* **Project title**: Improving techniques for estimating abundance and habitat use in nearshore marine habitats using environmental DNA
* **Principle investigators**: A. Ole Shelton (NWFSC), Correigh Greene (NWFSC), Ryan Kelly (University of Washington), Linda Park (NWFSC)
* **Affiliations:** NWFSC
* **Project duration and annual budget total(s)**: Apr. 2016-Mar. 2017 ($143K in FY2017)
* **Scope, objectives, and scientific merit of the work**: Early life history is a critical period for most commercially important fishes. For anadromous salmonids, the transition from freshwater to the marine environment is a key determinant of marine survival and fisheries productivity. Accurate estimates of juvenile salmon abundance in nearshore estuarine areas are critical to develop spawner-recruit curves that underlie stock assessment models. Equally important are data that relate variation in juvenile salmon abundance to measures of nearshore habitat quality. However, estimates of fish abundance in the nearshore habitats are difficult and expensive to obtain using traditional sampling methods; shallow water and vegetation interfere with acoustic surveys, turbid water often hinders visual surveys, and the presence of vegetation and other structures restricts the efficacy of some net survey techniques. Nonetheless, estimating smolt and other fish species abundance is especially important in light of (a) continued loss of foundational vegetated habitats such as seagrass beds, salt marshes, and other coastal wetlands, and (b) restoration efforts intended to mitigate such losses. Assessing the importance of nearshore habitats to salmonids in general—and the success of nearshore restoration efforts in particular—requires efficient methods for quantifying salmonid abuandance in of these habitats. **We propose to apply recently developed environmental DNA (eDNA) survey techniques to assess the salmonids across habitats that span a range of restoration actions, continuing work started in 2016.** We will focus on characterizing habitat use by salmonids along with other commercially valuable and ecologically important fishes. If successful, this project would adapt a rapidly-developing, innovative technology that could inform stock assessments nationwide.

*Objectives*: **(1)** Validate and improve existing molecular tools for rapidly detecting the occurrence and abundance of coastal fish; **(2)** Assess the efficacy of eDNA methods by comparing estimates of occurrence and abundance from traditional net sampling and eDNA methods across three estuaries; **(3)** Document and contrast costs and relative benefits of eDNA methodologies relative to traditional sampling methods.

*ASTWG Themes Addressed*: Our proposal addresses ASTWG themes **2** (Remote species identification and enumeration) and **5** (Efficient Ecosystem Surveys).

**Technical Approach:** We propose to develop general methods for rapid, spatially-explicit assessments of nearshore marine habitats using environmental DNA (eDNA). We will leverage existing beach seine sampling in 3 major estuaries of Washington state (Skagit, Nisqually, Elwha) to document the advantages and disadvantages of eDNA methods for providing estimates of occurrence, abundance, and habitat specific use by fish species. We will focus on providing estimates of fish species that are ecologically and commercially important but difficult to survey, including juvenile salmon (*Oncorhynchus* spp.) and forage fish (e.g. herring (*Clupea pallasi*) and smelt (family *Osmeridae*)). Herring and smelt have been estimated as the most numerically abundant fish but exhibit recent declines, while Chinook salmon populations in these river systems are all ESA-listed, but suffer from poor quantification.

We will collect three 1L water samples (containing eDNA) alongside traditional beach seine surveys across the three estuaries quarterly; sampling in off-season for each salmon run (late fall and winter) will serve as negative field controls. Each estuary is sampled with beach seines, and water samples will be taken in triplicate at each location during sampling. We will record environmental covariates at each sampling event to determine how environmental conditions may affect the concordance between eDNA and net sampling.

Year 1: Having received FY2016 funding in April 2016, we have already begun work to use existing mtDNA primers and high-throughput sequencing cross-validate eDNA techniques with beach seine samples. We have the necessary eDNA primers (both multi-taxon and species-specific) in hand, leveraging earlier work by co-PIs and USGS, and have the relevant bioinformatics pipeline up and running. Field sampling is underway, and our 2016 deliverables remain our achievable goals despite starting the project later in the season than originally anticipated. Deliverables: broad-scale report on species abundance, validated proof-of-concept, and molecular tools for future use.

Year 2: Measure temporal variability of the parameters measured in year 1; use validated tools from year 1 to improve quantification of focal species in space and time. Deliverables: Map of dynamic usage of habitats for focal species; accounting of assemblages of major associated species; rapid-assessment metrics for importance of nearshore vegetated habitats and success of restoration efforts in these habitats; cost-benefit comparison of eDNA and net methodologies.

**Expected Results:** Preliminary data: A growing number of publications have demonstrated the utility of extraction and sequencing of this eDNA from water samples to survey metazoan species and communities1-3. Millions of DNA sequences are generated from each sample, revealing a diverse suite of species that would otherwise be difficult or impossible to survey. Preliminary data in hand demonstrate eDNA’s feasibility, appropriate spatial scale, and suitable taxonomic breadth for the proposed project.



