eGC1 number: A56275

Proposal Title: Salmon senescence as a model for aging

PI: Steven Roberts
School of Aquatic and Fishery Sciences

Co-PI: Thomas Quinn
School of Aquatic and Fishery Sciences

Abstract
Aging is a complex process that is regulated by multiple cellular and molecular mechanisms. Given the clinical interest in better understanding senescence there has been significant research on human subjects and model organisms. Two primary processes that have been identified are telomere shortening and oxidative stress. While it is clear that these two processes are important in regulating senescence there still is a lack of understanding regarding *whether telomere shortening directly triggers senescence, what mechanisms control telomere length, and what are the molecular mechanisms governing energy allocation.* Pacific salmon, *Oncorhynchus* spp. are ideally suited organisms for the study of aging. There is a very high heritability for the date of reproduction in salmon, so longevity is not a random variable but rather an adaptation to breeding date within a population. In addition to the adaptive variation in longevity within populations and the links to other aspects of fitness such as energetic investment in gonads vs. body stores, there is also significant variation in senescence rates among populations. These facts indicate an adaptive, genetic basis for variation in senescence rates in salmon. The research objectives of this study are to 1) characterize transcriptomic differences in senescent and pre-senescent salmon and 2) develop assays for specific target processes involved in aging and senescence. An understanding of these mechanisms would not only be a breakthrough in the biology of these much-studied fishes, but it would also facilitate their use as a model system for the study of general processes of aging.

Description of Proposed Research

A. Introduction and Rationale

Aging is a complex process that is regulated by multiple cellular and molecular mechanisms. Aging is generally characterized by cellular damage exceeding the work capacity of repair mechanisms (Kim 2007) and the associated decline in cell replication (Hodes 1999). The accumulation of these age-related changes, ultimately leading to death, is referred to as senescence (Fitch 1994). During senescence an organism is more susceptible to disease and experiences an inability to carry out fundamental physiological processes (Chen 2007). Given the clinical interest in better understanding senescence there has been significant research on human subjects and model organisms including mice and nemotodes.

Several of the processes associated with aging and senescence are conserved from across taxa. Two primary processes that have been identified as regulating senescence are telomere shortening and oxidative stress. Telomeres are series of tandem nucleotide repeats that cap the ends of chromosomes protecting them from degradation and the loss of genetic information that directs cellular processes. As cells age telomeres shorten in length and it is hypothesized that a critical telomere length could be one possible trigger of senescence (Armanios 2009). Oxidative stress refers to damage that occurs when reactive oxygen species (byproducts of cellular metabolism) supersedes detoxification activity, causing damage to cells (Passos et al. 2007). While is is clear that these two processes are important in regulating senescence there still is a lack of understanding regarding ***whether telomere shortening directly triggers senescence, what mechanisms control telomere length, and what are the molecular mechanisms governing energy allocation***. To provide new insights into cellular and molecular mechanisms regulating senescence, several research groups have suggested taking a more comparative approach (Fitch 1994, Blackburn et al. 2006, Kim 2007). Recent technological advances now make it increasing easier to use non-model species to examine biological processes, including senescence.

Pacific salmon, *Oncorhynchus* spp. are ideally suited organisms for the study of aging. The five species found in North America (Chinook, coho, chum, pink and sockeye salmon) are all entirely semelparous. That is, all individuals mature and reproduce in one season and die thereafter. Their life cycles vary among species and populations but can be summarized as follows (Quinn 2005). Breeding occurs in the fall, when females select, prepare and defend a nest site from disturbance by other females, and males compete aggressively for the opportunity to breed with females. Within days or, at most, a few weeks, both males and females die, depleted of body energy (Hendry and Berg 1999) and have elevated levels of cortisol (Barry et al. 2001). Only under extremely controlled and artificial conditions do any individuals ever survive (Unwin et al. 1999). The fertilized eggs develop as embryos in the stream gravel environment during the winter and emerge as free-swimming fry in spring. Depending on the species and population, they spend some variable period of time growing in freshwater, then migrate to sea where they achieve the great majority of their final adult size. The processes of sexual maturation commence while the salmon are still at sea, months before they return to freshwater to spawn. Cued by

photoperiod, the salmon migrate from distant parts of the North Pacific Ocean and converge on their natal stream with great fidelity and temporal precision, complete the final stages of maturation, breed, and die (Quinn 2005).

