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Applications of environmental DNA data in support of aquaculture

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Abstract: With advances in analytical and computational technologies the data for environmental DNA or eDNA are becoming rapidly and increasingly available. eDNA data have been applied successfully to assess presence of fish species, impacts of human activities on benthic biota and more recently to a limited extent to assess biomass. Because of the relative ease with which eDNA data can be collected, the number of proposed applications is increasing rapidly; this includes applications to support aquaculture. Some of the applications of particular interest for aquaculture operations include measurement of eDNA as a surveillance tool for pathogens, protected species and escapees from *e.g.* net pens; as an indicator of benthic impacts and efficacy of stock enhancement operations; and health of cultured species.

Key words: environmental DNA, genetics, aquaculture, seafood, production

Introduction

Environmental DNA or eDNA is collected from the environment and not directly from organisms larger than about half a micrometer in size, as determined by pore size of filters used. The collection, extraction and analysis of eDNA have become a popular method in recent years. This was largely made possible by the development of technologies to analyze the genetic sequences of DNA and to the high-powered computing systems easing the analysis of the large data sets acquired.

Aquaculture in Japan is a well-established and thriving industry with an annual production in 2017 of about 700 thousand tons of freshwater and marine animal products; most of this is seafood. In Japan another approximately 500 thousand tons of aquatic plants are also produced annually (http://www.fao.org/fishery/countrysector/naso_japan/en). In contrast in the USA freshwater and marine aquaculture produced only 270 and 41 thousand tons, respectively, in 2017 and ranked 17th in global aquaculture production. Ironically the USA is the leading global importer of fish and fishery products; 90% of USA seafood is imported and half of this is from aquaculture.

Not surprisingly there is now a strong expectation that marine aquaculture will be a significant factor in increasing the USA's seafood productivity, thereby reducing the USA's reliance upon import of seafood and helping to assure food security for the nation. Toward this end, multiple applications exist for eDNA data to support aquaculture to increase seafood production in an environmentally friendly and sustainable manner. This short paper is a summary of a presentation at the US-Japan Bilateral Meeting in Okinawa, Japan, in 2019 and gives a brief overview of some of those applications.

Why environmental DNA?

There are several benefits, which make eDNA a promising research tool. Firstly, the sampling is non-invasive or only minimally invasive. If you can collect water, you can collect eDNA, *e.g.* from rivers, lakes, bays, surface and deep-sea water, and pore

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water in sediments. Further the set of eDNA data comprises a comprehensive overview of all living organisms which contributed DNA into the sampled volume of water in the recent past (generally up to a few days). Moreover, the methodologies for filtering the water, extracting the DNA, amplifying it, and sequencing the genetic material have advanced to the point that eDNA measurements are routine and taught even in high school courses. That said, there is continual (a) technological development to improve the sequencing and amplification of genetic material, (b) increasingly high powered computing systems to analyze millions of data points into Operational Taxonomic Units or OTUs, and (c) expanding reference libraries to correlate OTUs with species.

Quantitative polymerase chain reaction or qPCR can perform species-specific analyses on eDNA to provide quantitative findings of the target species. In contrast, next generation barcoding or metabarcoding provides an estimate of the relative abundance of the suite of organismal DNA collected on your filter. One of the major questions for researchers today is whether metabarcoding of eDNA can be used for quantitative species assessments. Another question is whether eDNA can be differentiated between different life-history stages of the same species. Answering these questions would significantly add to the value of eDNA as a field survey tool. Still even with limitations to using eDNA for biomass and aging estimates in the field, there are multiple ways in which eDNA can and is used to support fisheries and aquaculture.

