NOAA/NMFS Office of Science and Technology - Internal Proposal Review and Budget Allocation Process

Advanced Sampling Technology Working Group

FY 2017 Proposal

Improving techniques for estimating abundance and habitat use in nearshore marine habitats using environmental DNA. (FY17)

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Submission Date: 11/30/2016

2 years, 112.5K (Year 1), 115.5K (Year 2) (Here, requesting Year 2 funds)

1. Investigators

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2. Summary

Organisms of all kinds shed cells containing diagnostic DNA into the environment, which can be recovered and assigned to a taxon based upon its match to known sequences. Because DNA degrades under most ambient environmental conditions—the half-life of DNA in fresh- and saltwater is approximately 24-48 hours (1,2)—this environmental DNA (eDNA) provides a snapshot of the species recently present in the sampled habitat. However, while it is widely accepted that DNA can be collected and identified from a range of environmental samples, connecting field collections of eDNA with abundance surveys remains largely unexplored.

Here, we propose to develop eDNA survey methods to quantify fish communities (with a focus on salmon and forage species) in a nearshore estuarine habitat in Puget Sound. To compare the efficacy of eDNA and traditional methods, we will collect water samples in parallel with collections made via traditional net sampling methods, targeting nearshore fish communities that provide a range of spatial sampling scales (from meters to 100s of meters). We will use both quantitative PCR (qPCR) and massively parallel DNA sequencing technologies to provide eDNA data. We will then apply a newly developed statistical framework to provide field estimates of the relationship between species abundance and eDNA. Our replicated sampling design—using multiple field methods that span spatial scales—provides an opportunity to understand the appropriate spatial scale for eDNA sampling, and the potential value and pitfalls of eDNA surveys for understanding patterns of fish abundance.

3. Is funding needed for this project?

Y

4. Scope, Objectives and Merit

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. Similarly, ecologically important forage fish (e.g. herring, smelt, and surf perch) use nearshore and estuarine habitats as spawning and rearing grounds. Thus, accurate estimates of fish abundance in nearshore estuarine areas are critical to understanding early life-history survival and play an important, poorly understood role in driving stock assessment models. 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 salmon 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 and forage fish —and the success of nearshore restoration efforts in particular—requires efficient methods for quantifying abundance in these habitats. We propose to apply recently developed environmental DNA (eDNA) survey techniques to assess the fish communities across three habitats used at different points during the salmon migration from freshwater to the ocean. We will characterize the abundance of salmonids and other commercially valuable and ecologically important fish in three distinct estuarine habitats; these three habitats offer the additional benefit of comparing results across three different spatial sampling techniques. If successful, this project would adapt a rapidly-developing, innovative technology that could inform stock assessments nationwide.

This project has three main objectives: (1) Validate and improve eDNA methods for rapidly detecting the occurrence and abundance of ecologically important 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 existing sampling methodologies; (3) Compare costs and relative benefits of eDNA methodologies relative to traditional sampling methods. The project will improve abundance estimates for species that are ecologically and commercially important but difficult to survey, including juvenile salmon (Oncorhynchus spp.) and forage fish (e.g. herring (Clupea pallasi), smelt (family Osmeridae), and surfperch (family Embiotocidae)). Our first year of sampling has shown that surfperch are numerically dominant and widespread, herring and smelt are present but patchily distributed, and Chinook salmon populations in this river system present and widespread but at generally low densities (3,4).

We will focus on developing rigorous eDNA methods in a single estuary and will compare results from eDNA samples with three widely used net sampling techniques (Fyke net, beach seine, surface trawl). Each of these sampling methods occurs in distinct habitats and samples different cumulative areas (increasing from the scale of ~1 to 100s of meters); as such, we expect the match between eDNA and net sample to vary with each sampling method. Concordance should be highest for Fyke nets and lowest for surface trawls. However, the rate at which the concordance between eDNA and sampling scale changes is itself a useful metric because it has direct implications for the appropriate scale at which to apply eDNA methods in the field. Beyond individual sample-to-sample comparisons, we will calculate aggregative measures of fish density from traditional and eDNA methods. Such sample aggregation is a critical step in the development of abundance indices that feed directly into most stock assessments. Thus our research plan provides information on two pressing questions for the future use of eDNA in stock assessments: (1) at what spatial scale can eDNA accurately reflect local abundance? and (2) can eDNA provide integrated

metrics of abundance on scales useful for management? Due to mechanical failures with the surface trawl survey vessel in 2016 and a lack trawl samples, we did not sample eDNA in parallel with this method in 2016. We hope to be able to conduct eDNA sampling alongside surface trawls in 2017 (see also ASTWG progress report).
Developing quantitative applications, such as we propose here, is the key next step in the evolution of eDNA into a practically useful tool, pointing the way to such uses as stock assessments, counts of endangered or invasive species, and other quantitative surveys for species and communities of interest. Accordingly, the work we propose here applies NMFS-wide, and additionally has benefits that will ramify outside of the agency. For example, USGS and State agencies have expressed interest in surveying salmonids and protected species with eDNA (pers. comm.), work that the proposed project would directly inform. In short, we propose to lay the necessary methodological and quantitative groundwork to make eDNA useful for NMFS and others. An added benefit of eDNA methods for NMFS is the potential to bring down the future costs of survey work: on a per-sample basis, eDNA appears likely to become cheaper than many traditional sampling methods. Finally, the project would contribute to a durable collaboration between the NWFSC and UW researchers in the College of the Environment, leveraging NMFS's financial and human resources.

5. Defined Uncertainties

The idea that one can sample a volume of water, sequence the DNA present, and report what species are living nearby is widely accepted among microbial biologists (e.g 5). For fisheries ecologists that have historically use manual count data, eDNA has quickly become a potential new avenue through which to examine the world, but has yet to come into common practical use because of unknowns surrounding quantification. Preliminary data in hand demonstrate eDNA's feasibility, appropriate spatial scale, and suitable taxonomic breadth for the proposed project.
Explicitly linking eDNA to biomass in the field remains a key goal of eDNA application. In both lab and field contexts, recent work has shown correlations between eDNA copy number and fish biomass (6,7,8,9,10,11). While these results may accurately reflect a functional link between individuals and DNA in the environment, a diverse set of processes that separate the biomass of source animals and the observed DNA fragments means that there are a large number of ways to arrive at spurious correlations between eDNA and observed catches.
A pervasive concern in the eDNA literature is determining the appropriate spatial scale for eDNA studies. Current evidence suggests that 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 (1,12). 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.
At present, methods for eDNA are not sufficiently well developed to make full inference about density or biomass in an ecological community from eDNA alone. Similar challenges confront estimation of density and biomass based on traditional sampling methods (13,14,15), but do not prevent researchers from making the best approximations possible given existing knowledge and data. We will apply a newly developed Bayesian statistical framework to assess uncertainties in linking biomass to eDNA reads, leveraging a large body of statistical thought from the fisheries literature and analogizing eDNA to the use of a new "net" used to sample target fish species.

6. NMFS-Wide Concerns

eDNA has widespread applicability for ecosystem-based management in all NMFS regions because of its potential to assess many 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). The noninvasive nature of eDNA sampling should be especially useful for fisheries that have been curtailed due to overfishing—making existing 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; for example, preliminary eDNA surveys could maximize cost-effectiveness in the immediate future by highlighting spatial areas in which to focus manual sampling. One long-term potential application for this method would be the development of autonomous samplers that could be deployed to collect eDNA (water) repeatedly over time to provide a detailed temporal picture of fish abundance and movement.
Our proposal addresses ASTWG themes 2 (Remote species identification and enumeration) and 5 (Efficient Ecosystem Surveys), and is broadly applicable within NMFS, with potential uses in stock assessments, counts of endangered or invasive species, and other quantitative surveys for species and communities of interest.

7. Technical Approach

Our approach has three components: 1) Field collection of eDNA samples conducted in parallel with existing nearshore sampling using three sampling techniques. 2) Laboratory processing of eDNA samples to produce quantitative estimates of DNA abundance and 3) application of novel statistical approaches to generate defensible estimates of biomass from eDNA and comparison of estimates from eDNA to those derived from traditional sampling methods. We discuss each in turn.

