

## **Issue and Question**

I propose to identify adaptive genetic markers associated with the expression of alternative life histories (stream-resident and stream-lake migratory) in nonanadromous rainbow trout (*Oncorhynchus mykiss*). This will greatly expand our understanding of the genetic control over life history and migration pathways using *O. mykiss* as the model, and thereby improve conservation of genetically significant units below the species level of classification.

## **Context**

Rainbow trout (*O. mykiss*) span a wide distribution of native forms within North America, ranging from northwest Mexico to southwest Alaska, including several recognized subspecies (Behnke 1992; Quinn 2005). The best-known types of *O. mykiss* are the ocean-rearing (anadromous) steelhead and the freshwater-resident (nonanadromous) rainbow trout (Quinn 2005), which are the focus of nearly all studies of genetic differentiation between life history forms (Docker and Heath 2003; Narum et al. 2004; McPhee et al. 2007; Heath et al. 2008). Yet, “freshwater-resident” and “nonanadromous” are umbrella terms that do not recognize the diversity of life histories and patterns of migration in this species that likely contribute to its productivity and population stability (for example, compare Pacific salmon studies of Greene et al. 2010; Schindler et al. 2010).

After emergence in their natal stream, rainbow trout display an array of life history patterns that result in differential habitat use and trophic ecology. Juveniles of all types initially reside in streams and some stay within the natal stream for their entire life cycle (Quinn 2005). In river systems with small tributaries, trout may leave their natal stream to rear within the larger main stem river and return only to spawn (Quinn 2005). Certain rainbow trout leave their natal streams to rear in lakes, typically only returning in the spring for spawning (Holecek and Scarnecchia 2013) or to opportunistically forage in rivers. Finally, anadromous steelhead migrate to sea after 1-4 years of residence in river systems (Quinn 2005). This gradient of migration balances fitness trade-offs between potential size at reproduction, fecundity in females, and mortality (Holecek and Scarnecchia 2013).

The development of restriction-site associated DNA (RAD) sequencing and single-nucleotide polymorphism (SNP) genotyping now provides enhanced parentage assignment performance (Hauser et al. 2011) and population structure resolution (Seeb et al. 2011), enabling discovery of fine-scale genetic differentiation between sympatric ecotypes. Such studies of anadromous steelhead and nonanadromous rainbow trout have identified and located genes associated with their migratory patterns and life histories that affect functions including neuronal and muscle development, immune response, circadian rhythm, olfaction, and sexual differentiation (Hale et al. 2013; Baerwald et al. 2015).

Employing these advanced methods in the study of different nonanadromous life histories will improve our understanding of the adaptive genetic basis for their behavioral and phenotypic divergence. With an augmented knowledge of and a greater appreciation for the variety of alternative nonanadromous life histories utilized by rainbow trout, we may better conserve and protect the intraspecific diversity that sustains native *O. mykiss* populations across the watersheds of western North America (Keeley et al. 2007).

## **Thesis Context**

My thesis research aims to better describe cryptic life history forms of nonanadromous rainbow trout in order to better inform their conservation. I will use a population complex from Iliamna Lake in southwest Alaska as the model system because it has had no artificial propagation or transplanted populations, and currently is only subjected to a nominal subsistence fishery and catch-and-release recreation (Sepez et al. 2005). As a part of the Bristol Bay watershed, which is located at the beginning of the Alaska Peninsula, Iliamna Lake has minimal human development (Sepez et al. 2005) and no introduced species (Bond and Becker 1963).

Iliamna lake and its associated tributaries support large, diverse populations of nonanadromous rainbow trout. The local habitat diversity and corresponding environmental conditions indicate the presence of both stream-resident (fluvial) and stream-lake migratory (adfluvial) individuals, which is consistent with the documented plasticity of *O. mykiss* life history patterns (Holecek and Scarnecchia 2013). For these rainbow trout, which could easily migrate to the ocean, their lifelong presence in freshwater is attributed to the presence of ample food resources (Kline et al. 1993; Quinn and Myers 2004) that enable maturity at sizes comparable to those of steelhead elsewhere (Kuzishchin et al. 2007; Kendall et al. 2015). The Kvichak River system, of which Iliamna Lake is a part, does not contain steelhead (Behnke 1992), which makes it an ideal area in which to study the diversity of nonanadromous life history forms. Employing a multifaceted approach, I will assess the ecological, growth, and genetic differentiation of nonanadromous rainbow trout in Iliamna Lake and its associated tributaries.

#### *Life History Forms*

Stable isotope analysis of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  will facilitate assessment of trophic level (Minagawa and Wada 1984) and habitat use (Rau 1980), respectively, and ontogenetic shifts in both. The resident trout may share prey in the same habitats (suggestive of generalist foraging ecology) or may utilize different food resources in discrete habitats (indicative of ecotypic separation) (Robillard et al. 2011). Analysis of preliminary samples collected in 2015 shows that this is promising, and we will collect additional samples in 2016.

