UNIVERSITY OF CALIFORNIA
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**RELATIONSHIPS AMONG
ENVIRONMENT, MOVEMENT, GROWTH AND SURVIVAL
OF COASTAL RAINBOW TROUT (*ONCORHYNCHUS MYKISS)***

A dissertation submitted in partial satisfaction
of the requirements for the degree of

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in
ECOLOGY AND EVOLUTIONARY BIOLOGY
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ABSTRACT

Individuals of the same species can vary dramatically in their size, physiology or behavior, thereby influencing their movement, growth and survival. Through influencing demographics individual variation is important to population resilience in the face of environmental change in space and time. Understanding the ecological consequences of individual and population variation is timely considering the dramatic rates of extinction and anthropogenic alterations we are witnessing including global climate change. Coastal rainbow trout *(Oncorhynchus mykiss)* express a dizzying range of variability in movement and growth rate. I used acoustic telemetry to determine the diversity of *O. mykiss* movement patterns in an altered river. Movement distance and frequency ranged widely from no movement over the 218d study period to traveling 170km to the Pacific Ocean in 14d. However, most individuals did not move more than 0.5km. Movement correlated with size, season and location. Mortality correlated with the number of moves rather than distance moved suggesting movement regardless of scale influenced survival. In laboratory experiments I found more northerly *O. mykiss* populations had growth optimals at colder temperatures than more southerly populations. However, temperature-dependent growth varied among populations at very local scales. Temperatures previously experienced did not affect temperature-dependent growth which is beneficial for a species that may experience dramatic fluctuations in temperature through space and time. I used a diet-switch experiment and model fitting to quantify the nitrogen isotope tissue turnover rate and discrimination factor for seven *O. mykiss*

tissues. Among seven tissues, diet-tissue δ15N discrimination factors ranged from 1.3 to 3.4‰. Model supported tissue turnover half-lives ranged from 9.0 to 27.7 days. Using parameter estimates and their uncertainty, I developed stable isotope clocks to estimate the time since resource-shifts. A greater understanding of the extent and importance of individual and population-level variation in fundamental demographic (i.e. growth and survival) and life history (i.e. anadromy and thermal optima) parameters is key to the management and conservation of threatened species. This research revealed that *O. mykiss* populations vary in these fundamental parameters, implying the importance of local-scale management and conservation practices that ensure the continued diversity of these parameters within and among populations.

DEDICATION AND ACKNOWLEDGEMENTS

My dissertation is in memory of my mother Ina Claire Heady

and

dedicated to my two sons, Quincy Kai and Wylie Flynn Heady.

Each chapter of my dissertation has its own specific acknowledgement section, but in the following paragraphs I would like to elaborate on my gratitude for the help and support I received from many funding sources, academic advisors, administrative assistants, volunteers in the field and lab, and my beloved family and friends.

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I must thank my entire family. They have encouraged, and supported me through graduate school and praised my accomplishments. I cannot thank my family enough. Without their support I would not be finishing today. Furthermore, if not for my family I would not be inspired to work for my PhD in Ecology. To be aware of, and understand the natural world around you is a very important quality to a Heady. I am thankful that my family instilled that value in me. I am thankful that my family lived life such that I spent most of my childhood in the wilderness. I am also thankful that critical thinking and intelligence is so important to Headys. After all, dad **is** a rocket scientist! Well dad… I am not an engineer… but I **am** a doctor! Thank you!

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EXECUTIVE SUMMARY

Individuals of the same species can vary dramatically in their size, physiology or behavior, thereby influencing their movement, growth and survival. Individual variation forms the basis for natural selection and the diversity of organisms on earth (Darwin 1859). Through influencing demographic processes individual variation is also important to population resilience and sustainability in the face of environmental change in space and time (Greene et al. 2010; Bolnick et al. 2011). Furthermore, variation among populations has been demonstrated to confer species viability over landscapes that vary through time and space (Schindler et al. 2010; Moore et al. 2010). Our understanding of the ecological consequences of individual and population variation to species persistence is timely considering the dramatic rates of extinction and anthropogenic alterations we are currently witnessing including global climate change (Deutsch et al. 2008; Schindler et al. 2010).

Salmonids provide a model system to investigate both individual and population level variation and how these influence population and species persistence. Pacific salmon express incredible intraspecific trait variation allowing the persistence of populations across large ranges of variable habitats through space and time (Quinn 2004; Augerot and Foley 2005). Of Pacific salmon, coastal rainbow trout *(Oncorhynchus mykiss)* express the greatest degree of individual variation in life history (Quinn 2004; Augerot and Foley 2005). Life history variation in *O. mykiss* is

largely a reflection in the amount of movement expressed by individuals, and is thought to be related to juvenile growth rates (Shapovalov and Taft 1954; Mangel and Satterthwaite 2008; Satterthwaite et al. 2010, 2012). For example individuals may spend their entire life within one stream habitat (resident rainbow trout), while others born in this same stream habitat may migrate hundreds of kilometers to sea and back prior to spawning (anadromous steelhead). *O. mykiss* life history varies incredibly between these extremes arising from the great variation in timing and degree of movement among individuals (Shapovalov and Taft 1954; Quinn 2004; Augerot and Foley 2005). This high degree of individual variation presumably has allowed the persistence of *O. mykiss* across such a wide range of habitat variation. *O. mykiss* historically ranged from Baja California, Mexico, throughout coastal North America, and across into Asia (Augerot and Foley 2005). Furthermore, due to their extensive range *O. mykiss* currently exist in a range of habitat qualities from largely unaltered habitats of Alaska and Asia to dramatically altered aquatic habitat, including urbanized environments of Seattle, San Francisco and Sacramento. For example, anthropogenic alterations have eliminated 80% of the original anadromous *O. mykiss* habitat of California's Central Valley, and the remaining habitat is dramatically altered (Mcewan 2001; Lindley et al. 2006). The remarkable plasticity and individual variation expressed by *O. mykiss* would suggest resilience to anthropogenic alterations, however, most populations at the southern end of their range have a relatively high threat of extinction (Augerot and Foley 2005). Thus, in the United States *O. mykiss* is managed by NOAA Fisheries under the U.S. Endangered Species