Challenges of Interpreting eDNA Data

As illustrated in **Fig. 1**, the quantity of eDNA in seawater is affected by species properties including size, age, behavior such as spawning; as well as environmental parameters such as temperature, salinity, and UV light. Understanding the processes affecting your sample is critical to interpreting your data. Further, being aware of the biases inherent in the amplification process, *i.e.* some genetic sequences amplify more efficiently than others, is important (Kelly *et al.*, 2019). The extent to which these uncertainties are significant for your research depends much upon temporal and spatial scaling

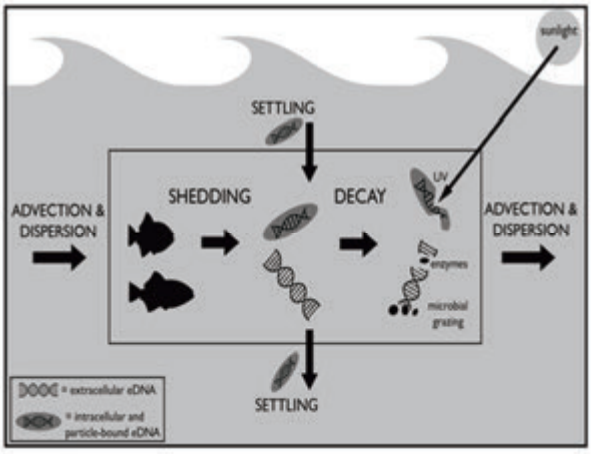

Fig. 1. Conceptual model of eDNA distribution in the water column. From Sassoubre *et al.* (2016).

requirements. Whether a decay rate of hake DNA is 2 days or 4 days is probably not significant for assessing distribution in the middle of the Pacific Ocean, but that same difference can be extremely important for estimating distribution of Atlantic salmon in a river in Maine or distribution of aquaculture escapees in an embayment.

Conceptually, to fully understand eDNA data from the field surveys, one would need to account for all the processes affecting eDNA distribution as shown **Fig. 1**, including shedding, enzymatic and other types of degradation, and physical processes such as advection, dispersion and export for example as feces. Realistically, we are likely to make faster progress in using eDNA as a survey tool by comparing field eDNA with trawl and acoustic survey data, and using the laboratory experiments to help in interpretation.

Ecosystem Services

Environmental DNA is often used to identify presence or absence of species. This can be *e.g.* an indicator of ecosystems services such as prey species' refugia or the impact of aquaculture on finfish diversity. In Milford, CT, USA, a team of researchers from the NOAA Milford laboratory has been measuring eDNA along with use of underwater video to evaluate the effect of caged oyster on fish diversity (Liu *et al.*, 2019). In the first year of investigation they identified 23 species from a total of 49 samples or 20 million reads from

