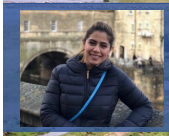


Development and Application of Genomic Tools for Tracking Viral Outbreaks in Fish

Shayma Alathari[1], Richard Paley[2], David V. Jeffreys[2], Charles R. Tyler[1], Ben Temperton[1]

[1] Biosciences, College of Life & Environmental Sciences, Geoffrey Pope Building, Stocker Road, University of Exeter, EX4 4QD. [2] Cefas , The Nothe, Barrack Road, Weymouth.

1. INTRODUCTION:

- **Tilapia** are the 2nd highest farmed fish globally after carp, contributing hugely to global food security & to the economies, especially of low- and middle- income countries.
- **2017-** Nile tilapia production in Ghana reached 53,000 tons.
- **2018-** Ghana reported mass mortalities in tilapia caused by **ISKNV** (Infectious spleen and kidney necrosis virus).
- Most farms suspended production; employees were laid off & locals income security was adversely impacted.

Fig.1 Culling tilapia fish during the ISKNV outbreak in Ghana.

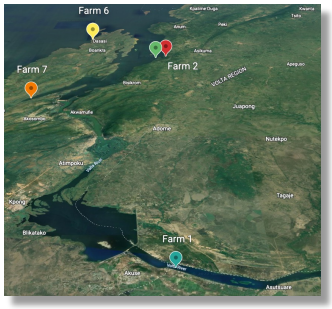


Fig.2 Map of the lower region of Lake Volta/ Ghana, showing farms where samples were collected.

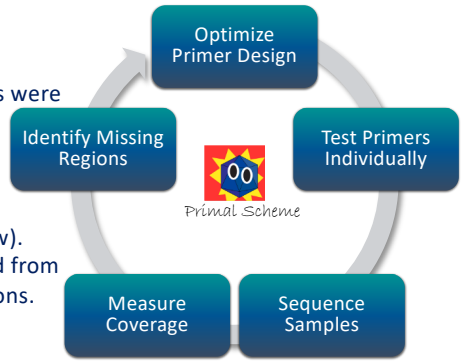
2. AIMS:

a) Develop a PCR-tiled sequencing approach for field-based whole-genome sequencing of dsDNA viral fish pathogens (adapting the **ARTIC-Network** protocol) to improve understanding of transmission and variant evolution.

b) Adapt this protocol for field-based real-time surveillance.

3. METHODS:

- 34 *Nile Tilapia* fish samples were collected from four farms on **Lake Volta 2018-2022**.
- Primers were designed.
- The final version **(V3)** was chosen for tiled PCR (below).
- 6kb primers were designed from **V1** to recover missing regions.



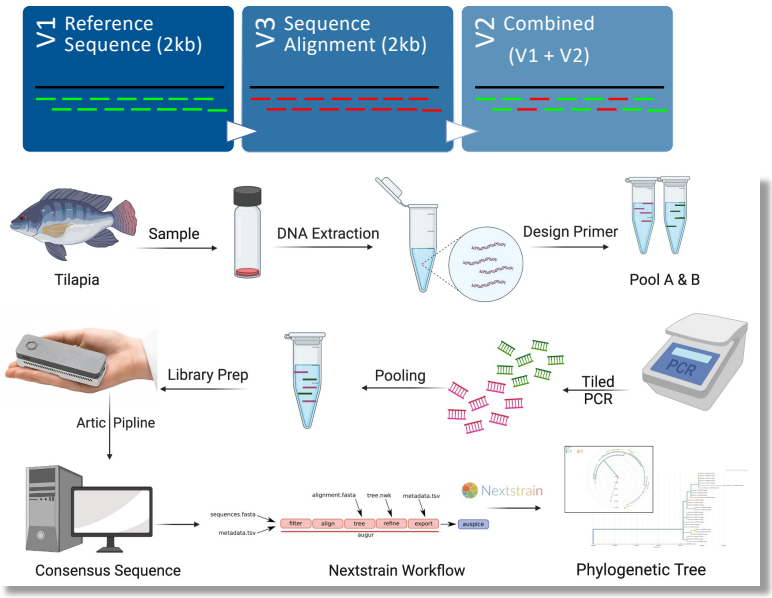


Fig.3 An illustration of processing tissue samples collected from infected fish, across Lake Volta (2018-2019).