Act (ESA) as a collection of Distinct Population Segments (DPS), where each DPS is discrete and significant to the overall species (USFWS and NMFS 1996).

For my dissertation research I used a combination of field studies, laboratory experiments and mathematical modeling to explore 1) the patterns, causes and mortality consequences of individual *O. mykiss* movement within one case study population; 2) how temperature-dependent growth varied among three *O. mykiss* populations; and 3) developed a new method to determine the timing of resource switches in *O. mykiss* using stable isotopes.

In the first chapter of my dissertation I used acoustic telemetry to investigate the patterns and causes of movement and consequences for survival of wild *O. mykiss* of the Mokelumne River. The Mokelumne River is a snow fed watershed that drains 1,624 km^2 of the Western Sierra Nevada Mountains and flows directly into the Sacramento-San Joaquin Estuary. Like most other streams of the Central Valley of California, the Mokelumne River now has several dams and diversions which greatly reduce the amount of streamflow in the river and its natural variability (Pasternack et al. 2004). Using a network of stationary acoustic receivers I determined the number of 78 individually tagged wild *O. mykiss* that emigrated from the freshwater rearing habitat as anadromous individuals. Using standardized transects within the freshwater rearing section of the Mokelumne River I investigated the degree of movement and survival of resident *O. mykiss* and how this related to individual size, habitat, streamflow and season. Movement distance and frequency ranged widely from no movement over the 218 day study period to traveling 170 km to the Pacific Ocean in

14 days. All six anadromous individuals emigrated in the spring and emigration was correlated with peak streamflows. Movement of resident fish was also highest in spring and summer relative to fall and winter. Like anadromous emigrants, smaller residents moved more in the spring and summer of 2008, perhaps due to a history of adaptation to the natural seasonal snow fed streamflow that was more apparent that year (Quinn 2004; Mellina et al. 2005). Larger fish moved more in the upper reaches in 2007, potentially exploring the habitat variability of these reaches to maximize growth at the reach scale (Kahler et al. 2001; Gowan and Fausch 2002; Höjesjö et al. 2007). *O. mykiss* that were observed to move more were more likely to suffer mortality. Thus, a trade-off exists between the potential growth benefits of other stream habitats or oceanic conditions against mortality associated with movement to realize these benefits (Gross et al. 1988; Northcote 1992; Quinn 2004).

Considering the potential for local adaptation in *O. mykiss* (Nielsen 1999; Moyle 2002; Quinn 2004), and the great variability of conditions throughout California (Moyle 2002; Lindley et al. 2006), populations may express optimal growth at different temperatures. By determining life history (Mangel and Satterthwaite 2008; Satterthwaite et al. 2010; Beakes et al. 2010) or conferring survival to reproduction (Bond et al. 2008; Hayes et al. 2011), juvenile growth rates influence population persistence. Growth rate in poikilothermic organisms generally increases with temperature to a certain point then drops precipitously as temperatures approach incipient lethal limits (Hoar et al. 1979; Myrick and Cech 2005). However, details of temperature-dependent growth are lacking for many *O. mykiss* populations

and highly sought after by managers (Myrick and Cech 2004, 2005). In my second chapter I conducted laboratory experiments testing *O. mykiss* growth at 14, 20 and 24^{o}C for three populations of *O. mykiss* among two DPS of California. I then compared my results to previous studies of other populations in California and Oregon. Furthermore, as individuals may experience varying water temperatures resulting from their varying degrees of movement (Chapter 1), or from seasonal habitat changes (Myrick and Cech 2004; Boughton et al. 2007; Bell et al. 2011), I investigated how the history of temperatures experienced by individuals affect temperature-dependent growth and how this varies among populations. Patterns of temperature-dependent growth varied among populations both within and among DPS. More southerly populations exhibited a relative growth optimum at higher temperatures. Patterns of temperature-dependent growth rate were not affected by controlled manipulations of the history of temperatures experienced. As such, individuals may attain growth related benefits (Bulkley 1967; Bond et al. 2008; Satterthwaite et al. 2012) from optimal temperatures, regardless of what thermal conditions they previously experienced, but these optimal temperatures vary among populations of *O. mykiss.*

