Evaluating environmental DNA detection alongside standard fish sampling in Great Lakes coastal wetland monitoring (Seed Project)

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Project background

Fish monitoring in the littoral zone of the Great Lakes is a critical part of ongoing efforts to improve Great Lakes health, restore coastal areas, and manage invasive species (Uzarski et al. 2005). While nearly all ecological monitoring programs currently rely on capture or observation of live organisms, recent developments in environmental DNA methods to detect aquatic macrofauna (Ficetola et al. 2008) suggest that this genetic approach may enhance monitoring programs. Environmental DNA (eDNA) is the genetic material from living organisms that can be detected by sampling the non-living environment. Aquatic macrofauna shed cellular material that diffuses in water, expanding the area and time window that can be sampled to detect an organism's presence. Such monitoring involves capturing DNA-containing particles from water, purifying the DNA, and then analyzing it for the presence and abundance of species-diagnostic genetic markers. Its application for large-scale aquatic monitoring programs is particularly promising because eDNA sample collection and genetic assays are so scalable – many species can be detected from a single water sample. As the number of sites or target species increases, economies of scale in genetic detection also provide substantial time- and cost-savings.

Project summary

Using this seed funding, the project team began investigating the performance of eDNA methods within an existing large-scale fish monitoring program. The Great Lakes coastal wetland monitoring program, directed by Dr. Don Uzarski of Central Michigan University, provided a framework onto which eDNA sampling was added. The goals were (1) to develop and apply eDNA sampling methods for detecting fish species and measuring eDNA concentration in these large, complex habitats and (2) to compare results between fish capture methods and eDNA methods. We aimed to use what we learned in support of new research proposals to other funding agencies, which is consistent with the goals of Illinois-Indiana Sea Grant under this program.

Overnight fyke net sampling is the standardized method for fish monitoring adopted by the Great Lakes Coastal Wetland Consortium, and net placement is designed to characterize the fish community at a given wetland site (GLCWC 2008). Given the size and varied habitat of these coastal wetlands, we concluded that existing methods for eDNA sampling (15 mL to 2 L grab samples) would provide inadequate coverage in terms of water volume and habitat area sampled. Indeed, preliminary testing in a small lake indicated substantial patchiness of fish eDNA (see results below). Thus, we developed an *in situ* filtration method wherein water is filtered through an eDNA capture device as the researcher moves through the site (e.g., on a boat). This spatially integrated sampling approach was deployed at 18 protected embayment wetland sites in Lake Michigan and Lake Huron, resulting in an average of 60 L of water filtered along a 175 m path over 65 minutes at each site. Fyke net sampling was conducted at 17 of these 18 sites within 4 days of eDNA sampling, on average. eDNA samples were analyzed for PCR inhibitors and subsequently for common carp (*Cyprinus carpio*) eDNA. Research developing and applying methods to detect and quantify eDNA of many other species is ongoing, supported by subsequently funded grants (see below).

PCR inhibitors in environmental samples

PCR inhibiting substances are ubiquitous in eDNA samples, as documented by the extensive literature from microbial ecology and forensics (Schrader et al. 2012; Alaeddini 2012). A PCR inhibitor is any substance co-purified with target DNA molecules that inhibits the amplification reaction, leading to inaccurate quantification or worse – complete failure to detect the target DNA when present. Common PCR inhibitors include non-target DNA, polysaccharides, humics, pigments and many other substances that are often not fully removed during DNA purification. None of the currently published research on eDNA detection of aquatic macrofauna has addressed or tested for PCR inhibition (Ficetola et al. 2008; Jerde et al. 2011; Dejean et al. 2011; Goldberg et al. 2011; Minamoto et al. 2012; Thomsen et al. 2012; Takahara et al. 2012; Dejean et al. 2012; Thomsen et al. 2012; Foote et al. 2012; Olson et al. 2012). We evaluated PCR inhibition in our samples using spectrophotometry (NanoDrop 2000, Thermo Scientific, Wilmington, DE) and an exogenous internal positive control qPCR assay (EIPC; Eurogentec, San Diego, CA). The concentration of total DNA in a sample was correlated with the magnitude of the PCR-inhibitory shift in the quantification cycle (ΔCq) detected by the EIPC qPCR assay (Figure 1). This result suggests that non-target DNA or another substance co-purified with non-target DNA directly inhibited the qPCR assay. To correct for the quantitative error introduced by this PCR inhibition we subtracted the ΔCq in the EIPC qPCR from the Cq of the common carp qPCR assay.