Field collections. This project aligns with the Skagit River Intensively Monitored Watershed Project, which tracks status and trends of species in the Skagit River estuary and Bay (Fig. 2), including all U.S. species of Pacific salmon and several forage fish species (16). The focal species is Chinook salmon, which is listed as threatened under the Endangered Species Act. Small (<50

mm) Chinook leave freshwater and rear in the estuary before migrating to the Pacific Ocean (3). Substantial variation in migration timing and fish movement complicates traditional estimations of abundance; nevertheless these data represent the longest time series of juvenile abundance Puget Sound salmon and are vital for determining stock status, trends, and responses to habitat restoration efforts.
The Skagit Intensively Monitored Watershed Project counts Chinook salmon at four life stages. We will focus on three stages: estuary residence in channels and impoundments, nearshore intertidal residence along beaches and in lagoons, and subtidal residence in Skagit Bay (Fig. 2, Table 1). Sampling in each habitat requires different sampling gear and complicates abundance estimates across habitats. In estuary channels and nearshore lagoons, capture efficiency can be very high (>50%), while efficiency in other habitats is much lower (< 10%). These various sampling procedures highlight the potential utility of eDNA for estimating local abundance, calibrating eDNA to each procedure individually.
Beyond salmonids, Pacific herring, surf smelt, stickleback, surfperch, and Pacific sandlance reside in Skagit Bay. The multi-species eDNA techniques we propose will capture these and other species inhabiting estuarine and nearshore habitats at various times of the year.
The spring and summer represent prime sampling windows when Chinook salmon and forage fish are abundant in the estuary and bay; winter and fall sampling will serve as negative controls. Water samples will be taken in replicate at each location. We will record environmental covariates to assess their effects on concordance between eDNA and net sampling.
Due to delayed funding 2016, we collected replicate water (eDNA) samples during summer (June) in 2016 alongside Fyke net and beach seines. We collected 6 1-L bottles for each seine and Fyke trap sites for eDNA. Our samples coincided with a range of Chinook salmon and forage species abundance providing sufficient statistiscal contrast for qPCR and metabarcoding testing (see ASTWG progress report for details).

Environmental DNA methods. There are two distinct approaches for eDNA analysis. In the first, the amount DNA from a single target taxon is quantified using quantitative PCR (qPCR; 12), by comparing the amplification rate of a field sample to one of a standard of known concentration. This protocol quantitatively assesses changes in single target-species' DNA concentrations in the field (17,18).
In the second technique a single locus is PCR-amplified from all genomes present in a sample, the resulting products (amplicons) are sequenced, and the sequences are matched to those of known species in a large database (19). Amplicon sequencing can provide data for a hundreds of taxa in the sampled community, but only provides information about relative abundance of DNA in the sample.
We propose to combine these two methods, using 1) qPCR to quantify the abundance of key species, and 2) amplicon sequencing to provide the relative abundance for dozens of species in the community, and then 3) linking qPCR and sequencing results to provide the first quantitative survey of an entire community (20).
For our focal species (salmonids and forage fish), there is sufficient published and unpublished genetic information to reliably identify taxa to the species level with both qPCR and sequencing approaches. 18 provides qPCR primers for Chinook salmon and colleagues at the US Geological Survey have developed qPCR primers specific for eight salmonid species (J. Duda pers. comm.). We have chosen Chinook salmon and shiner surfperch (Cymatogaster aggregata) as our focal species for qPCR, and are quantifying eDNA using replicate qPCRs from each of the triplicate water samples.
We will then use three sets of primers to generate mixed-species amplicons for sequencing. We will use primers with the following target genes and taxa: 16S mtRNA (16S; targeting fish and a diverse range of invertebrate taxa in Puget Sound; 20), 12S mtDNA (targeting vertebrates; 21), and we will use the software ecoPrimers (22) to develop a novel primer set that will amplify a region of mitochondrial cytochrome c oxidase I (CO1), which varies consistently between the focal species of Chinook salmon and shiner surfperch for qPCR.

Linking eDNA and net surveys. Matching eDNA field sampling and existing net sampling protocols will reveal the relationship between fish abundance and eDNA. While there are challenges for translating observations of eDNA into biomass, these are largely analogous to those faced by traditional sampling methods (Fig. 3; 20); we view eDNA sampling as a new "net" with a unique set of traits that can produce statistical biases in the estimation of abundance. Elsewhere the PIs have developed a statistical framework for quantifying the stages connecting biomass to observed eDNA counts (Fig. 3, 20). This proposal provides an ideal application of these new methods and in a management relevant setting.
An unresolved issue is how DNA is shed and disperses in the environment (18). Our multi-scale field sampling approach allows us to explore the relationship between eDNA and fish abundance across spatial scales. We expect the strongest eDNA-net correlations to be in estuarine channels, and weakest correlations to be in offshore trawls, consistent with the spatial scales of sampling in those habitats.
Beyond individual eDNA-to-net-sample comparisons, we will also compare aggregated estimates of abundance. In stock assessments, such abundance indices provide estimates of overall average density and trends through time; identical procedures can be performed with eDNA data. Thus while a single eDNA sample may not perfectly reflect abundance observed in an adjacent sample, on an aggregate basis both traditional and eDNA methods should provide equivalent estimates of mean abundance of the fish community. We expect strong concordance between aggregate estimates across all three scales of investigation.

8. Literature Cited

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9. Expected Results

Year 1 (in progress): Broad-scale report on fish communities from eDNA, validated proof-of-concept, and molecular tools for future use. Preliminary comparison of eDNA and net sampling for all three methods. On track to complete these objectives by end of Year 1 (May 2017; see also progress report). Year 2: Full description of traditional and eDNA samples for fish community. Comparisons of aggregate abundance metrics and indices of abundance. Cost-benefit comparison of eDNA and net methodologies.

10. Probability of Success

Year 1 deliverables have a high probability of success, and are partially completed (Fig. 1, Progress Report). eDNA validation with existing methods is straightforward using the statistical framework developed by the PIs in (20).
Year 2 deliverables are very likely, given preliminary results (see Year 1 progress report). The simultaneous use of multiple species-specific markers for use across multiple spatial scales can be unpredictable; unexpected interference between assays can affect accurate quantitation. We will endeavor to reduce these key uncertainties by continuing rigorous in silico and lab-based testing (for new markers) and by leveraging existing research with partners working in Monterey and Puget Sound to validate markers in development. Despite these uncertainties, marrying molecular and traditional surveys holds great promise for improving ecosystem surveys and stock assessments alike. Cost-benefit analysis is straightforward, with high probability of success.

11. Expertise of Principle Investigators and Partnerships

Park (NWSC) has extensive expertise developing and analyzing environmental and tissue-derived genetic samples for use in practical NMFS applications.

Kelly (UW) has experience developing and using eDNA amplicon-sequencing protocols for marine ecosystem surveys over the past several years in Monterey, CA, and Puget Sound, WA. Park and Kelly will oversee the molecular aspects of the study including proper sample collection, preparation, molecular techniques, and bioinformatics analysis.

Greene (NWFSC) will oversee field sampling, consistent with his role with the Skagit Intensively Monitored Watershed Project and will use his statistical expertise to aid the postdoc and Shelton in analyses.

Shelton (ERT) will be in charge of overseeing the statistical analysis of eDNA data and the development of new methods for application as necessary. Shelton has experience developing and applying new statistical methods to environmental and population data. Shelton will work with Greene to develop appropriate metrics for linking and comparing traditional and eDNA data.

Postdoc, O'Donnell (UW), will continue to carry out day-to-day project activities, and will be responsible for coordinating activities among the principal investigators. O'Donnell has shown remarkable success using molecular and bioinformatics techniques over a very short time.

12. Budget Justification

The proposed research will require personnel dedicated full-time to this project, thus the majority of the budget supports a postdoctoral researcher who will be performing field collections, laboratory procedures, and data analyses. Supply costs for field and laboratory work are included, with the largest non-personnel expense being the contract for next-generation sequencing services. While this expense might appear large the power of the technique lies in the tens of millions of sequences that will be generated and the actual cost per sample (and per sequence) is quite low. We have increased the costs slightly over our original budget request from 2016 to reflect unanticipated fees of 3.1% (ca. $3K) for the contract to UW to cover the postdoc salary.