Salmon cease feeding during the final period of their migration and reproduction, thus they are "capital breeders", a term referring to species whose entire energetic investment in reproduction is made in advance, with no "refueling" during reproduction. Thus they must allocate their limited supply of energy among competing demands: 1) migration to the spawning areas, which may be near to the ocean or far upriver, 2) gonads (ca. 20% of the mass in females and 5% in males), 3) courtship, nest building, territorial displays and battles, and other forms of behavior related to reproduction, and 4) longevity or lifespan in the stream itself. Fisheries biologists have long known that early arriving salmon tend to live longer than those that arrive later (Perrin and Irvine 1990) but the phenomenon was not put into an evolutionary context. However, Hendry et al. (1999) reported that not only did early arriving sockeye salmon (of both sexes) lived longer in the stream than late arriving ones but the late arriving fish allocated proportionally more energy to gonads and less to somatic stores needed for longevity. ***There is a very high heritability for the date of reproduction in salmon (Carlson and Seamons 2008), so longevity is not a random variable but rather an adaptation to breeding date within a population.*** Females breeding early need to guard their nest from disturbance by later arrivals, whereas those breeding at the end of the season have no such need. Males arriving early need to live as long as possible to maximize their breeding opportunities whereas those at the end of the season have no such selection for longevity (Carlson et al. 2004).

In addition to the adaptive variation of senescence within populations and the links to other aspects of fitness such as energetic investment in gonads versus body stores (Hendry et al. 2004), there is also significant variation in in-stream longevity (i.e., rate of senescence) among populations. That is, in some populations the in-stream longevity ranges from about two weeks for early arriving fish to one week for late fish whereas in nearby streams the range is from about four weeks to two weeks. This variation is related to the probability that the salmon will be killed early in their lives by bears, who kill up to 80% of the mature salmon in some small streams. In shallow, narrow streams, bears kill a higher proportion of the salmon than they do in larger streams (Quinn et al. 2001), and the bears also tend to kill newly arrived salmon because such salmon provide more energetic benefit as food for the bear (Gende et al. 2004). As Carlson et al. (2007) reported, salmon in streams where a high proportion are killed, and tend to get killed early in their breeding lives, show markedly faster senescence rates than salmon in streams where they are less likely to be killed, and to live most of their lives prior to being killed. Thus work by the Quinn Lab (Co-PI) on sockeye salmon in a series of streams has demonstrated consistent patterns of variation in senescence rate within and among populations, and has linked these patterns to strong, quantified selection mechanisms. Given the reproductive isolation among populations that arises from the strong homing fidelity of salmon, and the heritability of arrival and breeding date, there is strong, albeit indirect, evidence for an adaptive, genetic basis for senescence rates in these fishes.

Over the past year, PIs on the proposal have carried out some preliminary analysis on sockeye salmon in Alaska. Specifically, this involved sampling salmon at the mouth of Hansen Creek (pre-senescent) and senescent salmon upstream. Quantitative PCR was used to characterizing expression levels of genes associated with processes including stress, olfaction, reproduction, and leaning behaviour (Roberts et al. 2009). Of particular interest was NMDA expression that was significantly different in senescent and pre-senescent salmon. This gene encodes for a receptor that has been associated with aging and learning in other taxa. Together these preliminary data do provide insight into salmon physiology however it is unlikely that these genes are directly regulating senescence. Most recently, a graduate student in the lab of the PI was able to identify the telemorase gene in sockeye salmon using bioinformtic approaches. Briefly, she used next generation sequence data from another project (generously provided by Dr. Jim Seeb) and aligned these data with Atlantic salmon ESTs in GenBank with homology to characterized telomerase genes. A qPCR assay for this gene is currently being developed and will be used for the current project. Characterization of telomerase expression along with other research activity described in the following section will help us better understand the mechanisms behind the genetic basis for rapid senescence variation. ***An understanding of this mechanism would not only be a breakthrough in the biology of these much-studied fishes, but it would also facilitate their use as a model system for the study of general processes of senescence.***

B. Objectives

Pacific salmon offer an excellent model system to study senescence but there has been limited research into the senescence process in salmon. We therefore propose to characterize differences in salmon at different stages of aging to better understand fundamental processes regulating senescence. This research will focus on two populations of salmon, sockeye salmon in Alaska and Chinook salmon that return to the University of Washington's Fish Hatchery. The specific research objectives of the proposal are to:

1) Characterize transcriptomic differences in senescent and pre-senescent salmon
2) Develop and implement assays for specific target processes involved in aging and senescence

For the first objective, we will use next generation sequencing (NGS) as a tool to investigate gene expression pattern differences in senescent and pre-senescent sockeye salmon. This approach offers a broad, non-biased way to identify differences in gene expression that could provide valuable insight into physiological differences associated with senescence including regulatory pathways and a better understanding of resource allocation. For instance, this deep sequencing technique will allow us to characterize the relationship of fundamental physiological processes such as reproduction, growth, and immune function.