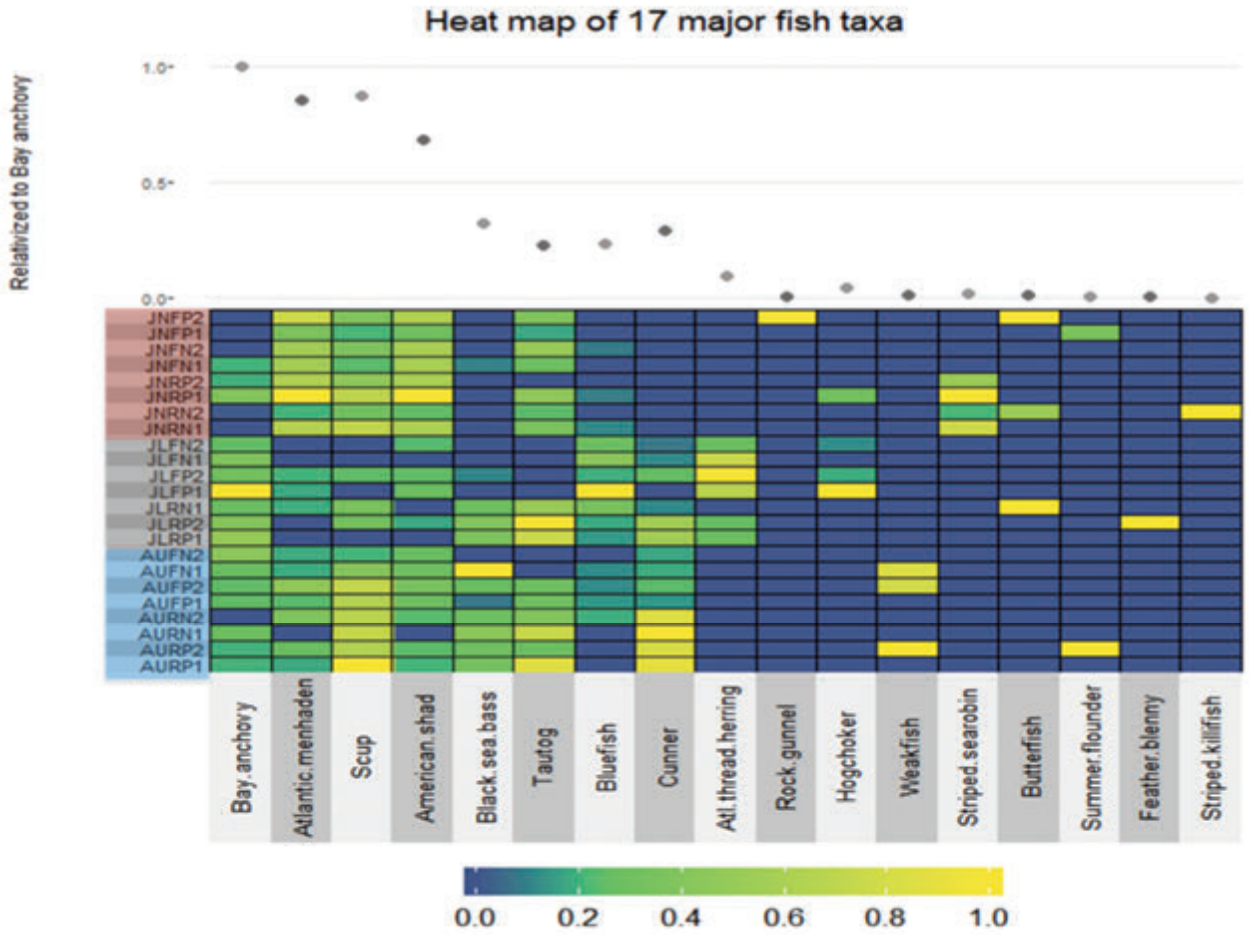

Fig. 2. Environmental DNA as a tool to assess spatiotemporal finfish distribution in relation to shellfish aquaculture operations. From Liu *et al.* (2019).

the sequencing data (**Fig. 2**). Notably, the species identified by eDNA overlapped but were not identical to the species identified with video, thereby leading researchers to conclude that employing both methods concurrently would give a more comprehensive result.

When is Quantity Important?

Of course scientists often want to know more than presence – absence of species in the field, and being able to estimate quantity even if in relative terms can be important. This is true, for example, for detection of pathogens in relation to critical thresholds for human consumption or permissible transport, estimating the frequency and magnitude of encounters of protected species with aquaculture gear, estimating the effect of aquaculture on the food web, or estimating the effect of aquaculture on benthic community composition. What is necessary to make eDNA a more useful quantitative survey tool?

As illustrated in **Fig. 1**, the quantity of eDNA in seawater is affected by species-specific rates of metabolism and physiology as well as environmental parameters. In collaboration with the Cold Spring Harbor Laboratory, University of Buffalo, Stanford and Monmouth Universities, NOAA scientists conducted laboratory experiments in a closed recirculating system at Sandy Hook, NJ, USA to determine species-specific shedding and decay rates of eDNA under different environmental conditions. To date we have run trials with adult black sea bass, juvenile winter flounder, and currently adult summer flounder. Some results from the black sea bass run in **Fig. 3** show eDNA in equilibrium and eDNA degradation. Most eDNA degraded within about two days.