13. Investigators and Affiliations

First Name	Last Name	Title	Role	Affiliation	Email	Phone 1	Phone 2
Correigh	Greene	Research Biologist	Co-investigator	NWFSC	correigh.greene@noaa.gov	206-860-5611	
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A. Ole	Shelton	ERT contractor	Co-investigator	NWFSC	ole.shelton@noaa.gov	206-860-3209	

14. Project Schedule

Task #	Schedule Description	Prerequisite	Planned Start Date	Planned Completion Date	Milestone
1	eDNA collections Perform preliminary comparison of eDNA and net sampling results		04/01/2017	08/31/2017	
2	Final statistical and bioinformatics analysis		09/01/2017	12/31/2017	
3	Complete analysis and write results for publication		01/01/2018	03/31/2018	

15. Cost Estimates

Cost Name	Cost Description	Cost Amount	Date Needed
Consultants/Contracts	Renew postdoc contract for laboratory and field work (incl. indirect)	$95000.00	04/01/2017
Other	Molecular supplies: DNA extraction supplies, primers for multiplexing, DNA library preparation and QA/QC, sampling bottles, filters, etc.	$10500.00	
Consultants/Contracts	Contract for sequencing service and associated supplies	$10000.00	04/01/2017
TOTAL COST		$115500.00	

16. Project Risk

Risk Description	Risk Impact	Risk Probability	Risk Mitigation Approach
The simultaneous use of multiple species-specific qPCR markers for use across multiple spatial scales can be unpredictable; unexpected interference between assays can affect accurate quantitation.	Interference between individual qPCR assays can result in the incorrect quantitation (relative and/or absolute) of DNA for certain species. Mapping of the spatial and temporal dynamics of key taxa would be compromised.	Low	We will endeavor to reduce these key uncertainties by continuing rigorous in silico and lab-based testing (for new markers) and by leveraging existing research with partners working in Monterey and Puget Sound to validate markers in development. We will test our assays in controlled combinations to detect interference and re-design and re-test assays when necessary.

17. Supporting Documents

"2016 Year 1 Progress Report", page 1

NOAA Advanced Sampling Technology Working Group

FY2016 Progress Report to the Office of Science and Technology – December 2016

TITLE Improving techniques for estimating abundance and habitat use in nearshore marine habitats using environmental DNA

INVESTIGATORS Linda Park (NWFSC), Correigh Greene (NWFSC), Ryan Kelly (School of Marine and Environmental Affairs, University of Washington, 3707 Brooklyn Ave NE, 206.616.0185, rpkelly@uw.edu), A. Ole Shelton (ERT Corp. contracted to NWFSC, 2725 Montlake Blvd. E. Seattle, WA. 206-860-3209, ole.shelton@noaa.gov)

GOALS

The overall project goal is to develop and assess a reliable method for rapidly detecting the occurrence and abundance of coastal fish using molecular tools.

PRIORITIES

Our project addresses ASTWG themes **2** (Remote species identification and enumeration) and **5** (Efficient Ecosystem Surveys).

APPROACH

We received funding in May 2016 and were able to hire a postdoctoral researcher who started in August 2016. For the first 4 months of our first year of funding, we focused on collecting water samples in collaboration with the Skagit River Intensively Monitored Watershed Project (SRP; Beamer et al. 2005) and developing single-species molecular tools for use with Chinook salmon. Despite not having a dedicated postdoc, we were able to collect replicate samples alongside both Fyke and beach seines in June 2016 and process these samples (extract DNA and archive samples). Since beginning in August, our postdoc has successfully tested single-species molecular markers for Chinook salmon, begun the process of developing primers for the most abundant species observed in field sampling (Shiner surfperch, *Cymatogaster aggregata*), and identified appropriate primers that can be used for metabarcoding (multi-species) eDNA approaches. Single-species primers for shiner surfperch and Chinook salmon are required to translate proportional composition estimates from metabarcoding into concentrations of DNA (see Shelton et al. 2016).

Looking forward to the end of Year 1 and into Year 2, we anticipate both field and laboratory components continuing. For the field component, we plan to accompany SRP researchers during the full sampling year from February to September 2017 and collect environmental DNA samples spanning the full seasonal range to capture early, peak, and late Chinook outmigration abundances in the Skagit river estuary. We will collect replicate samples from Fyke and beach seine sample locations on at least four dates in 2017 and take these samples to the laboratory for molecular analysis. We anticipate sampling alongside the surface trawl survey in 2017 as well, but we were unable to collect samples from trawls in 2016 due to events beyond our control (see *Work Completed and Results* below).

For the laboratory component, we envision using the remainder of the first year completing Chinook qPCR analysis of existing samples, developing, testing, and deploying single-species qPCR assays for shiner surfperch, and confirming the efficiency of metabarcoding primers for fish community assays. We will use Year 2 funding to process field collected samples using quantitative PCR (qPCR) and metabarcoding and comparing results to beach seine and Fyke net counts and biomass estimates. Once processed, we will use our recently published statistical framework (Shelton et al. 2016) to generate both quantitative estimates of local fish abundance and indices of estuary-wide abundance that are relevant to salmon and forage fish management.

WORK COMPLETED AND RESULTS

To date we have complete several components of our first year proposal. First we successfully hired a postdoctoral researcher to lead the field collections and molecular aspects of our proposal. We collected field water samples (six replicates per site) from 20 site-day combinations from Skagit Bay in late May and June 2016. All water samples were filtered and to date DNA has been extracted from 112 water samples (extractions include appropriate controls). We have identified primers that could be used for qPCR and independently confirmed their ability to solely amplify Chinook salmon DNA using 192 qPCR assays.

To test qPCR against field survey methods, we chose samples from 10 sites that span the full range of Chinook salmon abundances observed in the field. Figure 1 shows a preliminary comparison of Chinook salmon DNA concentration derived from pPCR and Chinook salmon captured in beach seines at 10 sites in Skagit Bay. Each point represents the number of Chinook salmon captured in beach seines at each site and the median DNA concentration estimated from three independent water samples. A linear model demonstrates the number of fish (log(x+1)) was a significant predictor of DNA concentration ($p = 0.000762$) and that the intercept cannot be distinguished from 0 ($p = 0.596$). This result shows that our approach to date has been successful and we are ready to expand our work to additional sites and samples.

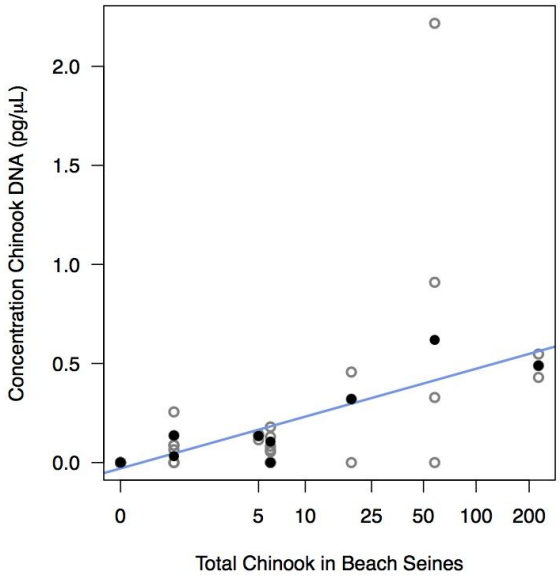

Figure 1: Skagit Chinook salmon eDNA concentration varies as a function of the total chinook caught in beach seines (N = 10 sites). Black points indicate site medians, grey points indicate individual replicate samples. Note that the black point indicating zero Chinook salmon and zero DNA represents identical results from two sites.

In addition to these successes using qPCR for Chinook salmon, we have identified candidate primers for using qPCR on surfperch. Beyond single-species primers, we have in hand a general metabarcoding primers that can be used for sampling the nearshore fish community in Puget Sound. We have tested eDNA samples from Puget Sound using these primers and successfully detected salmonids and other nearshore fish species under field conditions (O'Donnell et al. in review; Kelly et al. in press; Kelly et al. 2016).

We did not collect eDNA using our third sampling method - surface trawls - during the summer of 2016 due to vessel mechanical troubles beyond our control. The surface trawl surveys simply did not occur for almost the entirely of 2016. We intend to reassess the potential for collecting eDNA in concert with surface trawls early in 2017.

IMPACT/APPLICATIONS

The work has far-reaching application, insofar as environmental DNA tools are likely to be broadly useful for surveying field sites for known species (via qPCR) and for a cross-section of species of interest (via amplicons sequencing). In particular, if our project succeeds in quantitatively cross-validating the eDNA methods with the existing effort- and cost-intensive net-based methods of surveying fish species of management interest, we imagine eDNA methods could meaningfully complement—or eventually, even replace—such net sampling.

