The Next eDNA Target: Microparasites

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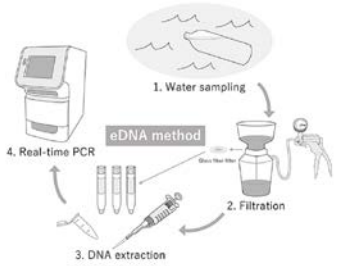
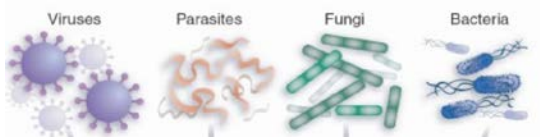
Definitions:
eDNA & Microparasites

- Environmental DNA (eDNA) is DNA extracted from environmental sources.
- eDNA is free or cell associated.
- Sources include soil, air, water, ingesta, and feces.



- Target is identified using nucleic acid assays such as qPCR, microarray, and sequencing.

- Microparasites include bacteria, viruses, protozoans, and fungi.
- May spend their entire lifestage with one host.
- Have the capacity to cause disease.



- Difficult to study due to their extensive diversity, microscopic size, and complex life cycles.

Why Filter Microparasite eDNA?

- Because we can! Microparasites of aquatic animals such as fish, mollusks, and crustaceans can be captured by filtration of water.
- Large-scale sampling of water has fewer biases and higher sensitivity than specimen-based methods (Bass et al. 2015).
- eDNA is detectable in varying degradation conditions (Huver et al. 2015).
- eDNA methods have already expanded the diversity of endoparasitic protistan lineages (Hartikainen et al. 2014; Okamura et al. 2015).

Bass, D., Stentiford, G.D., Littlewood, D.T.J., Hartikainen, H. 2015. Diverse applications of environmental DNA methods in parasitology. Trends Parasitol. 31:499–513.

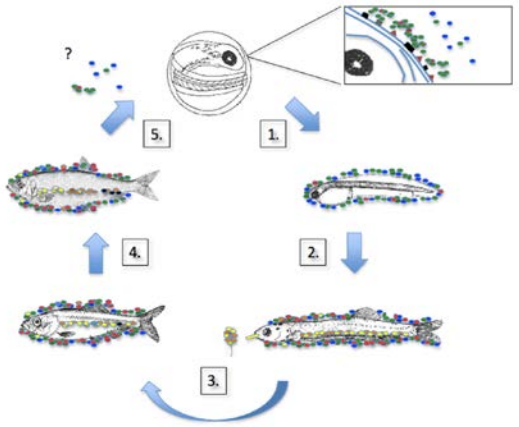
Hartikainen H, Ashford OS, Berney C, Okamura B, Feist SW, Baker-Austin C, Stentiford G, Bass D (2014) Lineage-specific molecular probing reveals novel diversity and ecological partitioning of haplosporidians. ISME J 8:177–186

Huver JR, Koprivnikar J, Johnson PTJ, Whyard S. 2015. Development and application of an eDNA methods to detect and quantify a pathogenic parasite in aquatic ecosystems. Ecol Appl. 25:991–1002.

Okamura, B., Gruhl, A. and Bartholomew, J.L., 2015. An introduction to Myxozoan evolution, ecology and development. In *Myxozoan evolution, ecology and development* (pp. 1-20). Springer International Publishing.

Additional advantages of eDNA?

- Quantification of both microparasites and hosts can resolve complex interactions and give novel insights into host-associated microbiomes, pathology, and etiology (Bass et al. 2015).
- Targets DNA (or RNA) from organisms present in a non-invasive sample.
- Knowledge of spatial and temporal abundances of infectious stages may provide early warning systems (Hartikainen et al. 2014).
- May allow assessment of disease risk when siting aquaculture facilities.