qPCR assay for common carp eDNA

We developed and optimized a qPCR assay to exclusively detect and quantify a 146 bp segment of the common carp (*Cyprinus carpio*) mitochondrial DNA (mtDNA) genome. To evaluate the diagnostic specificity of the qPCR assay (i.e., that the assay would amplify DNA only from common carp) the primers were tested for potential non-target amplification against the NCBI GenBank *nr* database using NCBI's Primer-BLAST tool with default settings. This *in silico* test detected no potential non-target amplification. The assay was tested *in vitro* using tissue-derived total genomic DNA from the target species and closely-related, potentially co-occurring species including goldfish (*Carassius auratus*) and grass carp (*Ctenopharyngodon idella*). No non-target amplification was detected. Finally, qPCR assay amplicons from the Great Lakes coastal wetland eDNA samples collected for this project were bi-directionally Sanger-sequenced and confirmed to match the target mtDNA segment from the target species in all cases.

Spatial patchiness of common carp eDNA

All of the currently published research on eDNA detection of aquatic macrofauna has utilized discrete water samples ranging in volume from 0.015 to 2 L (Ficetola et al. 2008; Jerde et al. 2011; Dejean et al. 2011; Goldberg et al. 2011; Minamoto et al. 2012; Thomsen et al. 2012a; Takahara et al. 2012; Dejean et al. 2012; Thomsen et al. 2012b; Foote et al. 2012; Olson et al. 2012). However, in a preliminary test of our common carp qPCR assay we discovered that among five 2-L lake water samples collected simultaneously from the same spot, the concentration of common carp eDNA ranged from 1,265 to 105,297 copies L^{-1}. This wide variation suggested that characterizing the eDNA concentration for a given species at a coastal wetland site would require either (A) an extremely large number of discrete samples or (B)

spatially integrated sampling. We opted for spatially integrated sampling because it greatly reduced the difficulty of sample collection, transport, and processing in the field and the cost of sample processing and analysis in the laboratory. Thus, each coastal wetland site was sampled using three filtration paths, each filtering an average of 20 L of water from the surface to 1 m in depth, for a total of 54 samples.

Common carp eDNA was detected in 35 of 54 samples (64.8%) and in at least one sample from all 18 wetlands (100%). When detected, the sample concentration ranged from 12 to 85,476 copies L^{-1}. Resulting qPCR amplicons from 1 sample at every site were bi-directionally Sanger-sequenced and confirmed to match the target mtDNA segment from the target species. Common carp were captured in 3 of 69 overnight fyke nets (4.3%) and in one net from 3 of 17 wetland sites (17.6%). The catch of common carp was 2 fish at one site and 1 each at two other sites. The average concentration of common carp eDNA at a site was not significantly higher where common carp were caught (Figure 2) and was not significantly correlated with the catch per unit effort (CPUE) of common carp (Figure 3). Presence and concentration of common carp eDNA often varied widely between the three samples from a site, confirming the heterogeneous distribution of DNA-containing material.

Conclusions and continuing work

Overall these results demonstrate that eDNA monitoring consistently detects common carp in Great Lakes coastal wetlands when they are detected by fyke net sampling and when they remain undetected by fyke net sampling. This is consistent with other work using eDNA to monitor the presence of fish (Jerde et al. 2011; Minamoto et al. 2012; Thomsen et al. 2012a; Takahara et al. 2012; Thomsen et al. 2012b) and is not surprising given that common carp are ubiquitous throughout the Great Lakes but are not captured efficiently with wetland fyke nets (M. J. Cooper pers. obs., D. G. Uzarski pers. comm.). The concentration of common carp eDNA was characterized by a patchy distribution that was not correlated with the local abundance of common carp as measured using fyke nets. Importantly, the low capture frequency of common carp may make this species a poor representative for comparison between fish capture and eDNA methods. Patchy distributions of common carp eDNA were consistent across the preliminary test on an inland lake and the coastal wetland sites. For example, the three samples from one particular site produced common carp eDNA concentrations of 0, 62, and 85,476 copies L^{-1}, respectively. This result contrasts with the conclusion of Thomsen et al. (2012a) that "DNA is homogeneously distributed in pond water." The patchy distribution we discovered may be influenced by the fish species, environment (e.g., pond versus lake), and/or the eDNA capture method (e.g., filtration versus precipitation). If this patchy distribution is general, however, it would suggest that reliable correlations between fish abundance and their eDNA concentration are unlikely. Nevertheless, these results demonstrate that eDNA methods produce highly sensitive detection of fish presence in a given water body. For segments of large water bodies such as coastal wetlands, it remains to be determined whether long-distance transport of fish eDNA will limit the location-specific inferences that can be made using eDNA.