TRANSITION TO OPERATIONS

The first year has focused on tool development and methods testing, and as indicated in the original proposal, the second year of fieldwork will focus on developing the quantitative relationship between field samples and DNA quantification. Our progress has been right in line with our proposed schedule and we look forward to sampling beginning in February 2017 and sampling across the entire range of the Chinook outmigration from the Skagit river.

ADDITIONAL INFORMATION

The ASTWG-funded project benefits from a set of collaborations between NOAA, the University of Washington, and affiliated researchers. Those projects include 1) Urbanization's effect on nearshore communities (with Ole Shelton, Jameal Samhouri, and others at NMFS; Kelly et al. 2016), 2) Assessment of nearshore habitat recovery after the removal of the dams on the Elwha River (with Jeff Duda, Marshal Hoy, and others at USGS), 3) Effects of oyster aquaculture on local biodiversity (with oyster industry representatives and scientists Jeff Cordell and Jason Toft at UW), and most immediately relevant, 4) Assessment of the quantitative relationship between qPCR assays and intensive manual counts of a sockeye salmon run in Hansen Creek (Lake Aleknagik, Bristol Bay, Alaska; see link) (with Mike Tillotson, Tom Quinn, and others at UW; Marshal Hoy and Jeff Duda at USGS). This last project has produced preliminary results that mirror those from the ASTWG-funded project (Fig. 2), with qPCR salmon concentrations varying as expected by time (left panel) and by manual salmon count (right panel). Importantly, these results are for counts of adult salmon returning to a small creek, not juvenile salmon in an open bay like our work in the Skagit estuary and are not attempting to move beyond a single-species to assess the nearshore fish community. Thus these results complement but do not replicate our work in Washington state.

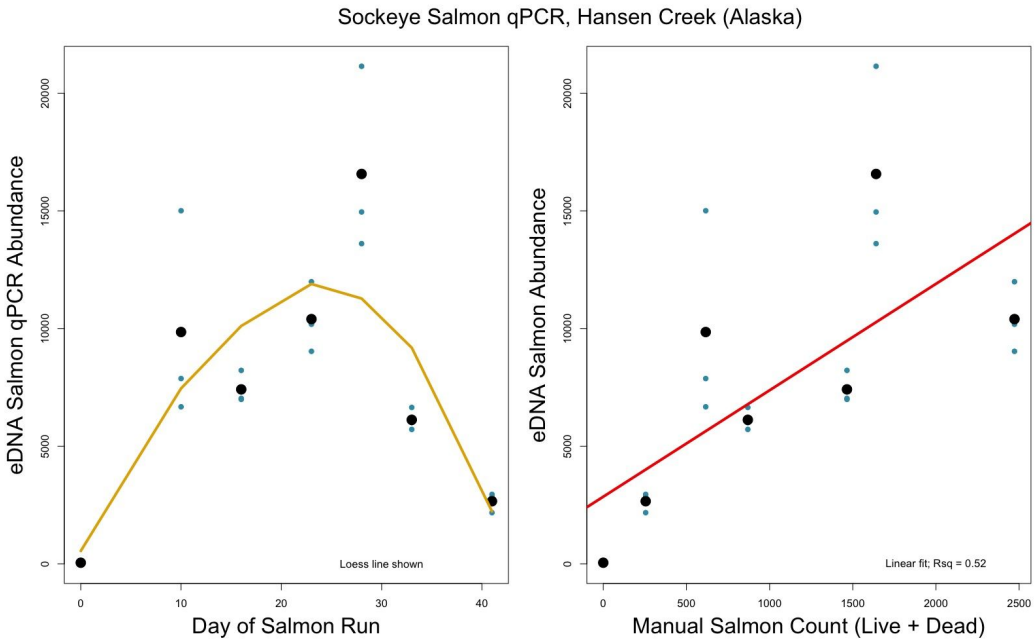

Figure 2: qPCR sockeye salmon concentrations by day of salmon run (left panel) and by manual count of salmon in Hansen Creek. Data courtesy Mike Tillotson (University of Washington) and Marshal Hoy (USGS). Black points represent means for each date (left panel) or manual sockeye count (right panel), with trendline fitted on these means to avoid pseudoreplication.

PUBLICATIONS AND PRESENTATIONS

Presentations:

O'Donnell, Corvallis, OR, December, 2016

Kelly, Western Society of Naturalists, November 2016

Kelly, Puget Sound Partnership, Puget Sound Ecosystem Monitoring Program Steering Committee, November 2016

Kelly, NOAA Science Advisory Board Ecosystem-Based Management Working Group, October 2016

Kelly, Ecological Society of America, August 2016

FY 2016 EXPENDITURES

Grant to University of Washington (for postdoc salary and sequencing): $102,000

Supplies for laboratory and field work: $10,525

LITERATURE CITED

Beamer, E., A. et al. 2005. Delta and nearshore restoration for the recovery of wild Skagit River Chinook salmon: Linking estuary restoration to wild Chinook salmon populations. Supplement to Skagit Chinook Recovery Plan, Skagit River System Cooperative, LaConner, WA. Available at: www.skagitcoop.org.

Kelly, R.P., C.J. Closek, J.L. O'Donnell, J.E. Kralj, A.O. Shelton, and J.F. Samhouri. *In Press*. Genetic and Manual Survey Methods Yield Different and Complementary Views of an Ecosystem. Frontiers in Marine Science. Preprint available at: http://journal.frontiersin.org/article/10.3389/fmars.2016.00283/abstract.

Kelly, R.P., J.L. O'Donnell, N.C. Lowell, A.O. Shelton, J.F. Samhouri, S.M. Hennessey, B.E. Feist, and G.D. Williams. 2016. Genetic Signatures of Ecological Diversity Along an Urbanization Gradient. PeerJ 4:e2444. Available at: https://peerj.com/articles/2444/.

O'Donnell J.L., R.P. Kelly, A.O. Shelton, J.F. Samhouri, N.C. Lowell, G.D. Williams. Spatial distribution of environmental DNA in a nearshore marine habitat. PeerJ Preprints 4:e2608v1. https://doi.org/10.7287/peerj.preprints.2608v1

Shelton, A.O., J.L. O'Donnell, J.F. Samhouri, N. Lowell, G. Williams, R.P. Kelly. A statistical framework for inferring biological communities from environmental DNA. *Ecological Applications* 26:1645-1659.

"Figure 1", page 1

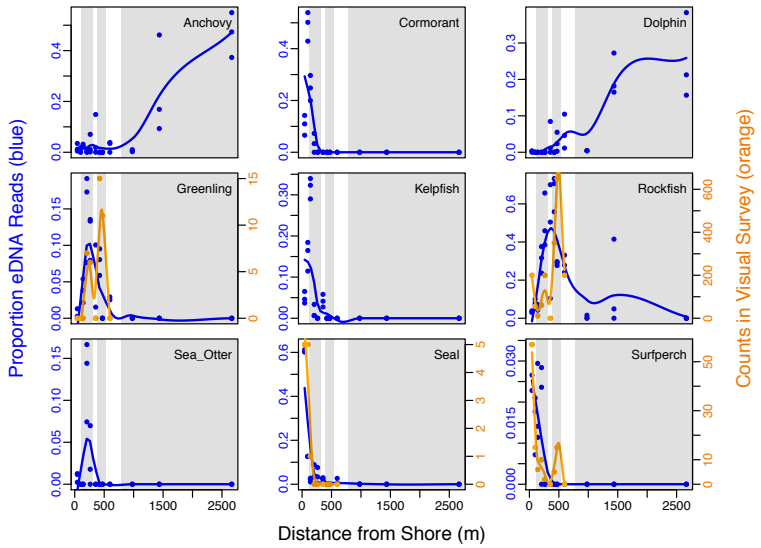

Figure 1. Spatial trends in eDNA and visual count data across a spatial transect through different marine habitats and sub-habitats in Monterey Bay, CA. eDNA counts (expressed as proportions of annotated reads) for three replicate samples are plotted for each sample site. eDNA counts were significantly associated with habitat for all taxa listed (KW, $P<0.05$). Visual counts are included for taxa seen on accompanying dive surveys. Loess curves for visual counts are only included for taxa with >15 counts total across all surveyed sites, and are not best-fit lines. Plot background (white and gray shading) distinguishes the following habitat types, moving away from shore: seagrass, kelp forest, shallow sandy bottom, rocky reef, deep sandy bottom, and open water. From *21*.