Coastal lagoons play a nursery role for *O. mykiss* by providing a far greater growth potential than upper watershed rearing habitats, thereby increasing marine survival (Hayes et al. 2008, 2011). In fact, *O. mykiss* can migrate multiple times between freshwater, lagoon and marine habitats (e.g., Hayes et al. 2011). The dramatic population consequences arising from individual variation in habitat use,

combined with the fact that much of these important habitats are altered by anthropogenic influences, makes the nursery role of lagoons to *O. mykiss* of particular interest (Shapovalov and Taft 1954; Bond et al. 2008; Hayes et al. 2011). Because stable isotope values vary significantly between freshwater, lagoon and marine habitats, they may prove useful in determining the timing of resource switches from freshwater to lagoon habitats (Phillips and Eldridge 2006; Klaassen et al. 2010). However, effective interpretation is predicated on laboratory validation (Deniro and Epstein 1981; Gannes et al. 1997; Martínez del Rio and Wolf 2005). Furthermore, many models used in stable isotope ecology rest on assumptions that, if not tested, provide an uncertain foundation for field application (Martínez del Rio and Wolf 2005; Martínez del Rio and Anderson-Sprecher 2008; Boecklen et al. 2011). For my third chapter I developed stable isotope clocks to track resource shifts in *O. mykiss*. I used a laboratory diet-switch experiment and model fitting to quantify the nitrogen isotope ($\delta^{15}N$) tissue turnover rate and discrimination factor for seven *O. mykiss* tissues: plasma, liver, fin, mucus, red blood cells, muscle, and scales. Among tissues, diet-tissue $\delta^{15}N$ discrimination factors ranged from 1.3 to 3.4‰. Model supported tissue turnover half-lives ranged from 9.0 (fin) to 27.7 (scale) days. I evaluated six tissue turnover models using AICc. The use of equilibrium tissue values was supported in all tissues and two-compartment models were supported in plasma, liver, and mucus. Using parameter estimates and their uncertainty, I developed stable isotope clocks to estimate the time since resource shifts. Longer turnover tissues (scale) provided accurate estimates of time since resource switch for durations

approximately twice their half-life. Faster turnover tissues (fin) provided even higher precision estimates, but only within their half-life post-switch. Averaging estimates of time since resource shift from multiple tissues, using multiple-tissue clocks provided the highest precision estimates of time since resource shift for the longest duration (up to 64 days).

My dissertation research reveals how individual variation within and among populations across varying landscapes affects fundamental demographic parameters such as growth and survival. I found individual movement of *O. mykiss* to be related to individual size, habitat, streamflow and season. Although differences observed between the years in seasonal movement may be a result of differences in the sizes of fish tagged, or the environment such as variability in streamflow, these results support the hypothesis that resident fish move more during periods of increased prey availability. The degree of instream movement and related survival were key missing information for this population (Sogard et al. 2012), and lacking for Central Valley *O. mykiss* in general (Lindley et al. 2007). Results from Chapter 1 provide insight into the causes of movement and effects on individual survival, as well as illuminates the remaining degree of life history variation important to the maintenance of this imperiled population (Greene et al. 2010). While high instream growth in the Mokelumne River, coupled with greater mortality associated with movement may explain the disproportionately low level of anadromy in the Mokelumne River relative to other watersheds, I observed a range of life histories based on movement patterns for future restoration efforts and management to maintain and build on.

Results of Chapter 1 imply the importance of adequate streamflow including peaks and variability to successful anadromous migrations and the maintenance of life history diversity in this imperiled population.

By investigating the variation in patterns (i.e. optima) of temperature-dependent growth among populations, I explored the scales of local adaptation in *O. mykiss*. With climate change imposing perhaps the largest anthropogenic perturbation to natural systems, understanding the physiological sensitivity of poikilothermic organisms to temperatures they may experience is crucially important (Deutsch et al. 2008). Results presented in Chapter 2 highlight the need for population specific management approaches when considering dam and water management, habitat criteria, and efforts to restore imperiled populations of *O. mykiss*. Future research examining the degree of within DPS variation in temperature-dependent growth for *O. mykiss* among the remaining twelve DPS could prove invaluable to the sound management and recovery of the species. Incorporating genetics into this continued research would illuminate the degree to which genotype x environment and plasticity each affect the variation of temperature dependent growth among *O. mykiss* populations.

While stable isotope ecology lends itself as a useful tool to investigate individual and population level variation, the field is still learning the degree to which stable isotope ratios vary among individuals and populations (Layman et al.; Boecklen et al. 2011). Chapter 3 provided a novel investigation of stable isotope tissue turnover models and their assumptions. While each model had been considered

independently, to my knowledge no study had ever explicitly compared all six. Furthermore, the extreme variation expressed in *O. mykiss* challenged many assumptions of previous isotope clock models (Phillips and Eldridge 2006; Klaassen et al. 2010). Thus, my explicit investigation of clock performance increased understanding of principles in stable isotope ecology, and provided confidence in the application to investigate resource switches in *O. mykiss*. Further research in the degree of variability of isotope turnover rates and discrimination factors among individuals and populations of *O. mykiss* is necessary to further establish the generality of stable isotope clocks among different populations and varying environmental conditions.

In summary, a greater understanding of the extent and importance of individual and population-level variation in fundamental demographic (i.e. growth and survival) and life history (i.e. anadromy and thermal optima) parameters is key to the management and conservation of threatened species. As revealed by this research, *O. mykiss* populations vary in these fundamental parameters, implying the need of local scale management and the importance of management and conservation practices that ensure the continued diversity of these parameters within and among populations.