The sequencing effort will also provide gene information to design quantitative PCR assays to examine specific target processes in greater detail, which is the basis of the second research objective of this project. For this objective, we plan on characterizing individual fish expression patterns for genes known to be involved in telomere shortening (telomerase), oxidative stress (superoxide dismutase and catalase), as well as targets identified to be significantly different in NGS analysis. Furthermore we will use quantitative PCR assays to determine mean telomere length. This is an important addition to characterizing gene expression as telomere length has been implicated in triggering the onset of senescence (Hemann et al. 2001) and can be used as an indicator of chronological age (Hoffmann et al. 2009). The analysis associated with the second research objective will be carried out on both sockeye salmon from Alaska and Chinook salmon at the UW Fish Hatchery in Seattle. This complementary approach will allow us to examine the intra- and inter-stream differences in Alaska (described above) and begin to examine the distinction between chronological age and senescence. Chinook salmon returning to the UW Fish Hatchery are commonly at various chronological ages (1-4 yrs), yet all undergo rapid senescence after their first reproductive season. This greater variation in age at maturity (compared to sockeye salmon) and the ease of sampling at the UW hatchery make this a very cost-effective and useful combined approach.

C. Procedure

Fish Sampling

Fish used for this research will be euthanized and sampled per IACUC regulations at the UW Fish Hatchery and the UW Alaska Salmon Program field stations at Hanson Creek and Lake Iliamna (Figure 1). Tissues harvested will include brain, liver, and gonad. Samples will be stored in RNAlater (Ambion). Fin clips will be taken and preserved in ethanol for telomore length analysis and any further genetic analysis. All fish will be photographed to easily quantify physical appearances and standard length measurements recorded.

Next Generation Sequencing

In order to characterize differential gene expression patterns in senescent and pre-senescent salmon we will examine differences in global gene expression patterns from pooled tissue libraries (liver and brain) from four group of salmon; 1) pre-senescent salmon at Hansen Creek, 2) senescent salmon at Hansen Creek, 3) pre-senescent salmon at Lake Iliamna, 4) senescent salmon at Lake Iliamna. For each library we will use 15 individuals and RNA will be pooled in equal quantities. RNA will be extracted using the Qiagen RNeasy kit and subsequent mRNA isolation carried out with the MicroPoly(A)Purist system (Ambion). A single lane will be sequenced for each library using the ABI SOLiD 3 system, generating approximately 40 million, 50bp reads per lane. The PI has used this platform on several occasions to sequence oyster (*Crassostrea gigas*) short read libraries. Compared to oyster data, assembly and gene annotation will be less challenging for sockeye given the wealth of genomic resources (e.g. ESTs). Sequencing will be carried out by the High-Throughput Genomics Unit (HTGU), Department of Genome Sciences, University of

Washington. Sequence assembly, annotation, mRNA-seq analysis, and statistical analysis will be carried out using the Genomics Workbench Server (CLCBio).

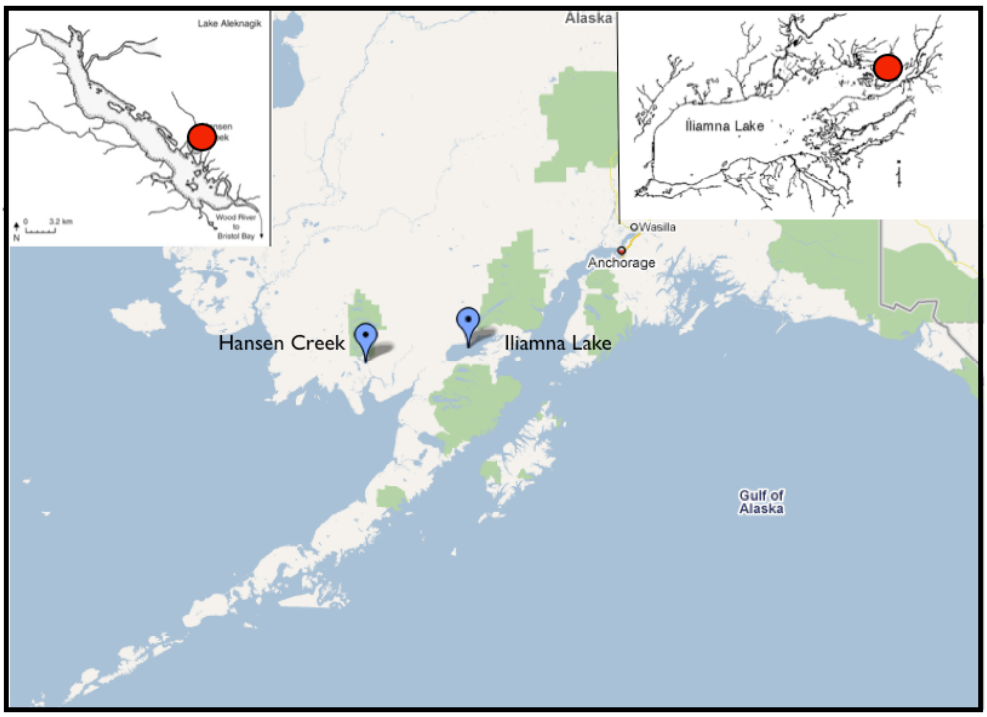

Figure 1. Location of field sites in Alaska where sockeye salmon will be sampled. Hansen Creek (inset left) salmon in-stream longevity ranges from about two weeks for early arriving fish to one week for late fish whereas in Lake Iliamna (inset right) the range is from about four weeks to two weeks.