"Figure 2", page 1

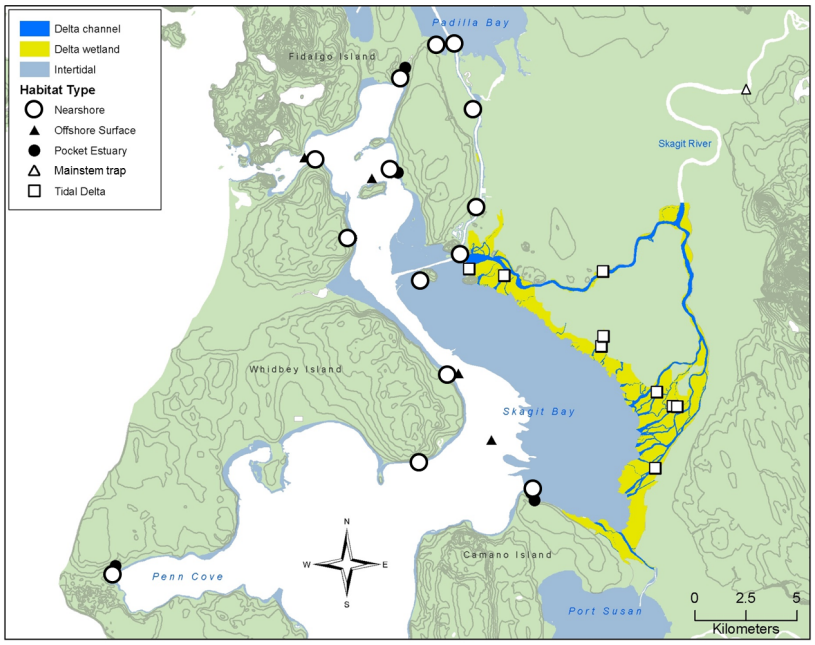

Figure 2. Map of the Skagit River (in Northern Puget Sound, WA) mainstem, estuarine tidal delta, and Skagit Bay, with sites sampled by different gear types indicated by different shapes. Squares indicate Fyke net sites, circles (both open and filled) indicate beach seine sites, and filled triangles indicate offshore trawl sampling sites. Only index sites are shown (site which are sampled during every sampling period).

"Figure 3", page 1

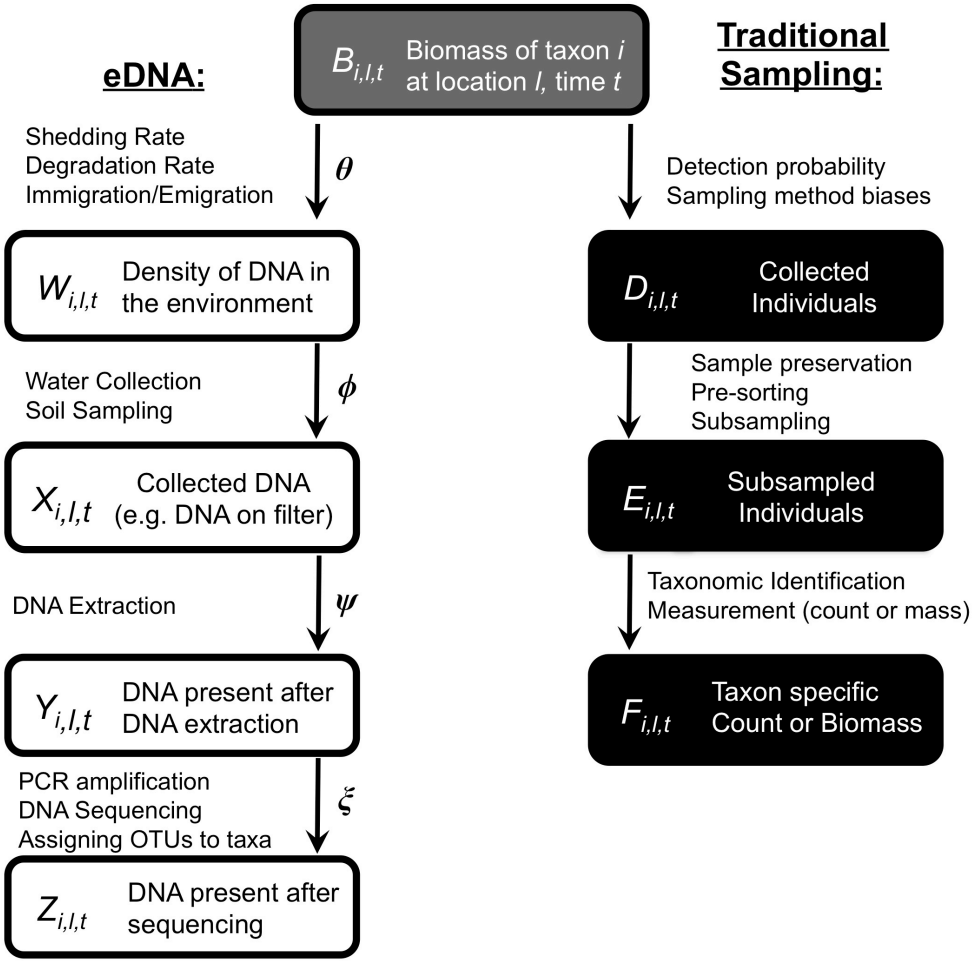

Figure 3: A schematic illustration of the process of sampling ecological communities using eDNA and traditional sampling methods. Boxes correspond to latent states, while arrows and greek letters represent process contributing to the transitions between states. While we present only one eDNA path and one traditional sampling path, recognize that there are many potential variations on the form of this figure depending on the details of a particular protocol. Note that the eDNA and traditional sampling branches of this schematic are not directly comparable (there are no arrows that connect white and black boxes). From *20*.

Table 1. Sampling efforts focused on Chinook salmon in the Skagit River Intensively Monitored Watershed Project.

Habitat	Sampling technique	Area sampled	Sampling months	Sampling frequency	Sites sampled[1]	Sampling efficiency
Estuary channels	Fyke trap	250-4700 m^2	Feb-July	Bi-weekly	11	20-60%
Bay beaches, lagoons	Beach seine	300-700 m^2	Feb-Sept	Bi-weekly	32	70-90%
Bay subtidal surface waters	Kodiak trawl	8-9000 m^3	Apr-Sept	Monthly	12	Unknown but low*

*camera-based observations have revealed substantial escapement of fish, particularly large size classes.

[1]Includes both index sites (sites repeated each sampling event) and random sites (sites sampled randomly with replacement each sampling event).

Curriculum Vitae A.O. Shelton

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Earth Resources Technology, Inc.
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Education

2009	Ph.D University of Chicago, Department of Ecology and Evolution
2005	M.Sc. University of Chicago, Department of Ecology and Evolution
2002	B.A. Biology. Brown University (Honors)

Positions Held

4/2016 – Present	Research Ecologist. Earth Resources Technology, Inc. Contracted to Conservation Biology Division. Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration.
4/2012 – 4/2016	Research Fisheries Biologist. Northwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration.
8/2009 – 4/2012	Post-doctoral scholar, Department of Applied Mathematics and Statistics and Center for Stock Assessment Research, University of California, Santa Cruz. Supervisor: Dr. Marc Mangel.
8/2002 – 8/2003	Research Technician, Hopkins Marine Lab, Stanford University. Supervisor: Dr. Fiorenza Micheli.

Selected Publications

Shelton, A.O., T. Francis, B.E. Feist, G.D. Williams, A. Lindquist, P. Levin. *In Press*. Forty years of seagrass population stability and resilience in an urbanizing estuary. Journal of Ecology. doi: 10.1111/1365-2745.12682

Kelly, R.P., C.J. Closek, J.L. O'Donnell, J.E. Kralj, A.O. Shelton, J.F. Samhouri. *In Press.* Genetic and manual survey methods yield different and complementary views of an ecosystem. Frontiers in Marine Science.

Shelton, A.O., J.L. O'Donnell, J.F. Samhouri, N. Lowell, G. Williams, R.P. Kelly. 2016. A framework for inferring biological communities from environmental DNA. Ecological Applications 26:1645-1659.

Ono, K., A.O. Shelton, E.J. Ward, J.T. Thorson, B.E. Feist, R. Hilborn. 2016. Space-time investigation of the effects of fishing on fish populations. Ecological Applications 26:392-406. DOI: 10.1890/14-1874

Ward, E.J., J.E. Jannot, Y-W Lee, K. Ono, A.O. Shelton, and J.T. Thorson. 2015. Using spatiotemporal species distribution models to identify temporally evolving hotspots of species co-occurrence. Ecological Applications. 25: 2198–2209.