LITERATURE CITED

Augerot X, Foley DN (2005) Atlas of Pacific salmon: the first map-based status assessment of salmon in the North Pacific. University of California Press

Beakes MP, Satterthwaite WH, Collins EM, Swank DR, Merz JE, Titus RG, Sogard SM, Mangel M (2010) Smolt transformation in two California steelhead populations: effects of temporal variability in growth. Transactions of the American Fisheries Society 139:1263-1275

Bell E, Albers SM, Krug JM, Dagit R (2011) Juvenile growth in a population of southern California steelhead *(Oncorhynchus mykiss)*. California Fish and Game 97:25-35

Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the use of stable isotopes in trophic ecology. Annual Review of Ecology, Evolution, and Systematics 42:411-440

Bolnick DI, Amarasekare P, Araújo MS, Bürger R, Levine JM, Novak M, Rudolf VHW, Schreiber SJ, Urban MC, Vasseur DA (2011) Why intraspecific trait variation matters in community ecology. Trends in ecology & evolution 26:183-192

Bond MH, Hayes SA, Hanson CV, MacFarlane RB (2008) Marine survival of steelhead *(Oncorhynchus mykiss)* enhanced by a seasonally closed estuary. Canadian Journal of Fisheries and Aquatic Sciences 65:2242-2252

Boughton DA, Gibson M, Yedor R, Kelley E (2007) Stream temperature and the potential growth and survival of juvenile *Oncorhynchus mykiss* in a southern California creek. Freshwater Biology 52:1353-1364

Bulkley RV (1967) Fecundity of steelhead trout , *Salmo gairdneri* , from Alsea River , Oregon. Journal of Fisheries Research Board of Canada 24:917-926

Darwin C (1859) On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life, 1st edn. John Murray, London.

Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR (2008) Impacts of climate warming on terrestrial ectotherms across latitude. Proceedings of the National Academy of Sciences 105:6668-6672

Gowan C, Fausch KD (2002) Why do foraging stream salmonids move during summer? Environmental Biology of Fishes 64:139-153

Greene CM, Hall JE, Guilbault KR, Quinn TP (2010) Improved viability of populations with diverse life-history portfolios. Biology letters 6:382-386

Gross MR, Coleman RM, Mcdowall RM (1988) Aquatic productivity and the evolution of diadromous fish migration. Science 239:1291-1293

Hayes S A., Bond MH, Hanson CV, Freund EV, Smith JJ, Anderson EC, Ammann AJ, MacFarlane RB (2008) Steelhead Growth in a Small Central California Watershed: Upstream and Estuarine Rearing Patterns. Transactions of the American Fisheries Society 137:114-128

Hayes SA, Bond MH, Hanson CV, Jones AW, Ammann AJ, Harding JA, Collins AL, Perez J, Macfarlane RB (2011) Down , up , down and "smolting" twice? Seasonal movement patterns by juvenile steelhead *(Oncorhynchus mykiss)* in a coastal watershed with a bar closing estuary. Canadian Journal of Fisheries and Aquatic Sciences 68:1341-1350

Hoar WS, Randall DJ, Brett JR (Eds) (1979) Fish physiology: Bioenergetics and growth, Volume VIII. Academic Press, New York

Höjesjö J, Økland F, Sundström LF, Pettersson J, Johnsson JI (2007) Movement and home range in relation to dominance; a telemetry study on brown trout *Salmo trutta*. Journal of Fish Biology 70:257-268

Kahler TH, Roni P, Quinn TP (2001) Summer movement and growth of juvenile anadromous salmonids in small western Washington streams. Canadian Journal of Fisheries and Aquatic Sciences 58:1947-1956

Klaassen M, Piersma T, Korthals H, Dekinga A, Dietz MW (2010) Single-point isotope measurements in blood cells and plasma to estimate the time since diet switches. Functional Ecology 24:796-804

Layman CA, Araujo MS, Boucek R, Hammerschlag-Peyer CM, Harrison E, Jud ZR, Matich P, Rosenblatt AE, Vaudo JJ, Yeager LA, Post DM, Bearhop S Applying stable isotopes to examine food-web structure: an overview of analytical tools. Biological Reviews doi: 10.11

Lindley ST, Schick RS, Agrawal A, Goslin M, Pearson TE, Mora E, Anderson JJ, May B, Greene S, Hanson C, Low A, Mcewan D, Macfarlane RB, Swanson C, Williams JG (2006) Historical population structure of Central Valley steelhead and its alteration by dams. San Francisco Estuary & Watershed Science 4:1-19

Lindley ST, Schick RS, Mora E, Adams PB, Anderson JJ, Greene S, Hanson C, May BP, McEwan DR, MacFarlane RB, Swanson C, Williams JG (2007) Framework for assessing viability of threatened and endangered Chinook salmon and steelhead in the Sacramento-San Joaquin Basin. San Francisco Estuary & Watershed Science 5:1-26

Mangel M, Satterthwaite WH (2008) Combining proximate and ultimate approaches to understand life history variation in salmonids with application to fisheries, conservation, and aquaculture. Bulletin of Marine Science 83:107-130

Mcewan DR (2001) Central Valley steelhead. In: Brown RL (ed) Contributions to the biology of Central Valley salmonids, Fish Bulletin 179(1). Scripps Institutions of Oceanography Library, San Diego, CA, pp 1-44

Mellina E, Hinch SG, MacKenzie KD, Pearson G (2005) Seasonal movement patterns of stream-dwelling rainbow trout in North-Central British Columbia, Canada. Transactions of the American Fisheries Society 134:1021-1037

Moore JW, McClure M, Rogers LA, Schindler DE (2010) Synchronization and portfolio performance of threatened salmon. Conservation Letters 3:340-348

Moyle PB (2002) Inland fishes of California. University of California Press, Berkeley