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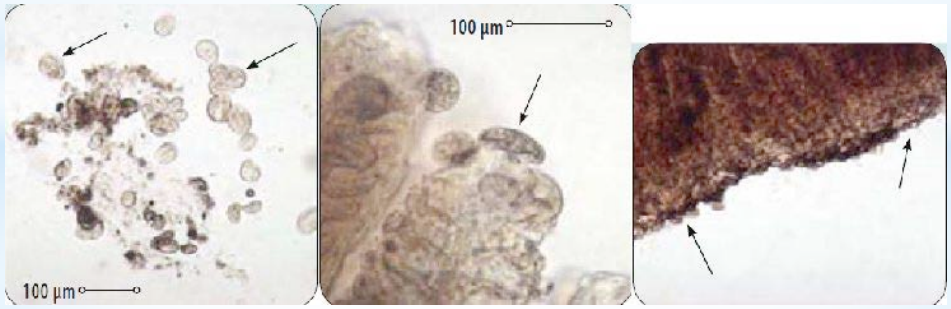
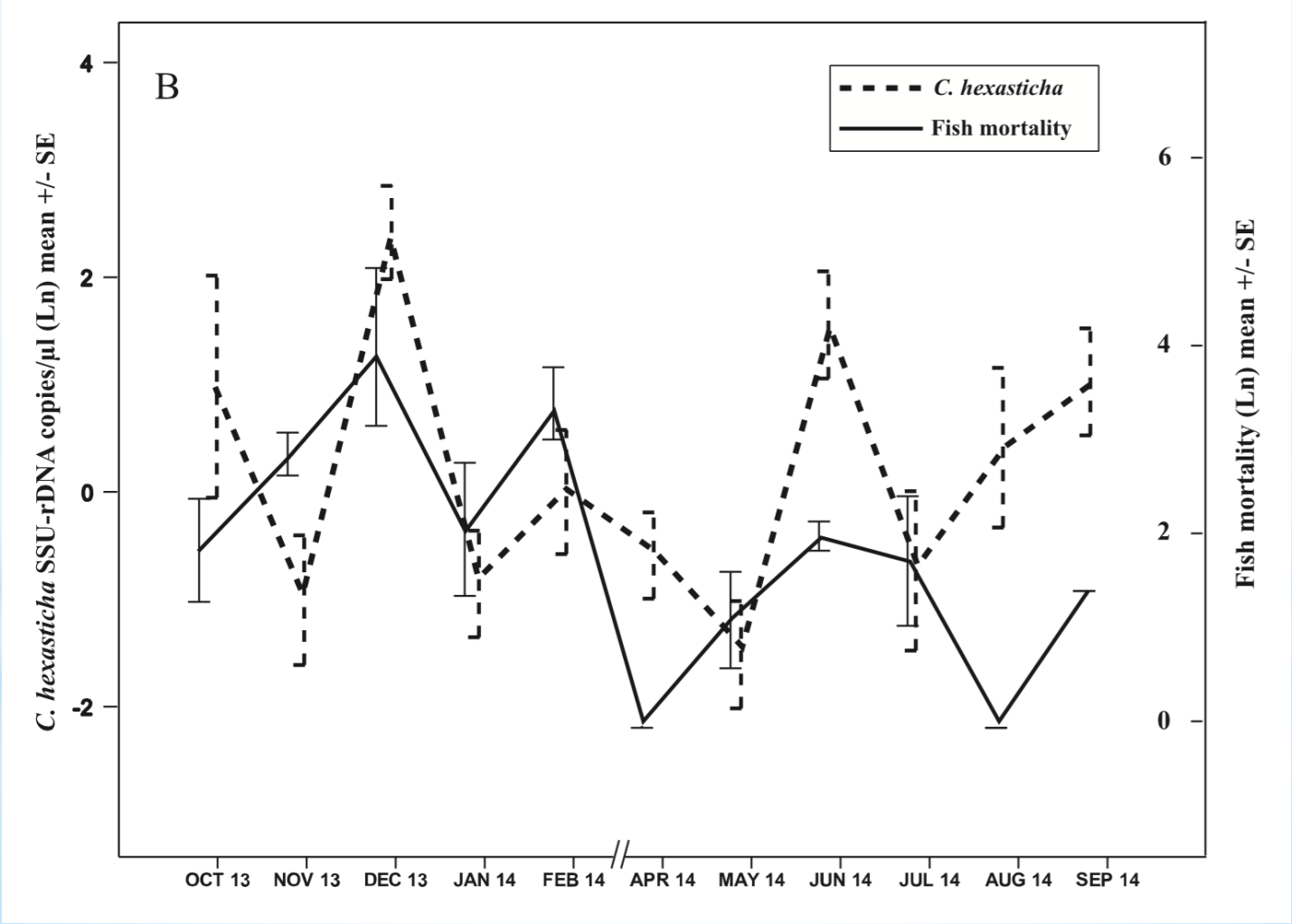
Examples of Microparasite eDNA Applications