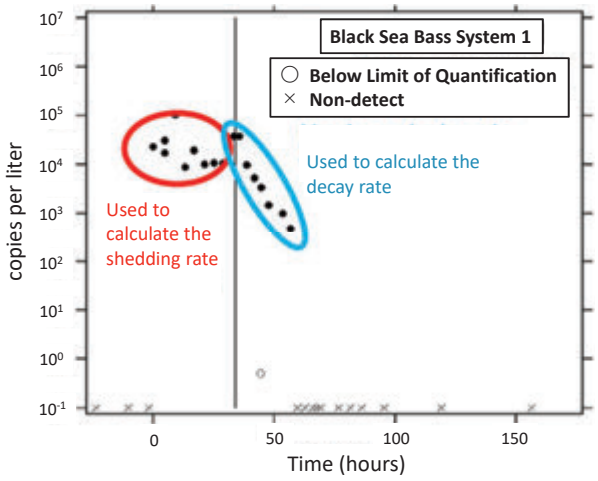

Fig. 3. Quantification of Environmental DNA shedding and decay rates for black sea bass. From Kirtane *et al.* (in preparation).

To apply such shedding and degradation rates to field investigations, Sandy Hook staff conducted sampling on cruises with the Marine Academy of Science & Technology (MAST) in NJ in early May, 2019, as well as on the Northeast Fisheries Science Center fall 2019 bottom trawl survey. On both cruises, water was filtered for later analysis; results are pending and should be available as the US response to the current pandemic permits.

One of the important next steps to making this a quantitative survey tool is to develop eDNA particle transport models, as has been done off Southern California. These models are intended to indicate the geographical origin of collected eDNA (Andruszkiewicz *et al.*, 2017). In addition to supporting stock assessments, this approach can be useful to tracking the distribution and abundance of *e.g.* escapees from penned cultured salmon and other species.

Notably, a promising application of environmental DNA in field surveys will be to pair this approach with acoustic surveys. This is particularly attractive because it would be relatively easy to develop a strategy for rapid sampling and rapid analysis, using eDNA species data to ground truth acoustic data. The development of towed midwater eDNA sampling equipment will significantly facilitate this methodology; several efforts are currently underway to develop this equipment. For example NOAA staff in Sandy Hook, NJ have partnered with MAST to construct a midwater eDNA sampler for deployment from small vessels.

Environmental DNA can be an important tool for benthic surveillance to assess habitat preferences (Takahara *et al.*, 2019) as well as impacts of caged cultured fish on sediments and associated biota. There are clear correlations reported for changes in OTUs and species as measured using eDNA in relation to input of organic waste, *e.g.* from aquaculture, to sediments (Keeley *et al.*, 2019). This has also been explicitly demonstrated for penned salmon (Dowle *et al.*, 2015). Notably even if species cannot be identified from the OTUs due to lack of appropriate reference libraries, the OTU response to a disturbance reflects a biological impact and can be analyzed without species identification. Thus eDNA is a promising tool to quickly measure biological impacts in sediments due to aquaculture and can be an important method to help identify Aquaculture Opportunity Areas and to monitor operations impacts.

It is also important to note that disease is one of the greatest impediments to successful aquaculture operations. Tracking and modeling the spread of disease will be key to managing the epidemiology of viruses, bacteria and parasites on cultured shellfish and finfish. eDNA is a tool which can be used for pathogen surveillance in the field. In conjunction with environmental and hydrographic information, this will assist modelers to predict rates and areas of spread of specific diseases. Notably, this also allow forecasts in relation to changing climate. Further, the potential is large for application of eDNA for seafood inspection, measuring not only fish and shellfish pathogens but also human pathogens such as Vibrio.

Aquaculture Operations Conditions and Fish Health

eDNA is potentially a valuable tool for studying health-related effects of aquaculture operations including use of pharmaceuticals and other chemicals on cultured fish and shellfish. Microbial diversity of gut microflora has been studied in various fish species collected from Japan's coastal waters using next-generation sequencing. In one study metabolites and bacterial eDNA in feces were

analyzed as indicators for fish health. The potential of this approach as a non-invasive inspection technique in aquaculture was suggested by Asakura *et al.* (2014).