Figure 1: Trends in the proportion of Rockfish (*Sebastes*) 12s environmental mtDNA recovered from a transect of water samples across nearshore habitats in Monterey, California. Blue points are data (in triplicate); black points are means for each sampling site; loess smoothing line shown. Data from JA Port, RP Kelly, et al. (submitted to *PNAS* 2015). The eDNA technique distinguished animal communities in the dynamic nearshore habitat with a minimum resolution of 60-100m.

As to spatial scale, eDNA can distinguish ecological communities at scales of 60-100m, even in a dynamic marine nearshore environment (Fig. 1), and is useful for detecting even rare species in both salt and freshwater3–5. While eDNA alone does not currently provide quantitative assessment of the abundance of source animals, our proposed work advances eDNA methods by providing a link between fish abundance and eDNA surveys and an application for rapidly assessing nearshore habitat use by fish.

As to taxonomic breadth, we have designed and tested primers to amplify a ca. 110bp fragment of the 16s mitochondrial DNA (mtDNA) from animal species from environmental samples. These successfully amplify and sequence diverse members of at least 7 phyla including Chordata (i.e., vertebrates), Arthropoda, and Mollusca from samples in Puget Sound, and are useful for characterizing animal communities. We have also used a small fragment of 12s mtDNA to focus on vertebrates specifically2; this tool detects and distinguishes bony fish well. Jeff Duda (USGS) and colleagues have developed salmon-specific molecular markers that would allow us to distinguish among all of the species, several of which have listed ESUs in Puget Sound. Others have used an existing 18s primer set to characterize a suite of metazoans from environmental samples, and we envision this as a supplementary source of data for the proposed work. Each of these tools is available and functional, but have not been used together in a synoptic way for the purposes we envision here.

**Probability of success:** Year 1 deliverables have a high probability of success, given preliminary data2 (Fig. 1) and validation with existing methods is straightforward. Year 2 deliverables are less certain, given unknown performance of additional molecular tools; mapping the spatial and temporal dynamics of key taxa is very likely to succeed, but the taxonomic specificity (genus-level vs. species-level) with which we can do this is not yet certain. However, the simultaneous use of multiple species-specific markers will add confidence to our estimates. We will endeavor to reduce these key uncertainties with rigorous *in silico* and lab-based testing (for new markers) and by leveraging existing research with partners working in Monterey and Puget Sound. Despite these uncertainties, marrying molecular and traditional surveys holds great promise for improving ecosystem surveys and stock assessments alike.

**NMFS-wide concern:** eDNA has widespread applicability for ecosystem based management in all NMFS regions because of its potential to assess all species present in an area, not just the target fishery species. More immediately, methods development for salmon have application to ESA-listed species in three regions (Northeast, Northwest, and Southwest). It should be especially useful for fisheries that have been curtailed due to overfishing, making current data on the status of the target species extremely limited, as well as for species where gear-avoidance or difficult habitats interfere with traditional assessment methods. The presence of eDNA is also independent of species or gear-type of a fishery; thus development of laboratory tools and analysis methods could be used to augment assessments of a wide range of species including forage fish, groundfish, and crustaceans. One long-term potential application for this method would be to develop of autonomous samplers that could be deployed to collect eDNA (water) repeatedly over time and provide a detailed picture of fish abundance and movement.

**Itemized Annual Budget FY2017**

Contract postdoc for laboratory and field work (incl. indirect) $120,000

Sampling bottles, filters, etc. 3,000

Barcoded primers for multiplexing 3,000

DNA extractions of samples (18 triplicate samples/site \* 3 sites) 1,500

Sample library preparation and QA/QC 5,000

Contract for sequencing services, MiSeq supplies 10,000

Sample shipping and handling costs 500

**Total** **$143,000**

1. Leray, M. & Knowlton, N. DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity. Proc. Natl. Acad. Sci.  112, 2076–2081 (2015).

2. Kelly, R. P., Port, J. A., Yamahara, K. M. & Crowder, L. B. Using environmental DNA to census marine fishes in a large mesocosm. PloS One 9, e86175 (2014).

3. Foote, A. D. et al. Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. PloS One 7, e41781 (2012).

4. Thomsen, P. F. et al. Monitoring endangered freshwater biodiversity using environmental DNA. Mol. Ecol. 21, 2565–2573 (2012).

5. Jerde, C. L., Mahon, A. R., Chadderton, W. L. & Lodge, D. M. ‘Sight-unseen’ detection of rare aquatic species using environmental DNA. Conserv. Lett. 4, 150–157 (2011)