- Bastos Gomes et al. (2017) rapidly assessed the background presence of a ciliate parasite in barramundi fish farms each month.
- Studied the relationship between eDNA of *Chilodonella hexasticha*, critical water parameters, and the occurrence of disease outbreaks.
- qPCR assay based on the small subunit ribosomal DNA gene was used to monitor the abundance of *C. hexasticha* in pond water.
- No correlations were found between abundance of this parasite and any of the water quality parameters measured (rainfall, water temperature and dissolved oxygen).
- Size of fish (smaller) and mortality were correlated though.



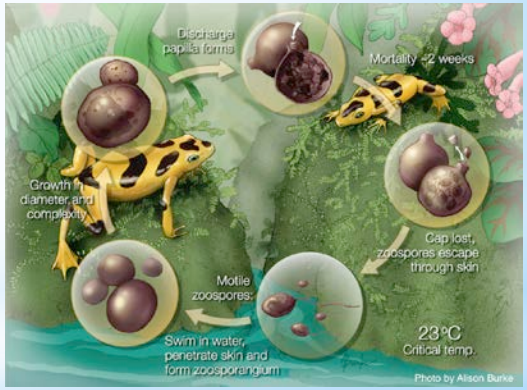
Bastos Gomes, Giana, Hutson, Kate S., Domingos, Jose A., Chung, Catherine, Hayward, Scott, Miller, Terrance L., and Jerry, Dean R. (2017) *Use of environmental DNA (eDNA) and water quality data to predict protozoan parasites outbreaks in fish farms.* Aquaculture, 479. pp. 467-473.

Chilodonella Abundance Correlated with Fish Mortality (r = 0.402; P < 0.001)



Examples of Microparasite eDNA Applications

- Methods exist to detect the chytrid fungus *Batrachochytrium dendrobatidis* (Bd) and Ranaviruses in water and sediment samples.
- Both pathogens have been identified as causes of amphibian declines.
- eDNA used to correlate the environmental conditions under which Bd becomes infectious, and the mechanisms by which it spreads.
- Kolby et al. (2015) performed a rapid response investigation to evaluate the presence and distribution of amphibian pathogens in Madagascar.
- Ranavirus was detected in water samples collected from two commercial amphibian export facilities.

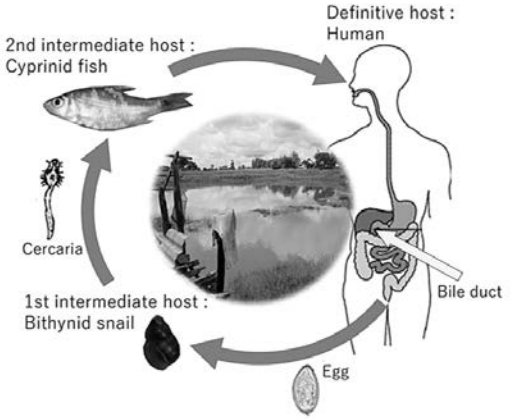


Kirshtein JD, Anderson CW, Wood JS, Longcore JE, Voytek MA. 2007. Quantitative PCR detection of *Batrachochytrium dendrobatidis* DNA from sediments and water. Dis Aquat Org 77:11-15.

