# Tracking Fish Viruses in the Field

## (On a Lake, On a Farm, In a Hotel Room & In the Dark)

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## Introduction:

- **Tilapia** fish contribute hugely to global food security & to the economies, especially of low- and middle- income countries.
- **2018-** Ghana reported mass mortalities in tilapia caused by **ISKNV** (Infectious spleen and kidney necrosis virus).
- Ghana is still experiencing high mortalities due to ISKNV.
- Water sampling could be an alternative to destructive sampling in fish.

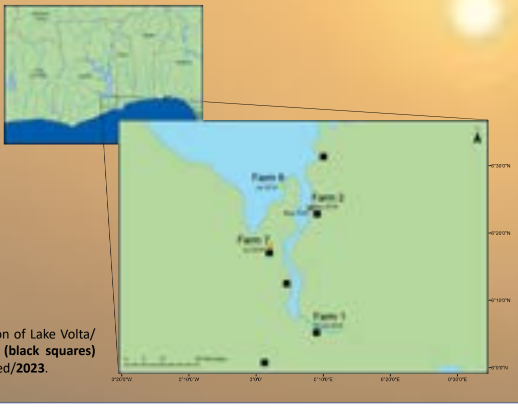

**Fig 1** Map of the lower region of Lake Volta/ Ghana, showing new farms **(black squares)** where samples were collected/**2023**.

## Methods:

To test our tiled PCR method developed for tracking viral outbreaks in fish:

- Primers were designed using the *Primal Scheme*
- We tested our method using samples collected from the first outbreak in **2018/2019**, and recent samples from **May/2022**
- We collected 72 *Nile Tilapia* fish samples from 6 distanced farms on Lake Volta **Jan/2023.**
- In-field sequencing was performed on the farms, following DNA extractions from fish spleen & liver and tiled PCR.
- Water samples were collected, and viruses were captured on 0.1mµ filters.
- ddPCR was performed to determine minimal input for genome recovery of ISKNV using the tiled PCR protocol.

## Results:

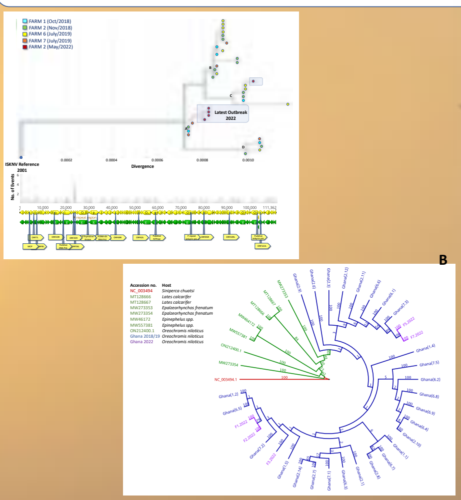

**Fig 2 a)** A phylogenetic tree focusing on genetic divergence [generated in ***Nextstrain***]. **b)** Phylogeny of whole ISKNV genomes of samples collected from Lake Volta (2019 in blue and 2022 in purple) with whole ISKNV genomes reported in the GenBank in green [MAFFT]. The tree was rooted to the ISKNV reference genome (NC_003494), shown in red.

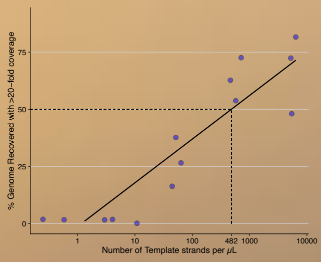

**Fig 3** Successful recovery of >50% of the ISKNV genome required 482 template strands per µL (2410 viral templates per 5 µL sequencing reaction), with at least 20-fold coverage. No. of viral templates measured by ddPCR of a serially diluted ISKNV template, which were subsequently sequenced

## Discussion:

- ISKNV is evolving, despite being a dsDNA virus.
- A mutation in the MCP discovered in samples collected in 2022, while another witnessed only in samples from Ghana.
- ISKNV collected from Ghana is distinct from all ISKNV samples infecting other hosts.
- The number of viral template needed to recover 50% of the genome was identified.
- Water monitoring to be used as a non-invasive alternative to track viral outbreaks in fish.
- Training vets and farmers to use the MinION to track viral outbreaks in their farms is possible.
- Take a Qubit!!!

**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] Ramírez-Paredes JG, Paley RK, Hunt W, Feist SW, Stone DM, et al. 2019. [6] Quick J, Loman NJ, Duraffour S, Simpson JT, et al. 2016. *Nature* 530: 228–32. [7] https://www.voltacatch.com/volta-catch-tilapia-tropo-farms . [8] https://www.ghanaweb.com. [9] https:// www.intrafish.com/aquaculture.