboration with NatureMetrics UK (Dr. Kat Bruce) and INBO in Belgium (Dr. Rein Brys and David Halfmaerten). We assessed samples from two large lakes in the UK one large lake in Sweden and mock communities originating from ponds and rivers in the UK and in Belgium.

2. PURPOSE OF THE VISIT

The main purpose of the visit was to form a close collaboration platform between the participants. The aim was to optimize and improve eDNA metabarcoding protocols and analysis pipelines for WFD (Water Framework Directive) monitoring of fish communities with a wider benefit for the DNAqua-Net community.

<u>The specific objectives were to compare:</u>

1) different primers regarding power to identify a wide range European freshwater fish species and how well the results correlate among primers. Primer choices (Riaz/Kelly, Kelly et al. 2014), MiFish (Miya et al 2015) and Teleo (Valentine et al. 2016)
2) different library preparation protocol especially testing whether 1st PCR indexing introduces additional variance by comparing PCR replicates with the identical and different indices
3) different bioinformatics pipelines

We agreed to

- create a set of samples with the aim to cover of as many fish species as possible. Workload: One primer in each lab using the very same samples which will be sent between the labs; Split would be Riaz/Kelly in Hull (Kelly et al. 2014) Micaela, Jilong Li, Bern), MiFish at NatureMetrics (Miya et al. 2015) and Teleo in Belgium (Valentini et al. 2016).

Furthermore we aimed to form a wider European fish metabarcoding working group embedded within DNAqua-net which has been initiated by Dr. Hänfling.

Prior to STSM

Prior to the STSM the water was collected in respective countries, filtered by Sterivex (Spens et al. 2016) and extracted during stringent conditions in laboratories - designed for eDNA only - by respective collaborators. The different collaborators found the STSM extremely useful for knowledge transfer, improvements of protocols and exchange of experiences regarding DNA extractions, metabarcoding and bioinformatics. Several discussions and email exchanges took place between the 4 institutions prior to the STSM in order to use the most suitable lab protocols during the STSMs

3. DESCRIPTION OF THE WORK CARRIED OUT DURING THE VISIT

The Hänfling laboratory was very welcoming. The first days were spent discussing and the different participants finalized and discussed the final protocols before initiating the project.

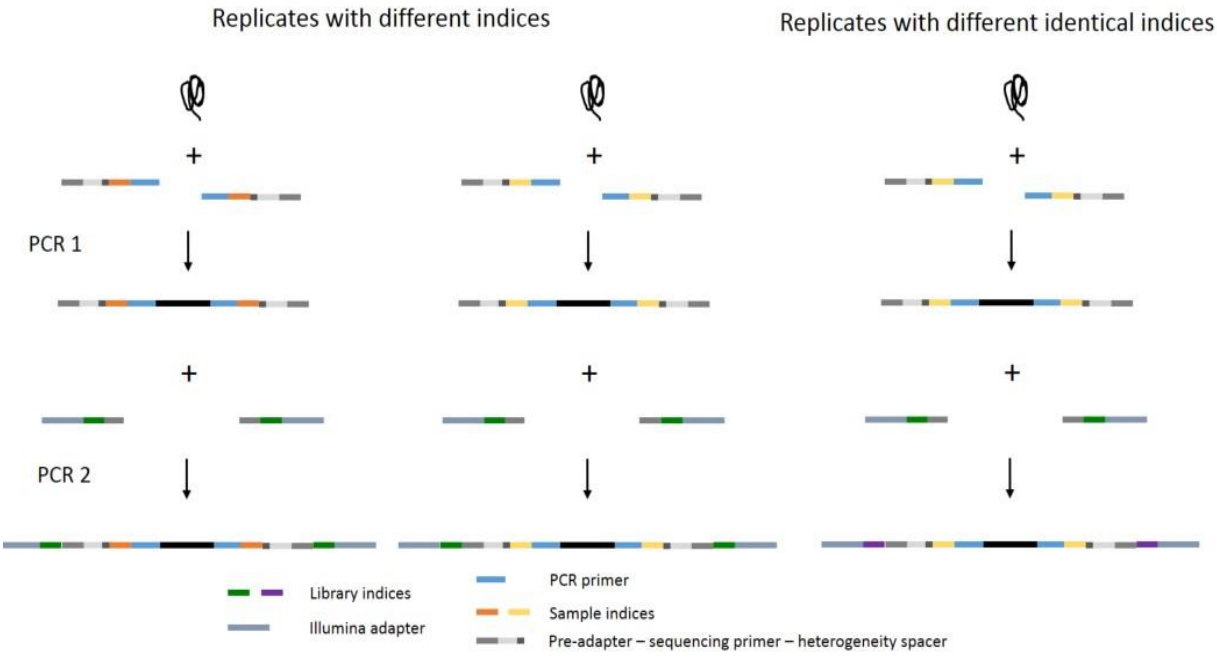


Figure 1. PCR setup and nature of primers and probes.