Quantitative PCR analysis

While NGS will provide a global high-throughput means to look at transcriptome variation, it will not be able to glean individual fish variation in expression. For this we will use quantitative PCR and target approximately eight genes that are likely candidates for controlling senescence. This will include telomerase (described above), genes identified in NGS effort, as well as other candidates involved in oxidative stress. Separate assays will be developed for sockeye and Chinook. Briefly, RNA will be isolated and reversed transcribed with the QuantiTect Reverse Transcription Kit (Qiagen), which also eliminates genomic DNA carry-over. Quantitative, real-time PCR reactions (25uL) will contain the following: 0.5uL cDNA, 0.04 uM forward/reverse primers, 1X Immomix Master Mix (Bioline) and 2uM SYTO13 (Invitrogen). An Opticon 2 thermocycler (Bio-Rad) will be used to quantify gene expression. Raw data will be analyzed using Real-Time PCR Miner Software (Zhao & Fernald, 2005).

Telomere Length assays

Genomic DNA will be extracted from samples using Qiagen DNeasy kit (Qiagen). Mean telomere length will be measured as the ratio of telomere PCR signal to the signal of a single copy gene (T/S ratio). The T/S ratio is proportional to the average telomere length per cell (Cawthon 2002, Gil et al. 2004). For this method, separate qPCR reactions are carried out for each sample, one amplifying telomere repeats using universal telomere primers for vertebrates (Cawthon 2002, Gil et al. 2004), and one amplifying the gene β-actin, for which primers have already been designed. Similar conditions will be used to carry quantitative PCR as described above with the primary difference that DNA will be the template material as opposed to cDNA. PCR signal will be determined using a standard curve generated from serial dilutions of a known template. This relative newly developed procedure has the advantage of being a cost effective, high-throughput, and a reproducible means of determining telomere length.

D. Time Schedule

Sampling sockeye salmon will occur during July and August of 2010, and sampling of Chinook salmon will occur in October and November of 2010. As we have archived samples from both species, development of qPCR assay and telomere length assays can begin immediately. Construction of NGS libraries and sequencing will take place in early Fall 2010 and all targeted assays will be complete by Winter 2011.

E. Need for RRF Support

We firmly believe that Pacific salmon are an excellent organism to study senescence, providing a unique comparative model system. In addition, a better characterization of the cellular and molecular mechanisms involved in salmonid senescence will help us better understand ecological consequences of varied selective pressure. This proposal addresses the mission of the Royalty Research Fund in multiple ways. Firstly, completion of this project will provide preliminary data that will significantly increase our ability to compete for major extramural funding (e.g., NSF and NIH). Furthermore, the project will give the PI (Roberts), a junior faculty at the University of Washington, an opportunity to explore a major new avenue of research (i.e., senescence and evolutionary ecological applications of genomic techniques) and to collaborate with a more senior faculty member (Quinn) whose field-oriented work might not otherwise be linked to genomics research. Conversely, it will allow Quinn to expand his evolutionary ecology work to understand the mechanisms behind the senescence phenomenon he has been studying on streams in Alaska.

Budget

01 Salary	
Steven Roberts, Assistant Professor (0.8 month summer salary)	6,431
Research Assistant, 50%, $1781 per month, 6 months, Fall 2010 and Winter 2011	10,686

03 Other Contractual Services	
Alaska Field Camp Fees ($124 x 21 days)	2,604

04 Travel	
Travel	2,000

05 Supplies and Materials	
Fish sampling supplies, dissection, RNA preservation	1,000
Sample Preparation and Sequencing	3,000
DNA and RNA extraction reagents, qRT-PCR kits	2,500
general consumables (e.g., plastics, tips, gloves, sample tubes)	1,500

07 Retirement and Benefits	
01-10 Assistant Professor (23.6%)	1518
01-40 Research Assistant (12.9%)	1378

08 Operating Fee/Tuition	
08-05 Research Assistant, Two quarters, 2010 rate	6,652

TOTAL BUDGET 39,270

Budget Justification

Salary

Funds are requested for one month of summer salary for Assistant Professor Steven Roberts. In addition to developing the experimental approaches, he will spend time sampling salmon in Alaska with co-P.I. Quinn, and compiling genomic resources for sequencing analysis. Funds are requested for 2 quarters of graduate student support. This Research Assistant will incorporate this into their thesis research; responsibilities will include sampling, assay validation, gene expression analysis, and telomere length characterization.