Myrick CA, Cech JJ (2004) Temperature effects on juvenile anadromous salmonids in California's central valley: What don't we know? Reviews in Fish Biology and Fisheries:113-123

Myrick CA, Cech JJ (2005) Effects of temperature on the growth, food consumption, and thermal tolerance of age-0 Nimbus-strain steelhead. North American Journal of Aquaculture 67:324-330

Nielsen JL (1999) The evolutionary history of steelhead *(Oncorhynchus mykiss)* along the US Pacific Coast: developing a conservation strategy using genetic diversity. ICES Journal of Marine Science 56:449-458

Northcote TG (1992) Migration and residency in stream salmonids - some ecological consideration and evolutionary consequences. Nordic Journal of Freshwater Research 67:5-17

Pasternack GB, Wang CL, Merz JE (2004) Application of a 2D hydrodynamic model to design of reach-scale spawning gravel replenishment on the Mokelumne River, California. River Research and Applications 20:205-225

Phillips DL, Eldridge PM (2006) Estimating the timing of diet shifts using stable isotopes. Oecologia 147:195-203

Quinn TP (2004) The behavior and ecology of Pacific salmon and trout. University of Washington Press, Seattle

Satterthwaite WH, Beakes MP, Collins EM, Swank DR, Merz JE, Titus RG, Sogard SM, Mangel M (2010) State-dependent life history models in a changing (and regulated) environment: steelhead in the California Central Valley. Evolutionary Applications 3:221-243

Satterthwaite WH, Hayes S a., Merz JE, Sogard SM, Frechette DM, Mangel M (2012) State-dependent migration timing and use of multiple habitat types in anadromous salmonids. Transactions of the American Fisheries Society 141:781-794

Schindler DE, Hilborn R, Chasco B, Boatright CP, Quinn TP, Rogers LA, Webster MS (2010) Population diversity and the portfolio effect in an exploited species. Nature 465:609-612

Shapovalov L, Taft AC (1954) The life histories of the steelhead rainbow trout *(Salmo gairdneri)* and silver salmon *(Oncorhynchus kisutch)* with special reference to Waddell Creek, California and recommendations regarding their management. California Department of Fish and Game Fish Bulletin 98:373p

Sogard SM, Merz JE, Satterthwaite WH, Beakes MP, Swank DR, Collins EM, Titus RG, Mangel M (2012) Contrasts in habitat characteristics and life history patterns of *Oncorhynchus mykiss* in California's Central Coast and Central Valley. Transactions of the American Fisheries Society 141:747-760

USFWS, NMFS (1996) Policy regarding the recognition of distinct vertebrate population segments under the Endangered Species Act. Federal Register (7 February 1996)61(26):4722-4725

CHAPTER 1

Movement and survival of wild coastal rainbow trout, Oncorhynchus mykiss, in a regulated California river

INTRODUCTION

Movement may affect an individual's growth, reproduction, and mortality by determining access to resources, avoidance of predators or environmental stressors, and facilitating social interactions including encounters with mates (Turchin 1998; Dingle and Drake 2007). Collectively, the consequences of individual movement are manifest in the distribution, structure, and dynamics of populations, connectivity of metacommunities and delivery of subsidies among ecosystems (Holyoak et al. 2005; Merz and Moyle 2006; Dingle and Drake 2007). For example, anadromous migrations involving large numbers of fish traveling thousands of kilometers through freshwater streams, across ocean basins and returning to their natal habitat illustrate the ecological and evolutionary significance of movement as a fundamental life history trait (Quinn 2004; Dingle and Drake 2007). In contrast, individuals of the same population may instead complete their entire life cycle within this same natal freshwater habitat (Northcote 1992; Quinn 2004). Resident stream fishes were long considered sedentary (e.g. the restricted movement paradigm; Gerking 1959; Gowan et al. 1994), yet advances in tracking technology now reveal variation in movement among individuals (Gowan et al. 1994). The causes and consequences of variation in spatial and temporal patterns of movement can provide insight into the evolution of

species, the ecology of populations, and the connectivity of communities and ecosystems.

Variability in movement among individuals within a single population likely depends on numerous parameters including individual size, season, habitat, and mortality risk. For example, in the stream environment, larger dominant trout (Salmonidae) are better able to defend optimal feeding stations, and thus may be more sedentary, forcing increased movement on smaller individuals (Northcote 1992; Keeley 2001). Size-dependent variation in movement may also reflect evolutionary consequences whereby in contrast to sedentary adults, movement in smaller sub-adults is seasonal, perhaps due to a history of selection facilitating emigration coincident with increased stream flow (Quinn 2004; Mellina et al. 2005). Alternatively, in response to habitat heterogeneity, larger trout may move more to maximize growth at the reach scale (Kahler et al. 2001; Gowan and Fausch 2002; Höjesjö et al. 2007). Thus, where a trout resides may affect how much it moves, depending on habitat quality and heterogeneity within its home range. Therefore, for salmonids, a cost-benefit comparison may explain movement variability across multiple scales, from small scales within rivers (Kahler et al. 2001; Keeley 2001; Gowan 2007) to large-scale migrations to sea (Gross et al. 1988; Northcote 1992; Mangel and Satterthwaite 2008).