Kolby JE, Smith KM, Ramirez SD, Rabemananjara F, Pessier AP, et al. 2015. Rapid Response to Evaluate the Presence of Amphibian Chytrid Fungus (*Batrachochytrium dendrobatidis*) and Ranavirus in Wild Amphibian Populations in Madagascar. PLOS ONE 10(7): e0134524.

Examples of Microparasite eDNA Applications

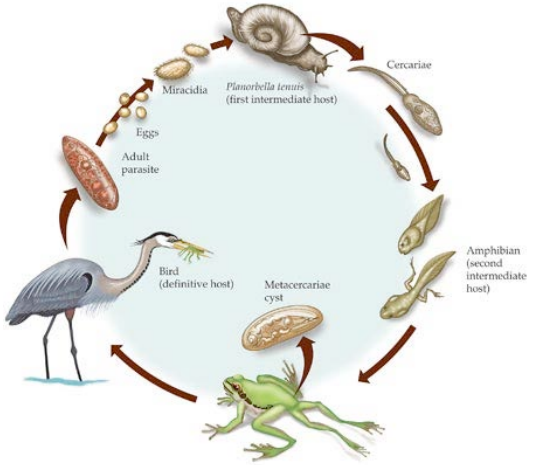
- eDNA analysis of water samples in Laos detected the Southeast Asian liver fluke, *Opisthorchis viverrini,* a major public health problem (Hashizume et al. 2017).
- Accurate and rapid monitoring of O. viverrini is crucial for disease prevention and containment.
- *O. viverrini* eDNA was detected in five samples by real-time PCR, indicating the presence of the fluke in the area and the risk of infection for individuals consuming fish from these water sources.



Hashizume H, Sato M, Sato MO, Ikeda S, Yoonuan T, Sanguankiat S, et al. 2017. Application of environmental DNA analysis for the detection of *Opisthorchis viverrini* DNA in water samples. Acta Tropica. 169:1–7.

Examples of Microparasite eDNA Applications

- eDNA method to examine the distribution and abundance of the trematode *Ribeiroia ondatrae*, a pathogenic parasite known to cause malformations in North American amphibians was developed (Huver et al. 2015).
- Compared this eDNA approach to classical host necropsy.
- Examined the detectability of *R. ondatrae* in water samples subject to different degradation conditions (time and temperature).
- Capable of detecting as little as 14 fg of this parasite's DNA (1/2500th of a single infectious stage) from field water samples.



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Examples of Microparasite eDNA Applications

- Six pathogenic fish viruses—three DNA and three RNA viruses—were monitored in two lakes in Yunnan, China (Minamoto et al. 2015).
- *Cyprinid herpesvirus 1, 2, and 3* as well as three rhabdoviruses, *Infectious hematopoietic necrosis virus, Viral hemorrhagic septicemia virus, and the Spring viremia of carp virus*, all of which have severe impacts on aquaculture, were targeted.
- Viruses in the pre-filtered lake water samples were trapped with Al^{3+}-coated HA electronegative filters (pore size = 0.45 microns and then concentrated from 15 mL to 200 uL with Amicon Ultra-15 Centrifugal Filter Units.
- Pathogen monitoring could be a powerful tool for predicting or preventing infectious diseases.
- Future research aims to relate the virus copy number to the infection risk.

Minamoto, T., Pu, X., Xie, J., Dong, Y., Wu, D., Kong, H., Yang, X., Takahara, T., Honjo, M.N., Yamanaka, H., Kawabata, Z., 2015. Monitoring of fish pathogenic viruses in natural lakes in Yunnan, China. Limnology 16:69–77.