Budget Justification continued

Other Contractual Services

Funds are requested to cover 21 days of camp fees at the UW Alaska Salmon Program Field site. This includes support for the PI (Roberts), co-PI (Quinn), and graduate student. These fees are a standard rate, set by the School of Aquatic and Fishery Sciences, to recover operational costs (food, fuel, boat and vehicle use, shipping, etc.) that are distributed among all scientific personnel using the camps.

Travel

$2000 is requested to partially cover travel costs associated with sampling salmon in Alaska and presenting research at local meetings.

Supplies and Materials

A total of $8000 is requested for materials and supplies to carry out the research objectives described as part of this project. $1000 is requested to cover costs of supplies for sampling fish both in Alaska and at the UW Hatchery in Seattle. $3000 is requested to cover library preparation and sequencing to be performed at the UW HTGU. Reagents necessary to carry-out targeted qPCR assays will cost $2500. Funds ($1500) are also requested to cover the common supplies and consumables used in work of this nature (i.e., gloves, pipettor tips, sample vials, PCR plates, etc)

CURRICULUM VITAE – STEVEN BEYER ROBERTS

Contact Information University of Washington
School of Aquatic and Fishery Sciences
Fisheries Teaching and Research Building
1140 NE Boat Street
Seattle, WA 98195
phone: 206.600.4495
email: sr320@u.washington.edu

Academic Experience Ph.D. – University of Notre Dame (South Bend, IN) – 2002
Integrative Cell and Molecular Physiology

B.S. – North Carolina State University (Raleigh, NC) – 1997
Natural Resources – Concentration in Marine and Coastal Resources

Professional Experience *2006-Present* · Assistant Professor
University of Washington, Seattle,WA

2006-Present · Adjunct Assistant Scientist
Marine Biological Laboratory, Woods Hole, MA

2003-2006 · Assistant Research Scientist
Marine Biological Laboratory, Woods Hole, MA

Select Publications

Goetz F, Rosauer D, Sitar S, Goetz G, Simchick C, Roberts S, Johnson R, Murphy C, Bronte C, Mackenzie S. (2010) A genetic basis for the phenotypic differentiation between siscowet and lean lake trout (*Salvelinus namaycush*). Molecular Ecology, 19 176–196

Defaveri J, Smolowitz R, Roberts S (2009) Development and validation of a real-time quantitative PCR assay for the detection and quantification of *Perkinsus marinus* in the Eastern oyster, *Crassostrea virginica.* Journal of Shellfish Research. 28(3):459-464

Roberts SB, Goetz G, White S, Goetz F (2009) Analysis of genes isolated from plated hemocytes of the Pacific Oyster, *Crassostrea gigas.* Marine Biotechnology. Jan-Feb;11(1):24-44

Roberts SB, Gueguen Y, de Lorgeril J, Goetz F. (2008) Rapid accumulation of an interleukin 17 homolog transcript in *Crassostrea gigas* hemocytes following bacterial exposure. Developmental and Comparative Immunology. Volume 32, Issue 9, Pages 1099-1104

Lyons MM*, Lau Y-T, Carden WE, Ward JE, Roberts SB, Smolowitz R, Vallino J, Allam B. (2007) Characteristics of marine aggregates in shallow-water ecosystems: Implications for disease ecology. EcoHealth. 4, 406–420

Select Publications continued

Hodgins-Davis A*, Roberts SB, Cowan D, Atema J, Avolio C, Defaveri J, Gerlach G. (2007) Characterization of SSRs from the American lobster, *Homarus americanus.* Molecular Ecology Notes. 7:330-332

Rodgers BD, Roalson EH, Weber GM, Roberts SB, Goetz FW. (2007) A Proposed Nomenclature Consensus for the Myostatin Gene Family. AJP- Endocrinology and Metabolism. 292(2):E371-2

Lyons MM*, Smolowitz R, Dungan C, Roberts SB. (2006) Development of a real-time quantitative PCR assay for the hard clam pathogen, Quahog Parasite Unknown (QPX). Diseases of Aquatic Organisms. 72(1):45-52

Roberts SB, Romano C, Gerlach G. (2005) Characterization of EST derived SSRs from the bay scallop, *Argopectens irradians*. Molecular Ecology Notes. 5: 567-568

Biga PR, Roberts SB, Iliev DB, McCauley LA, Moon JS, Collodi P, Goetz FW. (2005) The isolation, characterization, and expression of a novel GDF11 gene and a second myostatin form in zebrafish, *Danio rerio*. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 141: 218-230

Roberts SB, McCauley LAR, Devlin RH, Goetz FW. (2004) Transgenic salmon over-expressing growth hormone exhibit decreased myostatin transcript and protein expression. Journal of Experimental Biology. 207(Pt 21):3741-8