The tremendous variation in movement among individual coastal rainbow trout *(Oncorhynchus mykiss)* lends them as excellent models to understand the ecological causes and consequences of movement (Moyle 2002; Quinn 2004; Augerot

and Foley 2005). Individuals may spend their entire life within one stream habitat (resident rainbow trout), while others, born in this same habitat, may migrate hundreds of kilometers to sea and back prior to spawning (anadromous steelhead). Further, there is a diversity of expression of these life histories arising from differences in the timing and degree of movement among individuals (Shapovalov and Taft 1954; Quinn 2004; Augerot and Foley 2005). Like in other species, the degree of movement among individual *O. mykiss* likely depends upon individual size, differences in growth potential across spatio-temporal scales, and the risks associated with movements across these scales (Northcote 1992). The extreme variability in degree of movement among individuals has likely allowed the species to persist across such an extensive range (Augerot and Foley 2005); maintenance of this variability both among individuals and among populations may prove important to the sustainability of the species (Schindler et al. 2010; Greene et al. 2010).

The Sacramento-San Joaquin watershed, draining California's Central Valley, historically included a great diversity of aquatic habitats, including Sierran lakes and streams, large productive rivers and the largest estuary of Western North America (Conomos 1980; Hollibaugh 1996; Moyle 2002). These ecosystems supported a productive and diverse collection of native fishes with diverse life histories, including abundant *O. mykiss* (Moyle 2002). Dams and other hydrologic alterations have eliminated or otherwise made inaccessible 80% of original steelhead spawning and rearing habitat, resulting in dramatic population declines in this life-history strategy (McEwan 2001; Lindley et al. 2006). As a result, Central Valley steelhead

populations are listed under the US Endangered Species Act as threatened (NMFS and NOAA 2006). Yet there is surprisingly little known about wild *O. mykiss* of the Central Valley (Myrick and Cech 2004; Lindley et al. 2007; Sogard et al. 2012). Dams have also greatly simplified Central Valley rivers by stopping the delivery of larger coarse sediments downstream and buffering flows that would otherwise create and maintain habitat complexity across space and time (Merz et al. 2004, 2006; Kondolf et al. 2006). Habitat alteration may affect variability in growth potential both within rivers and the ocean, as well as alter the consequences of movement within rivers or en route to the ocean (e.g., mortality). Thus, habitat alteration may affect the cost-benefit ratio of movement at multiple scales. While it is clear that the elimination and alteration of habitat is largely responsible for declines of Central Valley *O. mykiss* (McEwan 2001; Lindley et al. 2006), the extent of variation in movement patterns in the remaining rearing habitat is unclear.

To determine the diversity of *O. mykiss* life history as a function of movement in a regulated river, and further investigate the causes and consequences associated with movement, I used acoustic telemetry to monitor the movement and survival of wild *O. mykiss* of the Mokelumne River, a highly modified river typical of the Central Valley. Telemetry has proven useful in monitoring movement patterns (Cooke et al. 2008; Semmens 2008), migration pathways (Perry et al. 2010; Del Real et al. 2012), reach-specific mortality rates along migration corridors (Skalski et al. 2001; Melnychuk et al. 2007; Welch et al. 2008) and investigating life history (Cooke et al. 2008; Melnychuk and Hausch 2011). I used standardized telemetry surveys along the

rearing section of the lower Mokelumne River to examine fine scale *O. mykiss* movement (<5 m resolution) and survival patterns over two varying flow years. Because this study was nested within a larger network of stationary acoustic receivers throughout the lower Mokelumne River and Sacramento-San Joaquin Delta (Perry et al. 2010; Del Real et al. 2012), I was able to document movement of wild *O. mykiss* ranging from less than 5 m to anadromous emigrations of over 150 km. I analyzed movement and survival patterns in relation to fish size, season, streamflow and reach scale habitat differences to answer the following questions: What is the proportion of and correlates with emigration in post-yearling anadromous wild *O. mykiss*? What environmental and individual traits correlate with movement of post-yearling resident wild *O. mykiss*? What are the seasonal movement patterns of post-yearling resident wild *O. mykiss*? How is survival of these individuals associated with size, movement or location?

METHODS

Study system

The Mokelumne River drains a 1,624 km^2 watershed of the western Sierra Nevada Mountains and flows directly into the Sacramento-San Joaquin Estuary (Figure 1). Typical of snow fed watersheds, Mokelumne River flow historically began increasing around October with increasing precipitation (and reduced evapotranspiration) and peaked around June at over 68 $m^3 s^{-1}$, with sustained high flows from snow melt throughout the summer, tailing off continually until the

following September (Pasternack et al. 2004). Like most other Central Valley rivers, the Mokelumne River is now highly regulated, with sixteen major water impoundments as well as many diversions for agricultural, municipal, energy production and flood control uses. This has resulted in a dampened hydrograph, with peaks half their historic levels (Appendix, Figure A1; Pasternack et al. 2004; Merz et al. 2006), and streamflows dependent upon the combined factors of human extraction and interannual variability in precipitation. Camanche Dam (elevation 72 m) is the most downstream barrier to anadromous fishes and reduces the available anadromous stream habitat to 12% of original (Lindley et al. 2006), with 103 km of river remaining available to anadromous fishes. By reducing peak flows and flow variability and by blocking the natural transport of stream sediments, regulation has dramatically simplified the remaining stream habitat available to *O. mykiss* in the lower Mokelumne River (Merz et al. 2006; Sogard et al. 2012). Although the alluvial plains of the river have been predominantly converted into vineyards and orchards (Merz 2002), the riparian habitat immediately adjacent to the lower Mokelumne River is surprisingly intact and supports an abundance and diversity of both aquatic and terrestrial wildlife (Merz and Moyle 2006). Woodbridge Irrigation District Dam (WIDD) is located 41 kilometers downstream from Camanche Dam (Figure 1). This relatively small, agricultural dam is passable to fish, and marks the boundary between riverine rearing habitats upstream and the tidally influenced portion of the lower Mokelumne River and the estuary downstream (Merz 2002).