In summary, we completed a preliminary single-species comparison of quantitative eDNA sampling with standard fish sampling conducted within the framework of an existing large-scale fish monitoring program. We improved upon existing macrofaunal eDNA methods by

developing a spatially integrated *in situ* filtration approach and by addressing PCR inhibition. Research on this project was conducted by Cameron R. Turner and Matthew J. Cooper (Ph.D. students at the University of Notre Dame) and Charles C.Y. Xu (an undergraduate student at Notre Dame) under the supervision of Drs. David M. Lodge and Gary A. Lamberti (Professors at Notre Dame). Dr. Donald G. Uzarski (Central Michigan University) supported the project by coordinating fyke netting and eDNA sampling and by providing fyke net data. The eDNA samples collected during this seed project will be analyzed using a multi-species metagenetic analysis method that we are developing through another grant (see below). Thus, genetic analysis of samples collected during this project will continue, allowing us to compare eDNA and traditional methods for all fish species captured during the study.

Research conducted as part of this project was instrumental in developing three recently funded proposals focused on eDNA methods:

- 2011-2013. Great Lakes Fishery Trust grant: $250,000. Estimating Asian carp abundance using environmental DNA. PI: David Lodge. Co-PIs: Lindsay Chadderton, Christopher Jerde, Andrew Mahon, Cameron Turner.
- 2012-2015. US Department of Defense, Strategic Environmental Research and Development Program grant: $1,470,582. Project ID: RC-2240. Development of an environmental metagenetics approach to for monitoring aquatic biodiversity. PI: David Lodge. Co-PIs: Christopher Jerde, Gary Lamberti, Andrew Mahon, Michael Pfrender, Cameron Turner.
- 2012-2014. Great Lakes Restoration Initiative grant: $599,931. Improving eDNA-Based Surveillance Programs for High-Risk Potentially Invasive Species. PI: Scott Egan. Co-PIs: David Lodge, Jeff Feder, Michael Pfrender, Christopher Jerde, Steve Ruggiero, Carol Tanner.

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Dejean, T., Valentini, A., Miquel, C., Taberlet, P., Bellemain, E., & Miaud, C. (2012). Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog Lithobates catesbeianus. *Journal of Applied Ecology*, 49(4), 953-959.

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Olson, Z. H., Briggler, J. T., & Williams, R. N. (2012). An eDNA approach to detect eastern hellbenders (Cryptobranchus a. alleganiensis) using samples of water. *Wildlife Research*. 39(7), 629-636.
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Figures

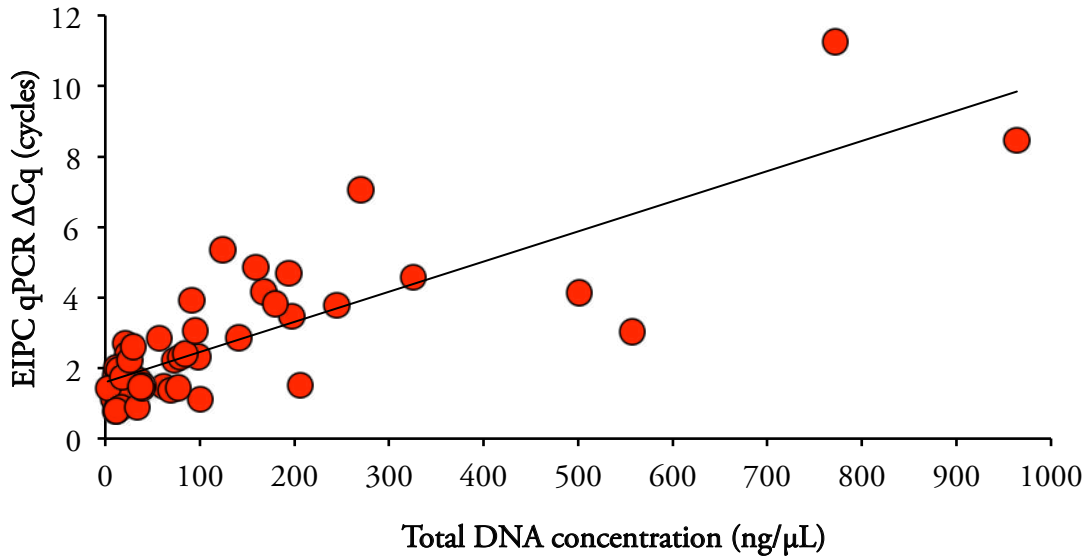

Fig. 1. Relationship between NanoDrop-measured concentration of total DNA in a coastal wetland eDNA sample and the difference between the sample and standard quantification cycle (Cq) from the exogenous internal positive control (EIPC) qPCR. Larger Cq differences reflect a larger PCR inhibitory effect in a given environmental sample compared to a standard preparation of the EIPC template DNA. r^2=0.67, P<0.001, n=54.