Kim H-W*, Mykles DL, Goetz FW, Roberts SB. (2004) Characterization of an invertebrate myostatin homologue from the bay scallop, *Argopecten irradians.* BBA – Gene Structure and Expression. 1679(2):174-9

* *indicates student author*

Recent Invited Presentations

Genomic approaches in aquaculture research. pre-BIO 2009 Symposium; Opportunities for UW / University of Queensland Australia Collaboration, Seattle WA, May 14 2009

Overview of Shellfish Activities at the University of Washington. USDA-WERA099: Broodstock Management, Genetics and Breeding Programs for Molluscan Shellfish. March 21, 2009. Savannah, GA

Analysis of genes isolated from plated hemocytes of the Pacific Oyster. National Shellfisheries Association Conference. March 24, 2009. Savannah, GA

Characterization of trout myostatin interacting proteins on primary muscle cells. Eighth International Congress on the Biology of Fish. Portland Oregon, July 29 2008

CURRICULUM VITAE

Name: Thomas Peter Quinn
Address: School of Aquatic and Fishery Sciences, Box 355020
Univ. of Washington
Seattle, WA 98195
Phone: (206) 543-9042
e-mail: tquinn@u.washington.edu

Degrees:
B.A. with Distinction in Biology, Swarthmore College, 1976
M.S. in Fisheries, University of Washington, 1978
Ph.D. in Fisheries, University of Washington, 1981

Employment:

2000-present	Professor, School of Aquatic and Fishery Sciences
1990-2000	Associate Professor, School of Fisheries,
1986-1990	Assistant Professor, School of Fisheries, Univ. of Washington
1984-1985	Research Associate, Department of Oceanography, University of British Columbia, Vancouver, B.C.
1981-1984	Post-doctoral Fellow, University of British Columbia and Pacific Biological Station, Nanaimo, B. C., Canada

Research Interests:
Behavior, ecology, evolution, and conservation of Pacific salmon and trout

Honors:
Distinguished Teaching Award, University of Washington, 1991
Marsha Landolt Distinguished Graduate Mentor Award, University of Washington, 2008
National Research Council panel on the Status of Pacific Northwest Anadromous Salmonids
College of Ocean and Fishery Sciences Distinguished Research Award 1998
Fulbright Fellowship to study in Ireland, 2000

Selected Relevant Publications

Hendry, A.P., O.K. Berg and T.P. Quinn. 1999. Breeding date, life history, and energy allocation in a population of sockeye salmon (*Oncorhynchus nerka*). Oikos 85: 499-514.

Quinn, T. P., M. J. Unwin, and M. T. Kinnison. 2000. Evolution of temporal isolation in the wild: genetic divergence in timing of migration and breeding by introduced chinook salmon populations. Evolution 54: 1372-1385.

Hendry, A.P., J. K. Wenburg, P. Bentzen, E. C. Volk and T. P. Quinn. 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. Science 290: 516-518.

Hendry, A. P., O. K. Berg and T. P. Quinn. 2001. Breeding location choice in salmon: causes (habitat, competition, body size, energy stores) and consequences (life span, energy stores). Oikos 93: 407-418.

Quinn, T.P., Hendry, A.P., and Buck, G.B. 2001. Balancing natural and sexual selection in sockeye salmon: interactions between body size, reproductive opportunity and vulnerability to predation by bears. Evolutionary Ecology Research 3: 917-937.

Quinn, T. P., L. Wetzel, S. Bishop, K. Overberg and D. E. Rogers. 2001. Influence of breeding habitat on bear predation, and age at maturity and sexual dimorphism of sockeye salmon populations. Canadian Journal of Zoology 79: 1782-1793.

Quinn, T.P., M.T. Kinnison and M.J. Unwin. 2001. Evolution of chinook salmon (*Oncorhynchus tshawytscha*) populations in New Zealand: pattern, rate, and process. Genetica 112/113: 493-513.

Quinn, T.P., S. M. Gende, G. T. Ruggerone and D. E. Rogers. 2003. Density dependent predation by brown bears (*Ursus arctos*) on sockeye salmon (*Oncorhynchus nerka*). Canadian Journal of Fisheries and Aquatic Sciences 60: 553-562.

Hilborn, R. T. P. Quinn, D. E. Schindler and D. E. Rogers. 2003. Biocomplexity and fisheries sustainability. Proc. Nat. Acad. Sci. 100: 6564-8568.

Seamons,T. R., P. Bentzen and T. P. Quinn. 2004. The mating system of steelhead (*Oncorhynchus mykiss*), inferred by molecular analysis of parents and progeny. Environmental Biology of Fishes 69: 333-344.