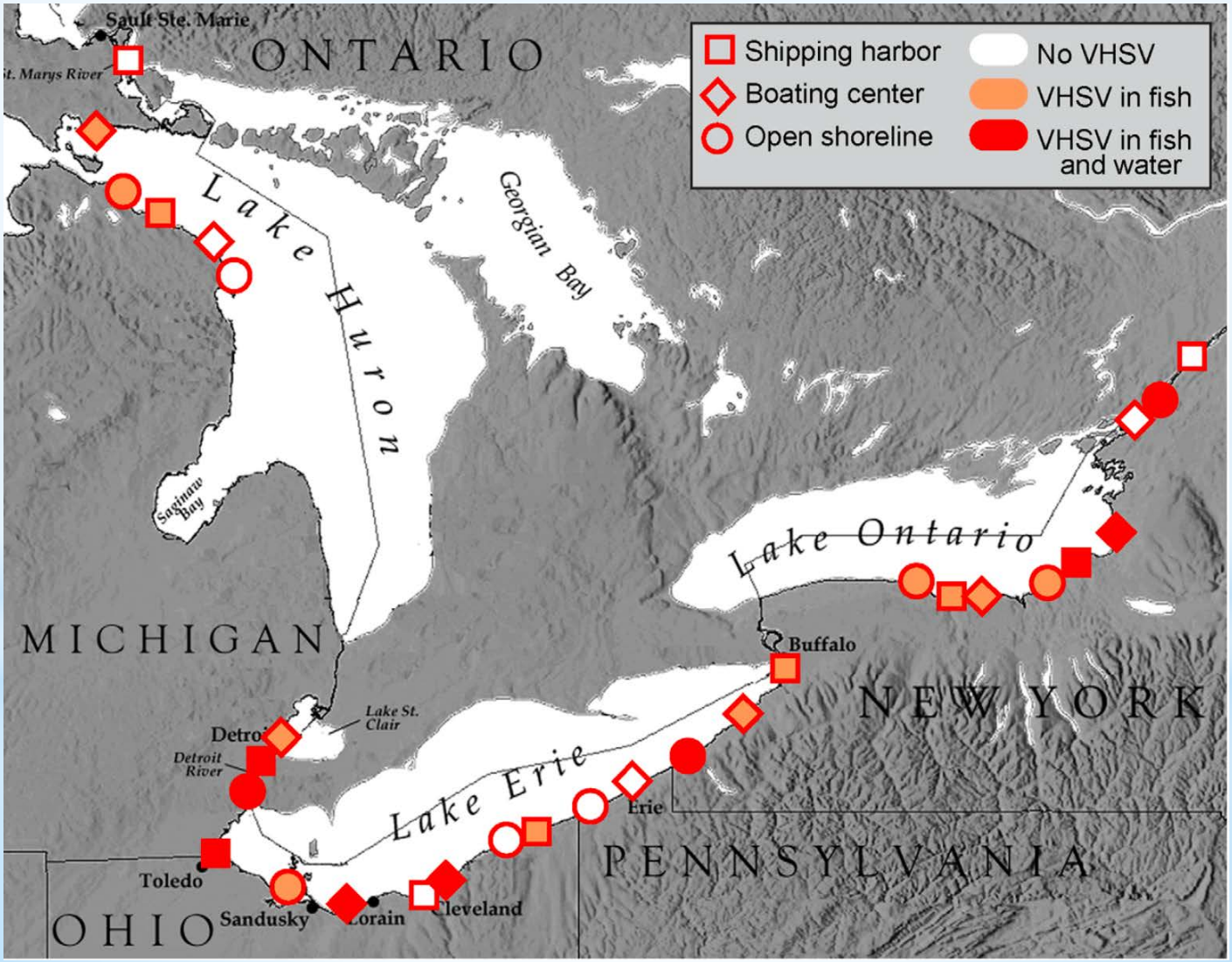
Minamoto, T., Naka, T., Moji, K., Maruyama, A., 2016. Techniques for the practical collection of environmental DNA: filter selection, preservation, and extraction. Limnology 17:23–32.