Previous researchers have considered the lower Mokelumne River to be comprised of six reaches, each defined by differences in substrate material and gradient (see Merz 2001, 2002). The three reaches between Camanche Dam and WIDD were the focus of my surveys. The furthest upstream reach, Reach 6, is of the highest gradient, dropping 30 m in the eight river kilometers from Camanche dam downstream to the Mackville Road Bridge (Figure 1). Reach 6 thereby has faster water velocity, a more complex habitat composition and substrate dominated by gravels with higher proportions of cobbles and boulders (East Bay Municipal Utilities District, EBMUD, unpublished data). Reach 5 is of intermediate gradient, dropping 10 m in the next 8.5 river kilometers downstream between Mackville Road Bridge and Elliot Road Bridge (Figure 1). Reach 5 contains an intermediate complexity of habitat types and water velocities, with substrates dominated by gravels and sands (EBMUD, unpublished data). Reach 4 is of very low gradient, dropping 18 m along the 24.5 river kilometers between Elliot Road Bridge and WIDD (Figure 1). Reach 4 has very low complexity of channel morphology among a series of continuously wide, deep, slow moving habitats with sand as the dominant substrate (EBMUD, unpublished data).

Depending upon flow release, Camanche Dam may also buffer stream water temperatures downstream (Merz et al. 2006). Stream temperatures within the rearing section vary seasonally from a low of approximately 10^{o}C in February to highs of approximately 14-16^{o}C from August through November (Sogard et al. 2012). As water moves downstream it is warmed during the day and cooled at night, resulting in

more extreme diel variation relative to upstream reaches. Below WIDD, the lower Mokelumne River continues as a low gradient river meandering through sandy alluvial plains until it flows into deeper estuarine habitats. However, due to extensive levees and the cumulative effects of upstream water extractions, it is often smaller, narrower and shallower than the three upstream rearing reaches.

Environmental data

Streamflow data as discharge and reach boundary elevations were collected from Camanche Dam from California Department of Water Resources California Data Exchange Center (CDEC). A GIS database habitat data including dominant substrate and habitat type was previously collected and compiled by the East Bay Municipal Utilities District (EBMUD, unpublished data).

Wild *O. mykiss* of the Lower Mokelumne River

A low proportion of anadromous individuals is hypothesized in the lower Mokelumne River due to anthropogenic alterations changing growth potentials combined with increased movement related mortality (Satterthwaite et al. 2010; Sogard et al. 2012; Del Real et al. 2012). Based on a cost-benefit analysis that modeled relative fitness gained by growth within the river relative to growth at sea, and considering movement related mortality, Satterthwaite et al. (2010) predicted low proportions of anadromy for *O. mykiss* within the lower Mokelumne River. These model predictions were most sensitive to instream mortality estimates due to uncertainty associated with insufficient mortality data (Satterthwaite et al. 2010). Del

Real et al. (2012) found low downstream migration of lower Mokelumne River *O. mykiss* and what downstream movement was observed was dominated by hatchery fish. However, the full range of movement expressed by wild *O. mykiss* within the lower Mokelumne River is still poorly understood (Satterthwaite et al. 2010; Sogard et al. 2012). Therefore, greater knowledge of the patterns, causes and consequences of movement and survival, specifically in relation to individual size, season, and environmental conditions, is critical to our understanding of the full spectrum of life histories in *O. mykiss* and potential management to maintain life history diversity and population stability (Greene et al. 2010; Moore et al. 2010; Sogard et al. 2012).

Tagging methods

We tagged a range of sizes of naturally produced or "wild" *O. mykiss* (all hatchery fish have been marked with an adipose fin clip since 1997; McEwan 2001) in 2007 and 2008 to investigate the various scales of movement expressed as well as correlates of movement and mortality including individual size (Appendix Figure A2). All fish used in this study were surgically implanted with both a Vemco V9-2L-69 kHz R64K coded acoustic transmitter and a passive integrated transponder (PIT) tag (Allflex). We did not tag fish under 180 mm fork length (FL) so as to not exceed a 5% transmitter-to-body-weight ratio (Del Real et al. 2012). As such, movement patterns described in this study are for post-yearling individuals only (Sogard et al. 2012; Del Real et al. 2012). Standardized tagging procedures were used for all surgical implantations (refer to Del Real et al. 2012 for further detail).

In February of 2007, 52 wild *O. mykiss* were captured using standardized boat electrofishing techniques (Meador et al. 1993; Del Real et al. 2012) in seven different sites among the three rearing reaches (Figure 1). Fish were transported in 1.9 m^3 tanks filled with river water to the Mokelumne River Hatchery and kept in raceways for approximately one week to monitor health prior to tagging. After tagging, fish were further monitored for another week, and then released in the same location as capture for each fish on February 20 and 21. An additional 8 wild *O. mykiss* were captured May 7-9. Surgery was performed on-site, and fish were immediately released following recovery in tanks filled with oxygenated river water. Roughly 50% (29) of the 60 tagged fish were either never detected or determined to be dead during initial surveys. This left 31 fish for which I had reliable resighting/recapture histories. Wet weight of tagged and monitored fish ranged from 100 to 1400 g (mean 372 g). Fork length ranged from 221 to 507 mm (mean 304 mm; Appendix Figure A2).