Carlson, S. C., H. B. Rich, Jr. and T. P. Quinn. 2004. Reproductive lifespan and sources of mortality for alternative male life history strategies in sockeye salmon, *Oncorhynchus nerka.* Canadian Journal of Zoology 82: 1878-1885

Dickerson, B. R., K. W. Brinck, M. F. Willson, P. Bentzen and T. P. Quinn. 2005. Relative importance of body size and timing of arrival to breeding grounds in reproductive success of pink salmon. Ecology 86: 347-352.

Quinn, T. P. 2005. The Behavior and Ecology of Pacific Salmon and Trout. University of Washington Press, Seattle. 378 pages.

Quinn, T. P., I. J. Stewart and C. P. Boatright. 2006. Experimental evidence of homing to site of incubation by mature sockeye salmon (*Oncorhynchus nerka*). Animal Behaviour 77: 941-949.

Seamons, TR, P Bentzen, TP Quinn. 2007. DNA parentage analysis reveals inter-annual variation in selection: results from 19 consecutive brood years in steelhead trout. Evol. Ecol. Res. 9:409-431.

Carlson, S. M. and T. P. Quinn. 2007. Ten years of varying lake level and selection on size-at-maturity in sockeye salmon. Ecology 88: 2620-2629.

Carlson, S. M., R. Hilborn, A. P. Hendry and T. P. Quinn. 2007. Predation by bears drives senescence in natural populations of salmon. PLoSOne 2(12): e1286.

Carlson, S. M., H. B. Rich, Jr., and T. P. Quinn. 2009. Does variation in selection imposed by bears drive divergence among populations in the size and shape of sockeye salmon? Evolution 63: 1244-1261.

Doctor, K. K. and T. P. Quinn. The potential for adaptation-by-time in sockeye salmon (*Oncorhynchus nerka)*: The interactions of body size and in-stream reproductive lifespan with date of arrival and breeding location. Can. J. Zool. (in press)

Seamons, T. R. and T. P. Quinn. Sex-specific patterns of lifetime reproductive success in single and repeat breeding steelhead trout (*Oncorhynchus mykiss*). Behavioral Ecology and Sociobiology (in press)

Current Research Support - Roberts
Title: Evaluation of putatively QPX-resistant strains of northern hard clams using field and genetic studies
Source of Support: USDA / Northeastern Regional Aquaculture Center
Award Amount: $263,490
Award Period: 03/01/2008 – 12/31/2010
Relationship: Similar technical approach

Title: Threats to bivalve aquaculture and fisheries: the influence of emerging diseases and environmental change
Source of Support: NOAA
Award Amount: $243,000
Award Period: 9/1/2009 - 8/30/2011
Relationship: Similar technical approach

Past Research Support - Roberts
Title: Development of genetic markers to assess disease resistance in the eastern oyster
Source of Support: USDA / Northeastern Regional Aquaculture Center
Award Amount: $154,066
Award Period: 9/1/2006-1/30/2008
Relationship: none

Title: Production of myostatin gene knockouts in zebrafish and the effects of specific myostatin interacting proteins
Source of Support: USDA - NRI
Award Amount: $195,862
Award Period: 1/1/2005 - 11/30/2008
Relationship: none

Title: Assessing withering syndrome resistance in California Black Abalone: Implications for conservation and restoration
Source of Support: California Sea Grant
Award Amount: $15,067
Award Period: 6/1/2007 - 5/30/2008
Relationship: none

Remaining Start-up funds: $90,732

Current Research Support – Quinn Co-PI

Evaluation of the reproductive success of wild and hatchery steelhead in natural and hatchery environments. Bonneville Power Administration. December 2006 – December 2010. $1,100,861.

Migration patterns in Dolly Varden in the Iliamna River. Alaska Department of Fish and Game. September 2008 to June 2010. $69,028.

Ecology and behavior of trout in the Cedar River. NOAA-Fisheries. Sept 2008 – April 2010. April 2010. $48,878

Quantify Bristol Bay fishery selection. Alaska Sustainable Salmon Fund. July 1 2009 – June 30, 2011. $75,259. Relationship: Salmon population targeted in current proposal

Success of Cedar River salmon colonization: A genetic assessment. City of Seattle. $65,322. November 1 2009 – October 31 2011. This contract will provide additional support for the doctoral dissertation work of Joseph Anderson, as part of a multi-year project funded by Washington Sea Grant and the City of Seattle.

Past Research Support – Quinn Co-PI

Migration patterns and residence of Chinook salmon in Puget Sound. NOAA Fisheries. June 1, 2006 – August 31, 2009. $225,124. Relationship: Salmon population targeted in current proposal

Re-colonization of the upper Cedar River by anadromous salmonids.. Washington Sea Grant Program. February 2007 - January 2010. $237,233.