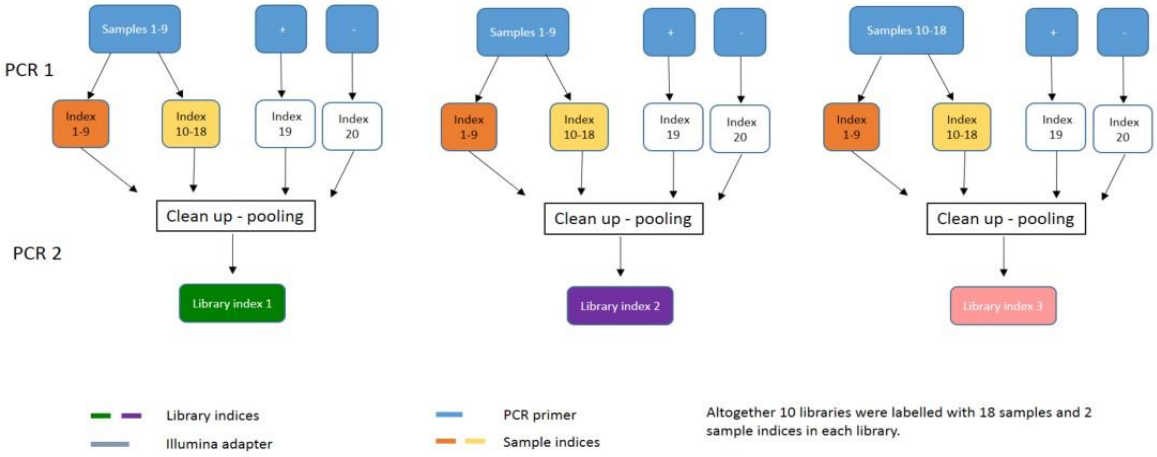


Figure 2. Setup of laboratory design per marker. Three out of ten libraries for one marker are shown in the figure.

The COST action gave me an opportunity to for the first time work on every single step in the pipeline ranging from sample collection to sequencing. The pipeline for the Bioinformatics was run in Hull after I left due to time constraints. However, we have discussed the preliminary data and the bioinformatics pipeline. The very same data from all four laboratories will be run through 4 different pipelines of bioinformatics. The lab work in Hull was very intense and was carried out together with PhD student Mr. Jianlong Li.

Mock community samples (18) originating from both mesocosms with known biomass, and from samples harbouring specific target species were provided by NatureMetrics and INBO. Additionally lake samples from the UK (27) and lakes never tested for eDNA is Sweden (9) were included. Altogether 10 libraries were prepared.

The DNA concentrations of the samples were measured. Aliquots from the Hull and AquaBiota laboratories were sent to the collaborators for testing their assigned primer sets. Furthermore eDNA extraction protocols were compared between the laboratories. During the course of the work and during discussions with other members of the Hänfling laboratory we developed and optimized the protocols. As each library was run on a separate PCR session altogether 30 libraries with 10 libraries per marker were prepared. The 10 Hull results from the 10 Kelly libraries were analyzed on a MiSew V2 (2*250 bp). The samples from the UK and Belgium had previously been tested in other assays whereas the samples from Sweden have not been analyzed with eDNA before.

4. RESULTS.

The results are still preliminary but show patterns and clear trends beyond what we dared to hope for. The read PF was 17.8 million and a first analysis showed that we have managed to target nearly

40 species of fish. This collaboration shed new light on the NGS sequencing of fish species in lacustrine ecosystems and will results in more scientific publications than initially anticipated.

5. FUTURE COLLABORATION WITH HOST INSTITUTION

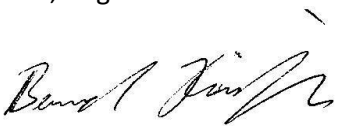
The COST action made this collaboration possible and both parties have started to work closely together with further development of the eDNA based monitoring techniques. The collaboration also includes research teams at INBO and NatureMetrics in the UK. Both institutes have started new collaborations related to the COST action, and focussing on development of DNA-based bioassessment techniques.

The work of the action will give rise to publications in peer reviewed journals and the participants have already initiated new research projects together with very promising results.

6. CONFIRMATION BY THE HOST INSTITUTION OF SUCCESSFUL EXECUTION OF THE STSM

Here I confirm that the goals of the STSM were reached during the visit by Dr. Micaela Hellström during the STSM visit in Hull. The project was carried out according to plan and has resulted in the generation of an excellent data set which allow us to explore the impact different primers and library preparation protocols. Our initial results are promising and will lead to more publications than initially anticipated. Crucially the COST action also has led to further collaborations of the participating parties and the initiation of a number of other eDNA projects.

Hull, August 29th 2017



Bernd Hänfling

7. PROJECTED PUBLICATIONS/ARTICLES RESULTING OR TO RESULT FROM THE STSM

The findings are being processed into manuscripts to be published in peer-reviewed journals and will be presented in national and international conferences.

8. THANK YOU

I would like to thank the COST DNAqua-Net for providing this amazing opportunity and I would also like to thank Dr. Bernd Hänfling and his communicative and skilled group for hosting me. A special thanks to Mr. Jianlong Li for sharing his insights in eDNA and for spending time with me in the laboratory during very long hours.

9. REFERENCES

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