Protected Species

In the USA, one of the greatest concerns impeding the permitting of offshore aquaculture is the risk of entanglement of protected species such as the right whale in the NE USA. To date, predicting species distributions and migrations have largely relied on models of food supply availability and habitat suitability of the species of interest such as right whales. Use of environmental DNA as a surveillance tool for protected species may enable us to go from modeling their distribution to actual observation for assessing encounter rates of aquaculture gear with target species. With the use of in situ deployed automated samplers and sequencers (*e.g.* from Monterrey Bay Aquarium and Research Institute) eDNA is a promising tool to actually record the presence of protected species or their prey in the direct vicinity of aquaculture sites. Getting more field observation data will be pivotal to informing and verifying the reliability of entanglement models.

Summary

Sampling and processing of environmental DNA samples are relatively rapid and inexpensive. eDNA data can support multiple applications to support aquaculture. The applications can be qualitative and quantitative. Applications are useful to describe species diversity; to monitor for finfish escapees, pathogens and protected species; to assess impact of aquaculture operations on habitats; to evaluate suitability of habitats for cultured shellfish; to assess the health of cultured species; and other applications.

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Liu Y., Wikfors G. H., Rose J. M., McBride R. S., Milke L. M., and Mercaldo-Allen R., 2019: Application of environmental DNA metabarcoding to spatiotemporal finfish community assessment in a temperate embayment. *Front. Mar. Sci.,* **6**, 674. (doi.org/10.3389/fmars.2019.00674)

Sassoubre L. M., Yamahara K. M., Gardner L. D., Block B. A., and Boehm A. B., 2016: Quantification of Environmental DNA (eDNA) Shedding and Decay Rates for Three Marine Fish. *Environ. Sci. Technol.*, **50(19)**, 10456–10464.

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Annotated Bibliography of Key Works

(1) Sassoubre L. M., Yamahara K. M., Gardner L. D., Block B. A., and Boehm A. B., 2016: Quantification of Environmental DNA (eDNA) Shedding and Decay Rates for Three Marine Fish. *Environ. Sci. Technol.,* **50(19)**, 10456–10464.

A key publication on experiments for shedding and decay rates of eDNA from marine finfish. Also includes a much cited conceptual model for processes in the field affecting concentrations of eDNA.

(2) Andruszkiewicz E. A., Starks H. A., Chavez F. P., Sassoubre L. M., Block B. A., and Boehm A. B., 2017: Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding. *PLoS ONE,* **12(4)**, e0176343. (doi.org/10.1371/journal. pone.0176343)

This paper is the first to my knowledge to apply numerical modeling to predict the origin of eDNA collected in the field. The outputs include levels of uncertainty for the calculations.

(3) Kelly R. P., Shelton A. O., and Gallego R., 2019: Understanding PCR Processes to Draw Meaningful Conclusions from Environmental DNA Studies. *Sci. Rep.,* **9**, 12133. (doi.org/10.1038/s41598-019-48546-x)

This paper presents guidelines for the use of PCR for the successful application of eDNA data to estimate biomass. The investigation is a modeling approach and describes how the proportional indices of amplicon reads capture trends in taxon biomass with high accuracy.

(4) Liu Y., Wikfors G. H., Rose J. M., McBride R. S., Milke L. M., and Mercaldo-Allen R., 2019: Application of environmental DNA metabarcoding to spatiotemporal finfish community assessment in a temperate embayment. *Front. Mar. Sci.,* **6**, 674. (doi.org/10.3389/fmars.2019.00674)

Describes the field investigations addressing the beneficial effect of caged oyster on finfish biodiversity in Long Island Sound, USA.

(5) Kirtane A., Sassoubre L., Wieczorek D., Phelan B., Nash B., and Noji T., in Preparation. Quantification of Environmental DNA (eDNA) Shedding and Decay Rates for Three Marine Fish in Support of Quantitative Field Investigations.

Quantification of Environmental DNA (eDNA) Shedding and Decay Rates for Three Marine Fish in Support of Quantitative Field Investigations. Describes the results from laboratory experiments on one pelagic fish species and two flatfish. Also relates these data to field surveys for these species with comparisons to trawl data.