Fitting mixture models of Von Bertalanffy growth curves to length-at-age data will allow for the identification of growth trajectories and asymptotic sizes characteristic of different life history forms (Woods et al. 2012). Similar growth trajectories may suggest that they experience the same environmental conditions, while dissimilar trajectories with different asymptotic sizes may indicate experience of discrete conditions (Woods et al. 2013). Additionally, non-overlapping age distributions in the stream and lake may indicate an ontogenetic shift from resident to migratory behavior by all trout, whereas overlapping age distributions in those two habitats would indicate that individual trout adopt stream-resident (fluvial) or stream-lake migratory (adfluvial) life histories (Al-Chokhachy and Budy 2008).

#### *Genetic Analyses*

The genetic analyses proposed are designed to enable the identification and genomic location of candidate markers associated with the expression of alternative life histories (Hale et al. 2013). Fluvial and adfluvial fish may exhibit minimal population differentiation at adaptive loci, suggesting weak selection, or significant differentiation at adaptive loci, indicating strong selection (Hale et al. 2013). Reproductive isolation of life history forms, resulting from spatial or temporal separation, in addition to mate selectivity (Jonsson et al. 1988), may result in differentiation at neutral loci (Limborg et al. 2012).

In concert, the three synergistic approaches described above will yield information that augments the ability of conservation and management efforts to recognize and protect the diverse life history forms of nonanadromous rainbow trout throughout their range.

**Methods and Approach**

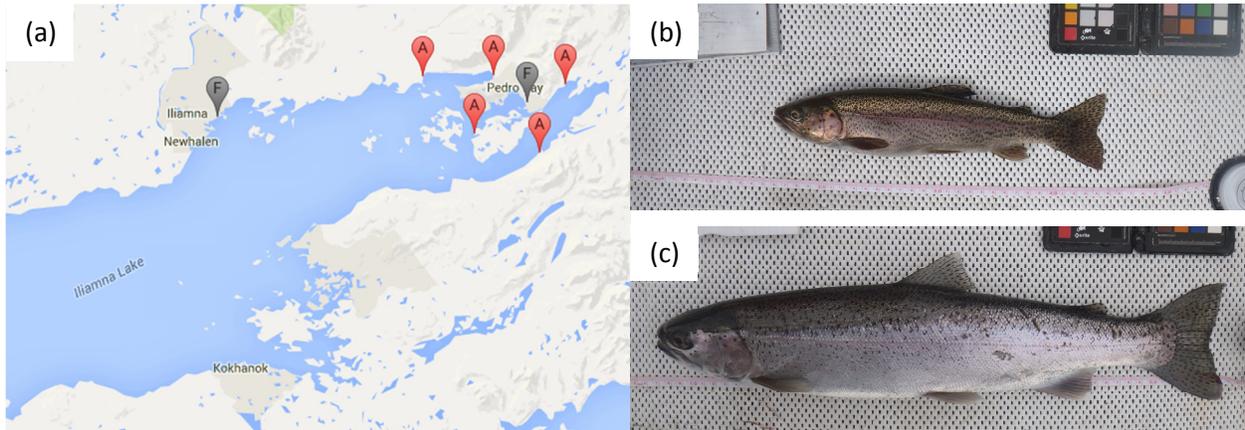


Figure 1 – (a) Sampling site map of eastern Iliamna Lake, AK (Google Earth). Gray “F” and red “A” marks show where fluvial and adfluvial rainbow trout, respectively, will be sampled. Representative fluvial (b) and adfluvial (c) individuals.

I will collect caudal fin clips from 96 rainbow trout in each of three primary sampling areas; Russian Creek (1 site), Roadhouse Creek (1 site), and Iliamna Lake (5 sites; Fig. 1). Fish in Russian and Roadhouse Creek represent stream-resident (fluvial) populations, whereas those in Iliamna Lake represent stream-lake migratory (adfluvial) populations (Fig. 1). All sampling will be conducted under an ADF&G fish resource permit, following IACUC Animal Care Protocol 3142-01.

Following the methods of Baird et al. (2008) and Everett et al. (2012), RAD libraries will be prepared for 192 fluvial and 96 adfluvial *O. mykiss* with the *SbfI* restriction enzyme. An Illumina HiSeq2000 (single-end, 100 bp target – SE:100) will be used for sequencing with 96 individuals pooled into each sequencing lane.

**Product**

The findings of this work will be submitted as a manuscript to Molecular Ecology and be presented at the Alaska Salmon Program Science Symposium in 2017.

**Timeline**

Table 1 – Quarterly timeline of proposed research

	2016		2017	
	Summer	Fall	Winter	Spring
Field sampling of fin clips	█			
Collect RAD data		█		
Genetic analysis			█	
Manuscript preparation			█	█

**Budget**

I am requesting \$6,000 for this project. Please see Table 2 for the itemized costs.

Table 2 – Itemized budget of RAD sequencing data collection costs for proposed identification and location of genetic markers associated with alternative life histories of nonanadromous rainbow trout.

Data collection costs	Item	No. items	\$/Item	Total
DNA extraction	96 well plate	3	254	762
University of Oregon sequencing	Sequencing lane	3	1800	5400
				\$6,162

### **Budget Justification**

The well plates and sequencing lanes are necessary for the proposed data collection and will allow for genetic sampling of up to 288 individual rainbow trout. I request no field personnel or travel funding, which will be covered by the Alaska Salmon Program and Dr. Quinn’s research budget, respectively. Dr. Quinn has also agreed to cover the difference between my request (\$6000) and the anticipated total cost (\$6162).

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