Thorson, J.T., J. Cope, K. Kleisner, J.F. Samhouri, A.O. Shelton, E.J. Ward. 2015. Giants' shoulders 15 years later: Lessons, challenges, and guidelines in fisheries meta-analysis. Fish and Fisheries 16:342-361

Lynch, H.J, J.T. Thorson, and A.O. Shelton. 2014. Dealing with under- and over-dispersed count data in life history, spatial, and community ecology. Ecology 95:3173–3180. http://dx.doi.org/10.1890/13-1912.1

Shelton, A.O., J.F. Samhouri, A.C. Stier, and P.S. Levin. 2014. Assessing trade-offs to inform ecosystem-based fisheries management of forage fish. Scientific Reports 4:7110 DOI: 10.1038/srep07110

Shelton, A.O., W.H. Satterthwaite, M.P. Beakes, S.B. Munch, S. Sogard, and M. Mangel. 2013. Individual variation in growth and its population consequences: separating intrinsic and environmental contributions. American Naturalist 181:799-814

Shelton, A.O. S.B. Munch, D. Keith, and M. Mangel. 2012. Maternal age, fecundity, egg quality, and recruitment: linking stock structure to recruitment using an age-structured Ricker model. Canadian Journal of Fisheries and Aquatic Sciences. 69: 1631–1641

Shelton, A.O. and M. Mangel. 2011. Fluctuations of fish populations and the magnifying effects of fishing. Proceeding of the National Academy of Sciences(USA) 108:7075-7080.

Shelton, A.O., E.J. Dick, D. Pearson, S. Ralston, and M. Mangel. 2012. Single-species landings and uncertainty estimates from multi-species fisheries landings data: hierarchical Bayesian models for California groundfish fisheries. Canadian Journal of Fisheries and Aquatic Sciences. 69:231-246.

Shelton, A.O. 2010. The ecological and evolutionary drivers of female-biases sex ratios: two-sex models of a perennial seagrass. American Natuarlis 175:302-315.

Shelton, A.O. 2010. The origin of female-biases sex ratios in intertidal seagrasses (*Phyllospadix* spp.) Ecology 91:1380-1390.

Shelton, A.O. 2008. Skewed sex ratios, pollen limitation, and reproductive failure in the dioecious seagrass genus *Phyllospadix.* Ecology 89:3020-3029.

Grants, Fellowships, And Awards

Advanced Sampling Technology Working Group (NOAA) 2015-16 (*$112,500*). "Improving techniques for estimating abundance and habitat use in nearshore marine habitats using environmental DNA." PIs: A.O. Shelton, R. Kelly, C. Greene, L. Park.

Habitat Assessment Improvement Plan (NOAA) 2012-13 (*$55,769*) "Integrating spatial habitat and fisheries effort data to improve abundance estimates of west coast groundfish" PIs: A.O. Shelton, E. Ward, J. Thorson, M. Bellman, B. Feist.

National Science Foundation Graduate Research Fellowship *2003-2006 (~$120,000)*

Workshops and Working Groups

***Participant*:** Ocean Modeling Forum, Pacific Herring Working Group. *2016-present*

PI: NCEAS Working Group*:* Applying Portfolio Effects to the Gulf of Alaska Ecosystem: Did Multiscale Diversity Buffer Against the Exxon Valdez Oil Spill?" PIs: K. Marshall, A. Beaudreau, R. Brenner, M. Hunsicker, A.O. Shelton, E. Ward

Participant , NCEAS Working Group on "Red flags and species endangerment: Meta-analytical development of criteria for assessing extinction risk**",** PI: R. Waples, J. Hutchings. *2010-13*

Collaborators over the past 48 months:

Marc Mangel (CSTAR, UC Santa Cruz), Steve Munch (SWFSC), Will Satterthwaite (SWFSC), Micheal Beakes (SWFSC), Phil Levin (NWFSC), Jameal Samhouri (NWFSC), Blake Feist (NWFSC), Sherri Dressel (Alaska Dept. Fish & Game), Ryan Kelly (U. Washington), Eric Ward (NWFSC), Jim Thorson (NWFSC), Kotaro Ono (AFSC), Tessa Francis (U. Washington), Anne Beaudreau (U. Alaska), Richard Brenner (ADFG), Mary Hunsicker (NWFSC), Kristin Marshall (U. Washington), Greg Williams (Pacific States), Adam Lindquist (WDFW), James O'Donnell (U. Washington).

Curriculum Vitae—Linda Park

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Phone: (206)860-3241
email: linda.park@noaa.gov

Education: B.S. Biology/Genetics, 1983, Cornell University, Ithaca, NY.
Ph.D. Population Biology, 1989, Washington University, St. Louis, MO.

Research Experience:
2010-present: Team Leader, Conservation Biology Molecular Genetics Laboratory, NWFSC, Seattle, WA.
2004-2010: Program Manager, Genetics and Evolution Program, NWFSC, Seattle, WA.
2000-present: Team Leader, Northwest Fisheries Science Center Forensic Laboratory, Seattle, WA.
1998-2004: Team Leader, Conservation Biology Molecular Genetics Laboratory, NWFSC, Seattle, WA.
1990-2004: Research Molecular Population Geneticist, NMFS, NWFSC, Seattle, WA.
1990: Post-Doctoral Researcher. Hopkins Marine Station, Stanford University, Pacific Grove, CA.

Selected Publications:

Waples, R.S., A. Elz, W.D. Arnsberg, J.R. Faulkner, J.J Hard, E. Timmins-Schiffman, and L.K. Park. Human-mediated evolution in a threatened species? Juvenile life-history changes in Snake River salmon. Accepted pending revision. Evolutionary Applications

Everett, M.V., L.K. Park, E.A Berntson, A.E. Elz, C.E. Whitmire, A.A Keller, and M.E. Clarke. Large-scale genotyping-by-sequencing indicates high levels of gene flow in the deep-sea octocoral Swiftia simplex (Nutting 1909) on the west coast of the United States. PLoS One 11(10): e0165279. doi:10.1371/journal.pone.0165279.

Ford,M.J., J. Hempelmann, M.B Hanson, K.L Ayres, R.W. Baird, C.K Emmons, J.I. Lundin, G.S Schorr, S.K. Wasser, and L.K. Park. 2016. Estimation of a killer whale (*Orcinus orca*) population's diet using quantitative sequencing analysis of DNA from feces. PLoS ONE 11(1): e0144956. doi:10.1371/journal.pone.0144956

Purcell, M. K., J. J. Hard, K. G. Neely, L. K. Park, J. R. Winton, and D. G. Elliott. 2014. Genetic Variation in Bacterial Kidney Disease Susceptibility in Lake Michigan Chinook Salmon and its Progenitor Population from the Puget Sound. J Aquat Anim Health, 26(1):9-18.

Phillips, R. B., L. K. Park, and K. A. Naish. 2013. Assignment of Chinook Salmon (*Oncorhynchus tshawytscha)* linkage groups to specific chromosomes reveals a karyotype with multiple rearrangements of the chromosome arms of rainbow trout (*Oncorhynchus mykiss*). G3, 3(12):2281-8.

Naish, K. A., R. B. Phillips, M. S. O. Brieuc, L. Newton, A. Elz, L. K. Park. 2013. Comparative genome mapping between Chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout (*O. mykiss*) based on homologous microsatellite loci. G3, 3(12):2289-95.

Faber-Hammond, J., R. B. Phillips, and L. K. Park. 2012. The Sockeye Salmon Neo-Y Chromosome is a Fusion between the Coho Y Chromosome and the Long Arm of Rainbow Trout Omy2. Cytogenetic and Genome Research, 136(1):69-74.

Myers, J. M., L. K. Park, K. G. Neely, P. Swanson, J. J. Hard, and A. Elz. 2011. Feeding ration, genetics, and reproductive traits in female coho salmon: is bigger better? Journal of the World Aquaculture Society, 42(6):812-823.

Metzger, D. C., D. G. Elliott, A. Wargo, L. K. Park, M. K. Purcell. 2010. Pathological and immunological responses associated with differential survival of Chinook salmon following Renibacterium salmoninarum challenge. Dis Aquat Organ. 2010 May 18;90(1):31-41.

