# *Introduction to eDNA & Pilot study to detect Pir-AB*

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# Introduction to eDNA

eDNA

"True environmental samples such as water, soil, sediment, biofilms." WOAH 2022

"Excludes direct host-derived material such as faeces, mucous, sloughed cells " WOAH 2022

eNA = Environmental Nucleic acid

eDNA = Environmental Deoxyribonucleic acid

eRNA = Environmental Ribonucleic acid

eDNA analysis is using water incubated filters to detect pathogen targets as a replacement for the analysis of tissue samples

The use of environmental DNA methods for detection of WOAH listed aquatic animal diseases

Original: English (EN)
February 2022

A discussion paper developed by the WOAH Aquatic Animal Health Standards Commission (Aquatic Animals Commission).

# Why use eDNA as a sampling approach ?

Detection of a Pathogen in the test environment (pre-infection)

Non-invasive, no tissue damage to stock

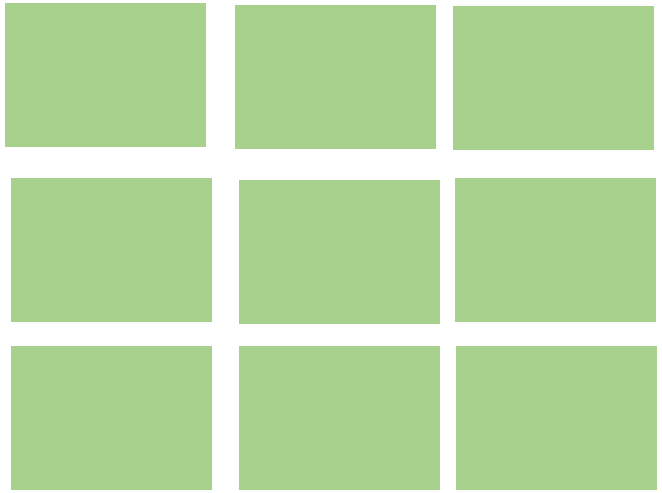
No sharps handling by staff (safety)

Staff aren't handling high load tissues (biosecurity risk)

Theoretically detect a pathogen before clinical signs

Significant cost saving in testing and time to collect samples

E.g. Recently used to detect SARS-Coronavirus in sewerage treatment plants



Sampling of a population of 500,000 to detect a pathogen with a 10% prevalence with 95% confidence requires sampling of 39 prawns per pond.
If tested as pools of 3 = 13 samples
13 samples x $44 per sample for analysis = **$572 each pond**.

eDNA sample collection of 2 incubated filters samples
2 x $44 per sample for analysis = **$88 per pond**. ( x6)

# Workflow of eDNA

eDNA Typical workflow

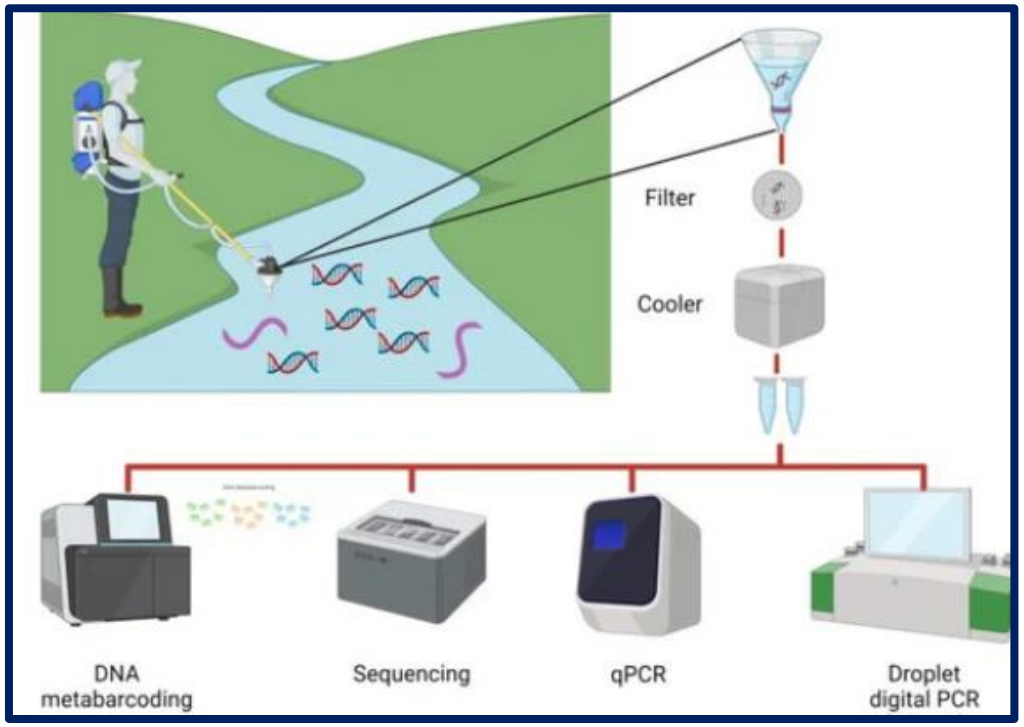
Collect water sample and filter or

Filter water at point of collection

Extract the nucleic acid (DNA, RNA or both)