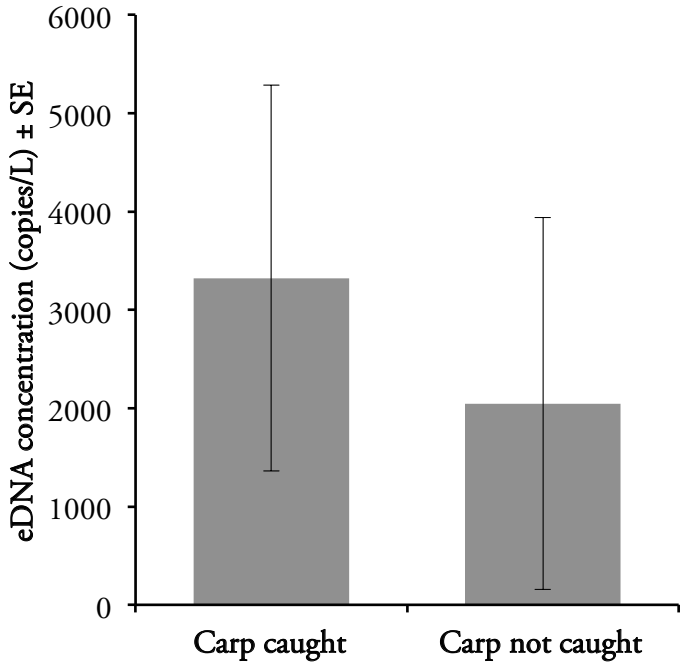

Fig. 2. Mean ± 1 SE concentrations of qPCR-measured common carp eDNA at coastal wetland sites where common carp were or were not caught in fyke nets. Carp were caught at 3 sites and not caught at 14 sites. $F(1,15)=0.06$, $P=0.81$.

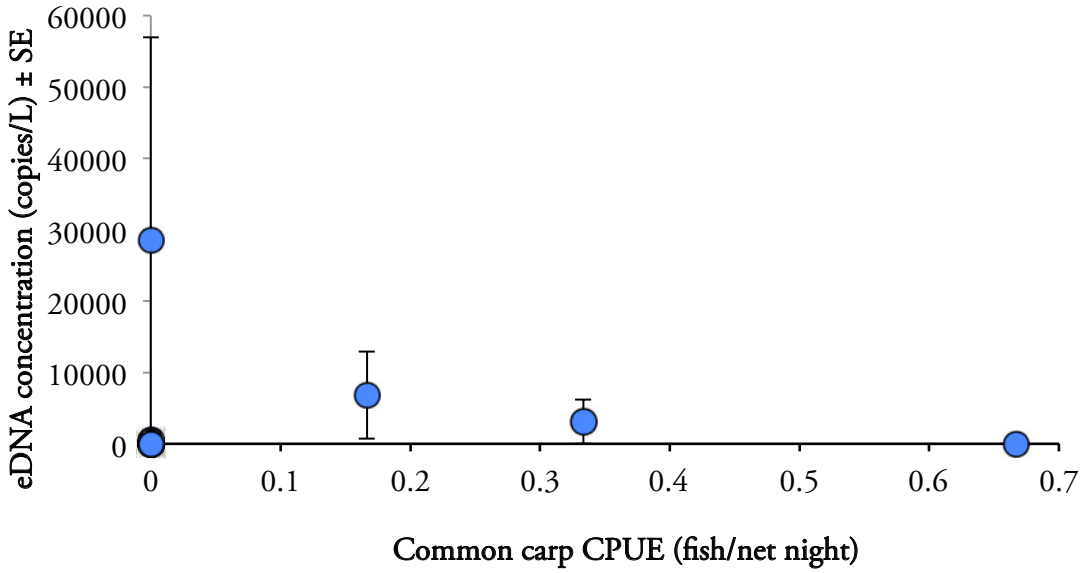

Fig. 3. Plot of common carp catch per unit effort (CPUE) and mean ± 1 SE concentrations of qPCR-measured common carp eDNA at coastal wetland sites. $r^2=0.0009$, $P=0.9$, $n=17$