Purcell, M.K., A.L. Murray, A. Elz, L.K. Park, S.V. Marcquenski, J.R. Winton, S.W. Alcorn, R.J. Pasco, and D.G. Elliott. 2008. Decreased mortality of Lake Michigan Chinook salmon (*Oncorhynchus tshawytscha*) following bacterial kidney disease challenge: evidence for pathogen-driven selection? Journal of Aquatic Animal Health, 20:225-235.

Swanson, P., Campbell, B., Shearer, K., Dickey, J., Beckman, B., Larsen, D., Park, L., and Berejikian, B. 2008. Application of Reproductive Technologies to Captive Breeding Programs for Conservation of Imperiled Stocks of Pacific Salmon. Cybium 32(2) suppl.:279-282.

Phillips RB, Dekoning J, Morasch MR, Park LK, Devlin RH. 2007. Identification of the sex chromosome pair in chum salmon (Oncorhynchus keta) and pink salmon (Oncorhynchus gorbuscha). Cytogenet Genome Res. 2007;116(4):298-304.

Tabor, R. A., B. Footen, K. Fresh, M. Celedonia, F. Mejia, D. Low, and L. Park. 2007. Smallmouth Bass and Largemouth Bass Predation on Juvenile Chinook Salmon and Other Salmonids in the Lake Washington Basin. J. Fish. Management 27:1174-1188

Hard, J. J., D. G. Elliott, R. G. Pascho, D. M. Chase, L. K. Park, J. R. Winton, and D. E. Campton. 2006. Genetic variation in disease resistance of chinook salmon (*Oncorhynchus tshawytscha*): are phenotypic indicators of *Renibacterium* infection correlated with disease resistance of their progeny? Canadian Journal of Fisheries and Aquatic Sciences. 63:2793-2808.

Schwenke, P, Rhydderch J, Ford, MJ, Marshall, A, and Park, L. 2006. Forensic identification of endangered Chinook Salmon (Oncorhynchus tshawytscha) using a multilocus SNP assay. Conservation Genetics 7:983-989.

Purcell, M., Nichols, K., Winton, J., Kurath, G., Thorgaard, G., Wheeler, P., Hansen, J., Herwig, R. and Park , L. 2006. Comprehensive gene expression profiling following DNA vaccination of rainbow trout against infectious hematopoietic necrosis virus. Molecular Immunology. 43(13):2089-106

Smith, C. T., L. Park, D. VanDoornik, L.W. Seeb and J. E. Seeb. Characterization of 19 single nucleotide polymorphism markers for coho salmon. 2006. Molecular Ecology Notes 6:715-720.

Devlin, R. H., L. Park, D.M. Sakhrani, J. D. Baker, A.R. Marshall, E., LaHood, S.E. Kolesar, M.R. Mayo, C.A. Biagi, and M. Uh. 2005. Variation of Y-chromosomal DNA markers in Chinook salmon (Oncorhynchus tshawytscha) populations. Canad. J. Fish. Aqua. Sci. 62(6): 1386-1399

Phillips, R. B., M. R. Morasch, L. K. Park, K. A. Naish, and R. H. Devlin. 2005. Identification of the sex chromosome pair in coho salmon (*Oncorhynchus* kisutch): lack of conservation of the sex linkage group with chinook salmon (*Oncorhynchus tschwytscha*). Cytogenetics and Genome Research 111(2):166-70

McClelland, E.K., J.M. Myers, J.J. Hard, L.K. Park, and K.A. Naish. 2005. Two generations of outbreeding in coho salmon (*Oncorhynchus kisutch*): effects on size and growth. Can. J. Fish. Aquat. Sci. 62: 2538–2547

Smith, C. T., J. Baker, L. Park, L. W. Seeb, C. Elfstrom, S. Abe and J. Seeb. 2005. Characterization of 13 single nucleotide polymorphism markers for chum salmon. Mol. Ecol. Notes. 5: 259-262

"Park CV 2016", page 3

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rpkelly@uw.edu 206.616.0185

Education

J.D. 2011, University of California, Berkeley. School of Law (Boalt Hall).
Ph.D. 2006, Columbia University, New York. Ecology, Evolution, and Environmental Biology.
M.Phil. 2005, Columbia University, New York. Ecology, Evolution, and Environmental Biology.
M.A. 2003, Columbia University, New York. Ecology, Evolution, and Environmental Biology.
B.Sc. 2000, University of California, Los Angeles. Ecology and Evolution.

Employment History

March 2013 - Present: Assistant Professor, University of Washington, School of Marine & Environmental Affairs.
September 2011 - March 2013: Fellow, Center for Ocean Solutions, Stanford University.
August 2009 - May 2011: Graduate Student Instructor, UC Berkeley.
May - August 2009: Sierra Club Environmental Law Program, Summer Associate.
September 2006 - August 2008: Postdoctoral researcher, Stanford University.
August 2002 - May 2006: Graduate student and teaching assistant, Columbia University.
August 2003 - May 2005: Science enrichment teacher, 7^{th} and 8^{th} Grades, New York City.
Spring 2002: Staff Assistant, United States Senator Barbara Boxer, Washington, D.C.

Research Interests

My interests span the divide between hard scientific data and policymakers' use of those data. My research joins genetic and ecological research with real-world implementation in law and policy, particularly with respect to environmental monitoring, resource management, endangered species, and ocean acidification.

Research Experience

March 2013 - Present: Principal Investigator, University of Washington. Research focuses on environmental monitoring using environmental DNA, ocean acidification policy, environmental management using ecosystem thresholds, and related topics.
August 2008 - March 2013: Independent and collaborative work at Stanford University and U.C. Berkeley on ocean policy, marine genetics, and the intersection of scientific data and law.
September 2006 - August 2008: Postdoctoral work at Stanford University's Hopkins Marine Station in the laboratory of Stephen R. Palumbi, on comparative population genetics in Pacific nearshore species.
August 2002 - May 2006: Doctoral research at Columbia University in the laboratory of Rob Desalle at the American Museum of Natural History in New York City, on molecular phylogenetics and population genetics of Pacific chitons (Polyplacophora) and other marine invertebrates.

Recent Awards, Grants, and Fellowships

2016 - 2018: Packard Foundation: Assessing the Effects of Ocean Acidification in the Field Using Environmental DNA
2016: United States Geological Survey, Using eDNA to Assess Restored Elwha River Outflow

2016: Resources Legacy Fund, Comparing Ocean Acidification Policymaking Processes in Washington and California
2015 - 2016: University of Washington Royalty Research Fund, Making eDNA Quantitative
2012 - 2014: Packard Foundation: Environmental DNA as Next Generation Monitoring Tool

Relevant Publications

O'Donnell, J.L., **R.P. Kelly**, A.O. Shelton, J.F. Samhouri, N.C. Lowell, G.D. Williams. 2016. Spatial Distribution of Environmental DNA in a Nearshore Marine Habitat. (*submitted*, PeerJ).

Kelly, R.P., C.J. Closek, J.L. O'Donnell, J.E. Kralj, A.O. Shelton, and J.F. Samhouri. 2016. Genetic and Manual Survey Methods Yield Different and Complementary Views of an Ecosystem. (*in press*, Frontiers in Marine Science).

Kelly, R.P., J.L. O'Donnell, N.C. Lowell, A.O. Shelton, J.F. Samhouri, S.M. Hennessey, B.E. Feist, and G.D. Williams. 2016. Genetic Signatures of Ecological Diversity Along an Urbanization Gradient. PeerJ 4:e2444.

Kelly, R.P. 2016. Making Environmental DNA Count. Molecular Ecology Resources 16: 10-12.

Lowell, N. and **R.P. Kelly.** 2016. Evaluating Agency Use of "Best Available Science" Under the United States Endangered Species Act. Biological Conservation 196:53-59.

Port, J.A., J.L. O'Donnell, O.C. Romero-Maraccini, P.R. Leary, S.Y. Litvin, K.J. Nickols, and **R.P. Kelly**. 2016. Assessing the Vertebrate Community of a Kelp Forest Ecosystem using Environmental DNA. Molecular Ecology 25(2):527-541.

O'Donnell, J.L., **R.P. Kelly**, N. Lowell, and J.A. Port. 2016. Indexed PCR Primers Induce Template-Specific Bias in Large-Scale DNA Sequencing Studies. PLoS One 11(3): e0148698.

Shelton, A.O., J. L. O'Donnell, J.F. Samhouri, N. Lowell, G.D. Williams, and **R.P. Kelly**. 2016. A Framework for Inferring Biological Communities from Environmental DNA. Ecological Applications 26(6): 1645-1659.