Analyse the nucleic acid

Positive/Negative detection & copy number (load)




Farm management action

Pathogen population dynamics

# Issue with traditional workflow of eDNA

Filtering is typically clear water systems

Collect water sample and filter or

Filter water at point of collection

Farm management action

Pathogen population dynamics

Purpose of the testing requires the process to be standardised i.e., completed the same way every time it is conducted. The standardisation is easily achievable (500ml) in clear water systems but…..

The algal blooms in pond systems create the challenge in the number of filters required for a standardised volume e.g (500ml). Variable filter numbers reduce the comparability of multiple samples

Standardize by incubation time of the eDNA filter (passive eDNA)

# eDNA Pilot: Can Pir-AB be detected using eDNA?

Pir-AB (Photorhabdus insect related toxin gene A & B components)

Pir-AB : the toxin gene hosted by *Vibrio parahaemolyticus* to cause AHPND

Pir-AB : the toxin gene is also hosted by other Vibrio species involved in hepatopancreatitis

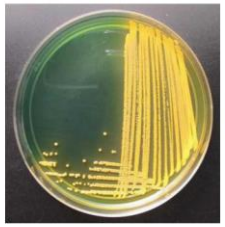
Current detection relies on collection of HP, gut samples > cultured > qPCR

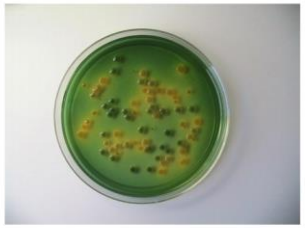
Vibrio bacteria are free-living and common in marine systems so should theoretically be detectable in water

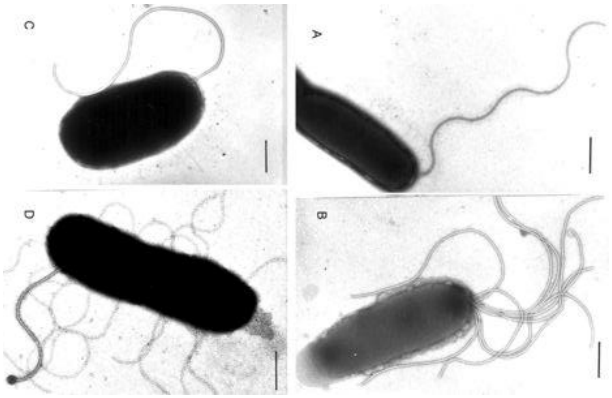
First step? Can Pir-AB be detected in prawn pond water during a hepatopancreatitis event?

i.e. Can we detect Pir-AB with eDNA at a time when we know it is present in prawns?

Hepatopancreatitis occurs sporadically and relatively infrequently







# eDNA Pilot: Can Pir-AB be detected with eDNA ?

2022: Confirmed hepatopancreatitis on prawn farm

Management considerations : 1. Impact of Pir-AB could be reduced with management

Management considerations: 2. Harvest is limited by processing capacity

Management considerations: 3. Prioritized harvest best achieved with ranking of load

Management considerations: 4. Can degrade to a rapid onset of mortality

Previous study: demonstrated Pir-AB could be detected on pleopods without culture

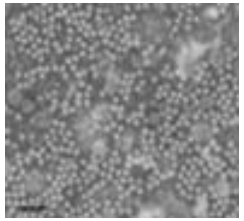
This study: Piloted eDNA approach on 7 ponds

Incubated eDNA filters for 2 durations: 24 and 48 hours> tested in triplicate >qPCR