4. RESULTS:

- (85-96%) of the ISKNV genome was recovered, using 2kb amplicons.
- 6kb amplicons recovered missing regions.
- Samples from different farms clustered separately.
- ISKNV from recent outbreak (2022) is genetically distinct from ISKNV collected in 2018, despite sampling from the same farm.
- Variants of ISKNV are host specific.

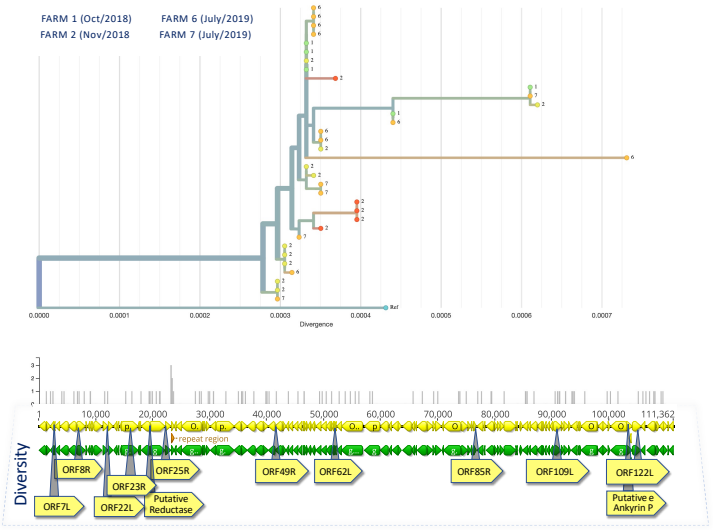


Fig.4 A rectangular phylogenetic tree focusing on genetic divergence [generated in ***Nextstrain***]. Divergence between Lake Volta samples is shown [below] in structural and non-structural proteins.

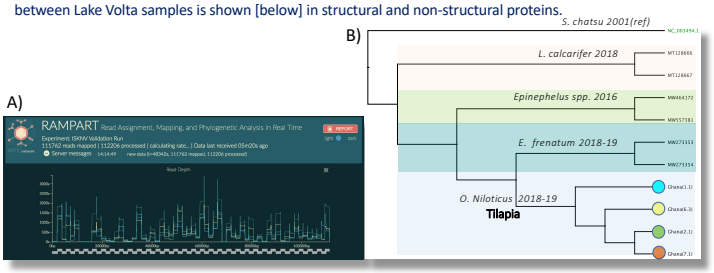


Fig.5 A) Real-time full genome sequencing [visualized in ***Rampart***]- read depth & coverage for Lake Volta samples. **B)** Phylogenetic tree [***Geneious Prime***] comparing ISKNV infecting tilapia with other hosts, showing phylogeographic signal & variants cluster according to host.

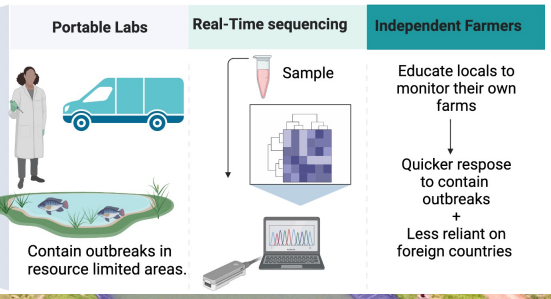
5. CONCLUSIONS & FURTHER DIRECTIONS:

(i) PCR tiled sequencing approach was successful with ISKNV, a large dsDNA virus.

(ii) A phylogeographic signal was captured across a short period of time.

(iii) This approach can be applied in the field to track other important fish viruses threatening aquaculture.

(iv) Water monitoring as a non-invasive alternative to track viral outbreaks in aquaculture.



References: [1] ARTIC Network https://artic.network/. 2020. [2] Augur https://docs.nextstrain.org/projects/augur/en/stable/.html. [3] FAO. The state of world fisheries and aquaculture. 2018. [4] Geneious Prime v 11.09+11 https://www.geneious.com. [5] RAMPART https://artic.network/rampart. [6] Ramírez-Paredes JG, Paley RK, Hunt W, Feist SW, Stone DM, et al. 2019. [7] Quick J, Loman NJ, Duraffour S, Simpson JT, et al. 2016. *Nature* 530: 228–32. [8] https://www.voltacatch.com/volta-catch-tilapia-tropo-farms . [9] https://www.ghanaweb.com. [10] https://biorender.com [11] https://www.intrafish.com/aquaculture.