Marshall, K., A. Stier, J. Samhouri, **R.P. Kelly**, E. Ward. 2015. Conservation Challenges of Predator Recovery. Conservation Letters 9(1):70-78.

Selkoe, K.A., T. Blenckner, M.R. Caldwell, L. Crowder, A. Erickson, T. Essington, J. Estes, R. Fujita, B.S. Halpern, M. Hunsicker, C.V. Kappel, **R.P. Kelly**, J.N. Kittinger, P.S. Levin, J. Lynham, M. Mach, R. Martone, L. Mease, A. Salomon, J. Samhouri, C. Scarborough, A. Stier, C. White, J. Zedler. 2015. Principles for Managing Marine Ecosystems Prone to Tipping Points. Ecosystem Health and Sustainability 1(5):17.

Kelly, R.P., A.L. Erickson, and L.A. Mease. 2015. How Not to Fall Off a Cliff, or, Using Tipping Points to Improve Environmental Management. 41 Ecology Law Quarterly 843.

Kelly, R.P. 2015. Will More, Better, Cheaper, and Faster Monitoring Improve Environmental Management? 44 Environmental Law 1111-1147.

Kelly, R.P., J.A. Port, K.M. Yamahara, R.G. Martone, N. Lowell, P.F. Thomsen, M.E. Mach, E. Prahler, M.R. Caldwell, and L.B. Crowder. 2014. Harnessing DNA to Improve Environmental Management, Science 344 (6191): 1455-1456. doi:10.1126/science.1251156.

Kelly, R.P., J.A. Port, K.M. Yamahara, and L. Crowder. 2014. Using Environmental DNA to Census Marine Fishes in a Large Mesocosm. PLoS One 9(1): e86175. doi:10.1371/journal.pone.0086175.

Kelly, R.P., A. Erickson, L. Mease, W. Battista, J. Kittinger, R. Fujita. 2014. Embracing Thresholds for Better Environmental Management. Philosophical Transactions of the Royal Society B 370: 20130276.

Kelly, R.P., and M. Caldwell. 2013. "Not Supported by Current Science" : The National Forest Management Act and the Lessons of Environmental Monitoring for the Future of Public Resources Management. 32 Stanford Environmental Law Journal 151.

Kelly, R.P., M.M. Foley, W. Fisher, R. Feely, B.S. Halpern, G.G. Waldbusser, and M.R. Caldwell. 2011. Mitigating Local Causes of Ocean Acidification with Existing Laws. Science 332: 1036-1037.

Correigh Greene, PhD

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Northwest Fisheries Science Center, NOAA
2725 Montlake Blvd, Seattle WA 98112

Professional Preparation

Ph.D. in Animal Behavior, UC Davis (Davis CA), 2001
MS in Wildlife Ecology, University of Michigan, (Ann Arbor MI), 1995
BS in Environmental Studies and Pscyhobiology, Tufts University (Medford MA), 1992

Appointments

2002 – present: Research biologist within the Watersheds Program
2003 – 2005: Team leader, Fish Habitat Relationships Team within the Watersheds Program
2001-2002: National Research Council Postdoctoral Research Associate, Northwest Fisheries Science Center

Recent Publications (last 5 years)

Naman, S. M., **Greene, C. M.**, Rice, C. A., Chamberlin, J., Conway-Cranos, L., Cordell, J. R., et al. 2016. Stable isotope-based trophic structure of pelagic fish and jellyfish across natural and anthropogenic landscape gradients in a fjord estuary. Ecology and Evolution. DOI: 10.1002/ece3.2450

Oyafuso, Z.S., A.E. Baxter, J.E. Hall, S.M. Naman, **C.M. Greene**, and L.D. Rhodes. 2015. Widespread detection of human- and ruminant-origin Bacteroidales markers in subtidal waters of the Salish Sea in Washington State. Journal of Water and Health 13:827-837. DOI 10.2166/wh.2015.253.

Greene, C. M., L. Kuehne, C. Rice, K. Fresh, and D. Penttila. 2015. Forty years of change in forage fish and jellyfish abundance across greater Puget Sound, Washington (USA): Anthropogenic and climate associations. Marine Ecology Progress Series 525: 153-170.

Zimmerman, M.S., J.R. Irvine, M. O'Neill, J.H. Anderson, **C.M. Greene**, J. Weinheimer, M. Trudel, and K. Rawson. 2015. Spatial and temporal patterns in smolt survival of wild and hatchery Coho Salmon (*Oncorhynchus kisutch*) in the Salish Sea. Marine and Coastal Fisheries: Dynamics, Management, and Ecosystem Science 7:116-134. DOI: 10.1080/19425120.2015.1012246.

Reum, J.C.P., R.A. Hovel, and **C.M. Greene**. 2015. Estimating continuous body size-based shifts in δ15N-δ13C space using multivariate hierarchical models. Marine Biology 162: 1-10. DOI 10.1007/s00227-014-2574-8.

Greene, C.M., K. Blackhart, J. Nohner, A. Candelmo, and D.M. Nelson. 2014. A national assessment of stressors to estuarine fish habitats in the contiguous USA. Estuaries and Coasts. 1-18.

Reum, J.C.P., T. E. Essington, **C. M. Greene**, C. A. Rice, and K. L. Fresh. 2013. Biotic and abiotic controls on body size during a critical life history stage of a pelagic fish. Fisheries Oceanography 22:324-336.

Rice, C., J. Duda, **C.M. Greene**, and J.R. Karr. 2012 Geographic and seasonal patterns of fish and jellyfish assemblage composition in Puget Sound surface waters. Marine and Coastal Fisheries 4:117-128.

Reum, J.C.P., T.E. Essington, **C.M. Greene**, C.A. Rice, and K.L. Fresh. 2011. Multiscale influence of climate on estuarine forage fish: the role of coastal upwelling, river discharge and local water temperature and salinities. MEPS 425: 203–215.

Rhodes, L.D., C.A. Rice, C.M. Greene, D.J. Teel, S.L. Nance, P. Moran, C.A. Durkin, and S.B. Gezhegne. 2011. Nearshore ecosystem predictors of a bacterial infection in juvenile Chinook salmon. MEPS 432: 161–172.

Other Publications

Busch, D. S., **C. M. Greene**, T. P. Good. 2014. Estimating impacts of tidal power and climate change on threatened and endangered marine species and their food web. Conservation Biology.

Rice, C.A., **C.M. Greene**, P. Moran, D.J. Teel, D.R. Kuligowski, R.R. Reisenbichler, E.M. Beamer, J.R. Karr, and K.L. Fresh. 2010. Abundance, length, and stock origin of marked and unmarked juvenile Chinook salmon in the surface waters of greater Puget Sound. Transactions of the American Fisheries Society 140:170-189.

Greene, C.M., J.E. Hall, K.R. Guilbault, and T.P. Quinn. 2010. Improved viability of populations with diverse life history portfolios. Biology Letters 6: 382-386.

Greene, C.M., D.W. Jensen, E. Beamer, G.R. Pess, and E.A. Steel. 2005. Effects of environmental conditions during stream, estuary, and ocean residency on Chinook salmon return rates in the Skagit River, WA. Transactions of the American Fisheries Society, 134:1562-1581.

Greene C.M., and T.J. Beechie. 2004. Consequences of potential density-dependent mechanisms on recovery of ocean-type chinook salmon (Oncorhynchus tshawytscha). Canadian Journal of Fisheries and Aquatic Sciences, 61: 590-602.

Synergistic Activities

National Fish Habitat Action Plan (NFHAP). NFHAP is an assessment of the nation's fish habitats, which involves academia, state agencies, the US Fish and Wildlife Service, US Geological Survey, and the National Marine Fisheries Service. I am co-leader for the nation's estuary and coastal assessment. November 2008-present.

NOAA Fisheries' Habitat Assessment Improvement Plan. Coauthor of a national research plan to advance habitat science of marine fish stocks, address fisheries management, and initiate graduate education opportunities. July 2008-present.

National Center for Ecological Analysis and Synthesis. Panel member of the Moore Foundation-funded Salmon and Climate Change Project, which involves integration of regional and local climate models to predict population changes in Pacific salmon. April 2009-present.

Skagit Climate Science Consortium (SCSC). SCSC is composed of representatives from academia, tribes, and state and federal agencies in order to provide expert advice on the effects of climate change in the Skagit River watershed, the largest watershed in Puget Sound and Seattle's primary source of hydropower. November 2008-present.