Literature Citations

Armanios, M., J.K. Alder, E.M. Parry, B. Karim, M.A. Strong, and C.W. Grider. 2009. Short telomeres are suffcient to cause the degenerative defects associated with aging. The American Society of Human Genetics 85(6): 823-832.

Barry, T. P., M. J. Unwin, J. A. Malison, and T. P. Quinn. 2001. Free and total cortisol levels in semelparous and iteroparous chinook salmon. Journal of Fish Biology 59:1673-1676.

Blackburn, E.H., C.W. Grider, and J.W. Szostak. 2006. Telomeres and telomerase: the path from maize, *Tetrahymena* and yeast to human cancer and aging. Nature Medicine 12(10):1133-1138.

Carlson, S. M., R. Hilborn, A. P. Hendry, and T. P. Quinn. 2007. Predation by bears drives senescence in natural populations of salmon. Public Library of Science One 2(12):e1286.

Carlson, S. C., H. B. Rich, Jr, and T. P. Quinn. 2004. Reproductive lifespan and sources of mortality for alternative male life history strategies in sockeye salmon, *Oncorhynchus nerka*. Canadian Journal of Zoology 82:1878-1885.

Carlson, S. M., and T. R. Seamons. 2008. A review of quantitative genetic components of fitness in salmonids: implications for adaptation to future change. Evolutionary Applications 1:222-238.

Cawthon, R. M. 2002. Telomere measurement by quantitative PCR. Nucleic Acids Research 30(10):e47.

Chen, J., C.N. Hales, S.E. Ozanne. 2007. DNA damage, cellular senescence and organismal ageing: casual or correlative? Nucleic Acids Reseaerch 35(22):7417-7428.

Fitch, C.E. 1994. Longevity, Senescence, and the Genome. University of Chicago Press, Chicago, USA.

Gende, S. M., T. P. Quinn, R. Hilborn, A. P. Hendry, and B. Dickerson. 2004. Brown bears selectively kill salmon with higher energy content but only in habitats that facilitate choice. Oikos 104:518-528.

Gil M.E. and T.L. Coetzer. 2004. Real-time quantitative PCR of telomere length. Molecular Biotechnology 27:169-172.

Hemann, M.T., M.A. Strong, L. Hao, C.W. Grider. 2001. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. Cell 107(1):67-77.

Hendry, A. P., and O. K. Berg. 1999. Secondary sexual characters, energy use, senescence, and the cost of reproduction in sockeye salmon. Canadian Journal of Zoology 77:1663-1675.

Hendry, A. P., O. K. Berg, and T. P. Quinn. 1999. Condition dependence and adaptation-by-time: breeding date, life history, and energy allocation within a population of salmon. Oikos 85:499-514.

Hendry, A. P., Y. E. Morbey, O. K. Berg, and J. K. Wenburg. 2004. Adaptive variation in senescence: reproductive lifespan in a wild salmon population. Proceedings of the Royal Society of London B 271:259-266.

Hodes, R.J. 1999. Telomere length, aging, and somatic cell turnover. The Journal of Experimental Medicine 190(2):153-156.

Hoffmann, J., Y. Erben, A.M. Zeiher, S. Dimmele, and I. Spyridopoulos. 2009. Telomere length-heterogeneity among myeloid cells is a predictor for chronological ageing. Experimental Gerontology 44(5):363-366.

Kim, S.K. 2007. Common aging pathways in worms, flies, mice, and humans. The Journal of Experimental Biology 210:1607-1612.

Passos, J.F., G.Saretzki, and T. von Zglinicki. 2007. DNA damage in telomeres and mitochondria during cellular senescence: is there a connection? Nucleic Acids Research 35(22):7505-7513.

Perrin, C. J., and J. R. Irvine. 1990. A review of survey life estimates as they apply to the area-under-the-curve method for estimating the spawning escapement of Pacific salmon. Canadian Technical Report of Fisheries and Aquatic Sciences 1733:1-49.

Quinn, T. P. 2005. The behavior and ecology of Pacific salmon and trout. University of Washington Press, Seattle.

Quinn, T. P., L. Wetzel, S. Bishop, K. Overberg, and D. E. Rogers. 2001. Influence of breeding habitat on bear predation, and age at maturity and sexual dimorphism of sockeye salmon populations. Canadian Journal of Zoology 79:1782-1793.

Roberts, S. B., C. Storer, and T. P. Quinn. 2009. Fishing for mechanisms regulating senescence in salmon. presentation - Alaska Salmon Program Symposium. Dec 2009. http://www.slideshare.net/sr320/roberts-alaska-sen

Unwin, M. J., M. T. Kinnison, and T. P. Quinn. 1999. Exceptions to semelparity: postmaturation survival, morphology and energetics of male chinook salmon (*Oncorhynchus tshawytscha*). Canadian Journal of Fisheries and Aquatic Sciences 56:1172-1181.