Collected pleopod tissues from 39 animals from each pond >qPCR

# Results: Number of positive and qPCR Ct value of samples

| Pond Number | Sample | Number of tests | Pir-A | | Pir-B | |
|---|---|---|---|---|---|---|
| | | | Number of +ve | Average Ct of +ve | Number of +ve | Average Ct of +ve |
| A | 24hr eDNA | 3 | 1 | 37.65 | 0 | nd |
| | 48hr eDNA | 3 | 0 | nd | 0 | nd |
| | pleopods (pool of 3) | 13 | 0 | nd | 0 | nd |
| B | 24hr eDNA | 3 | 0 | nd | 0 | nd |
| | 48hr eDNA | 3 | 0 | nd | 0 | nd |
| | pleopods | 13 | 0 | nd | 0 | nd |
| C | 24hr eDNA | 3 | 3 | 33.54 | 3 | 34.25 |
| | 48hr eDNA | 3 | 3 | 35.09 | 3 | 35.47 |
| | pleopods (pool of 3) | 13 | 4 | 38.52 | 0 | nd |
| D | 24hr eDNA | 3 | 0 | nd | 0 | nd |
| | 48hr eDNA | 3 | 3 | 33.46 | 3 | 33.56 |
| | pleopods (pool of 3) | 13 | 0 | nd | 0 | nd |
| E | 24hr eDNA | 3 | 3 | 30.95 | 3 | 31.13 |
| | 48hr eDNA | 3 | 1 | 28.63 | 1 | 29.52 |
| | pleopods (pool of 3) | 13 | 6 | 38.52 | 0 | nd |
| F | 24hr eDNA | 3 | 2 | 36.06 | 2 | 36.10 |
| | 48hr eDNA | 3 | 0 | nd | 0 | nd |
| | pleopods (pool of 3) | 13 | 0 | nd | 0 | nd |
| G | 24hr eDNA | 3 | 0 | nd | 0 | nd |
| | 48hr eDNA | 3 | 3 | 38.97 | 3 | 37.97 |
| | pleopods (pool of 3) | 13 | 1 | 38.70 | 0 | nd |
| | Grand Total | 133 | 30 | 35.94 | 18 | 34.38 |



eDNA approach detected Pir-A and Pir-B

Detection of Pir-A more frequent than Pir-B

Copy number in eDNA was higher than in prawn pleopod

Indicate higher priority harvest of Pond E in schedule

Indicate lower priority harvest of Pond B in schedule

# eDNA Pilot: Can other pathogen targets be detected?

| Pond | Sample | Prawn Pathogen target genes | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | GAV | IHHNV | HPV | Zon occludens toxin | Hemolysin D toxin | Repeats-in Toxin | Pir-A | Pir-B |
| A | 24hr eDNA | 3 | 0 | 0 | 1 | 0 | 3 | 1 | 0 |
| | 48hr eDNA | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | pleopods | 13 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| B | 24hr eDNA | 3 | 0 | 0 | 1 | 0 | 3 | 0 | 0 |
| | 48hr eDNA | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | pleopods | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C | 24hr eDNA | 3 | 0 | 0 | 3 | 0 | 3 | 3 | 3 |
| | 48hr eDNA | 3 | 0 | 0 | 0 | 0 | 3 | 3 | 3 |
| | pleopods | 13 | 1 | 0 | 0 | 0 | 0 | 4 | 0 |
| D | 24hr eDNA | 2 | 0 | 0 | 1 | 0 | 3 | 0 | 0 |
| | 48hr eDNA | 3 | 0 | 1 | 3 | 0 | 3 | 3 | 3 |
| | pleopods | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| E | 24hr eDNA | 2 | 0 | 0 | 2 | 0 | 3 | 3 | 3 |
| | 48hr eDNA | 2 | 0 | 0 | 1 | 0 | 1 | 1 | 1 |
| | pleopods | 13 | 0 | 0 | 0 | 0 | 0 | 6 | 0 |
| F | 24hr eDNA | 3 | 0 | 0 | 3 | 0 | 2 | 2 | 2 |
| | 48hr eDNA | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | pleopods | 13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| G | 24hr eDNA | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 48hr eDNA | 3 | 0 | 0 | 3 | 1 | 3 | 3 | 3 |
| | pleopods | 13 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |

GAV was detected in eDNA noting was in high prevalence in tissue

IHHNV was not detected in eDNA noting low prevalence in tissue

HPV was detected in eDNA not tissue

Toxin genes detected in eDNA more often than tissue

eDNA results from this pilot indicate the potential of the approach and further investigation is on-going

# eDNA Pilot: Further work

In theory Vibrio toxin genes should be monitorable using eDNA

El Nino represents a higher risk of increased Vibrio (temperature, salinity, algal bloom, pH fluctuation).

Validation of eDNA for Pir-AB requires comparison with tissue during positive detection (hepatopancreatitis event)

If can be validated, presents an additional tool for Pir-AB detection which is simple to deploy; short duration; able to sample whole farm in one day > farm management

If can be validated, presents a tool for better understanding the fluctuation of Vibrio-associated genes (toxins or others) in the farm environment > ecology of pathogenic Vibrio

The use of environmental DNA methods for detection of WOAH listed aquatic animal diseases

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February 2022

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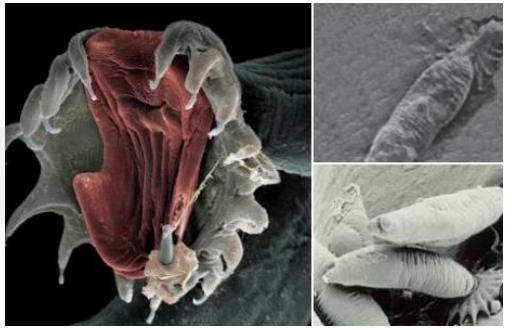
The WOAH recognizes eDNA as a valid approach for monitoring *Gyrodactylus salaris*



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