Examples of Microparasite eDNA Applications

- Both invasive round goby and a deadly rhabdovirus originated near Lake St. Clair on the US Canadian border. The goby were found in 1990.
- The *Novirhabdovirus,* viral hemorrhagic septicemia virus (VHSV), was discovered in muskellunge samples in 2003.
- In 2008, the Cornell Aquatic Animal Health Program, specifically Dr. Jim Casey, filtered 10-liter water samples collected from Lake Huron all the way to the St. Lawrence River.
- He used 0.22 um Tuffryn membranes (Pall Life Sciences) at 8 psi. The effluent was concentrated to 300 ml by tangential flow through a hollow fiber cartridge rated at 100,000 NMWC (GE Healthcare).
- VHSV was detected in 9 water samples from the Detroit River south of Lake St. Clair, Lake Erie, Lake Ontario, and the St. Lawrence River.

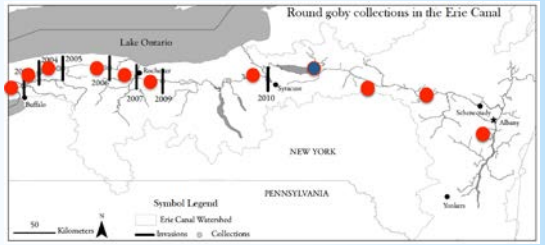
Bain, M. B., E. R. Cornwell, K. M. Hope, G. E. Eckerlin, R. N. Casey, G. H. Groocock, R. G. Getchell, P. R. Bowser, J. R. Winton, W. N. Batts, A. Cangelosi, and J. W. Casey. 2010. Distribution of an invasive aquatic pathogen (viral hemorrhagic septicemia virus) in the Great Lakes and its relationship to shipping. PLoS ONE [online serial] 5:e10156.

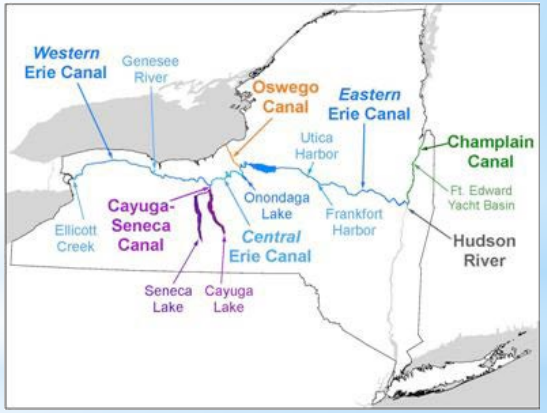
Examples of Microparasite eDNA Applications



Examples of Microparasite eDNA Applications

- The slow migration of round goby through the Erie Canal system ultimately led to a VHSV-induced fish kill at King Ferry and Long Point on Cayuga Lake in May 2017.
- Limiting the movement of round goby to new waters may slow the spread of VHSV (Cornwell et al. (2014).
- Our current efforts focus on how VHSV emerges from reservoirs like round goby to infect other fish.
- Citizen scientists have been trained to collect eDNA from water samples to allow researchers to track the movements of the invasive round goby throughout New York State.





Cornwell, E.R., A. Primus, P.T. Wong, G.B. Anderson, T.M. Thompson, G. Kurath, G.H. Groocock, M.B. Bain, P.R. Bowser, and R.G. Getchell. 2014. Round gobies are an important part of VHSV genotype IVb ecology in the St. Lawrence River and eastern Lake Ontario. Journal of Great Lakes Research 40:1002-1009.

Field-based eDNA Tools

- Collection and processing of aquatic eDNA samples for rapid field detection has been marketed by Smith-Root and Biomeme.
- The Australian Fisheries Research and Development Corporation is working on a DNA-based point of care device for on-farm detection of pathogens.
- The project ISMOTool, recently financed by the Research Council of Norway, hope to combine eDNA-based assays and an Environmental Sampling Processor to detect diseases and parasites which are causing substantial challenges for the aquaculture industry.
- Since 2011, scientists with WHOI have worked to develop a regional array of robotic instruments that draw water samples and analyze them for the DNA of particular HAB species.

http://www.smith-root.com/edna
https://www.jcu.edu.au/news/releases/2016/march/fish-farm-innovator-receives-national-award
http://www.iris.no/home/a-dna-based-tool-for-tackling-operational-and-environmental-challenges-in-aquaculture

Questions?

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