In 2008, all tagging occurred on site; tagged fish were held in tanks of oxygenated river water until full recovery from anesthesia and then released at the capture site. We captured 51 fish using standardized boat electrofishing techniques (Meador et al. 1993; Del Real et al. 2012) at six different sites among the three rearing reaches. Release dates were February 4, April 2 and 8, and May 8 and 9. Of the 51 fish, 9 were either never detected or determined to be dead during initial surveys, leaving 42 fish with reliable resighting/recapture histories. Wet weight ranged from 100 to 1150 g (mean 253 g) and fork length ranged from 198 to 498 mm (mean 268 mm; Appendix Figure A2).

In addition to the fish captured by electrofishing, 17 wild *O. mykiss* were captured using two rotary screw traps in the lower reaches of the lower Mokelumne River. These fish were tagged and released between February 4 and April 10. Only 5 of the 17 fish were subsequently detected. Because these 5 fish were captured in the lower rotary screw trap at WIDD, at the lower boundary of the rearing section (Figure 1), they were potentially undergoing emigration to the ocean, and were therefore not included in analyses of presumed resident behavior. Wet weight ranged from 83 to 231 g (mean 126 g) and fork length ranged from 202 to 411 mm (mean 268 mm).

Monitoring anadromous movements of wild *O. mykiss*

To monitor anadromous migrations of tagged *O. mykiss*, I collected data from the network of stationary acoustic receivers (Vemco VR2W-69 kHz) monitored in collaboration with EBMUD and the California Fish Tracking Consortium (CFTC; see Perry et al. 2010; Del Real et al. 2012). One receiver was placed at WIDD, to document any tagged *O. mykiss* leaving the freshwater rearing section and entering the tidally influenced section of the lower Mokelumne River. Other receivers were strategically placed downstream to monitor the migration pathways and timing of anadromous migrations throughout the lower Mokelumne River, Sacramento River system, and San Francisco Estuary. An array of stationary acoustic receivers was maintained at the Golden Gate Bridge to record any tagged *O. mykiss* successfully entering the Pacific Ocean. I defined any individual that passed the stationary acoustic receiver at WIDD as an anadromous emigrant. Conversely, I considered any

individual that was tagged above WIDD and never passed the receiver a resident for the duration of this study.

Monitoring movement and mortality of wild resident *O. mykiss*

To investigate movement patterns and mortality, I surveyed the rearing section using a hand held hydrophone. I mounted an omnidirectional hydrophone (Vemco, VH165) to an articulating boom over the gunnel of a 4 m aluminum skiff with 15 hp gas-powered motor. The hydrophone was connected to a Vemco acoustic receiver (VR60 in 2007, VR60 or VR100 in 2008) that decoded the individual identifier of each acoustically tagged wild *O. mykiss*. I used a handheld gps unit and recorded the time and location of each fish observation to 5 m accuracy. With an assistant, I surveyed the complete 41 km of the rearing section in a downstream direction from Camanche Dam to just above WIDD. Each survey included the entire rearing section, completed in one day, typically between the hours of 8 a.m. and 6 p.m.. To maintain high and equal detection probability among individuals and among observations, we established a standardized survey protocol. We conducted detection range tests with and without rain, and with and without the boat motor running for a variety of streamflows and habitat conditions, using test tag moorings placed in different habitats (Melnychuk and Christensen 2009). Tags were set on a 60 second nominal delay, meaning that they would emit signals on random sequences every 30 to 90 seconds. Therefore, to ensure equal probability of tag detection along the varying conditions of one survey as well as temporally among surveys, we remained within a detection area for at least 180 seconds. Detection areas ranged

from 5 m due to turbulent water or obstructions such as wood, boulders or gravel bars, up to 200 m stretches of straight, unobstructed river. We surveyed around habitat structures such as fallen submerged trees, throughout large habitats and within each sub-channel of braided sections of the lower Mokelumne River to ensure complete coverage. I estimated an overall mean detection probability of 94% (90% in 2007 and 95% in 2008) using methods outlined in Williams et al. (2002) and Melnychuk et al. (2007).

To eliminate the possibility of false-positive detections, we monitored at least two consecutive signals per observation (Melnychuk and Christensen 2009; Perry et al. 2010). If we could discern movement during this time, we assumed the detections were from a live fish. However, many fish were observed in the same location on multiple surveys without notable movement. In these scenarios we conducted a "splash test" to ensure that the detection was of a live fish, and not a shed tag lying on the river bottom. To do this, signals of a detected fish were monitored for at least two cycles, and then I would enter the river and either splash the surface of the water or swim toward the detection location in an attempt to alter the fish's behavior. I maintained this disturbance for at least 180 seconds while my partner monitored acoustic signals. Subsequent acoustic signals were monitored for another 180 seconds. If we received at least two strong signals prior to disturbance, then signal strength was lower or there was a lack of detection during disturbance, and this decreased or lack of signal strength persisted after the disturbance ended (to ensure the disturbance altered the fish behavior rather than physically interfering with

detection), we assumed that the fish was alive. We then either monitored two full detections after the fish returned to its previous location or sought the fish out in its new location. From these results we assumed that during disturbance the tagged fish either moved further into cover, thereby affecting its tag's signal strength, or moved out of range of detection. Often, simply slipping into the water as unobtrusively as possible altered the behavior of the tagged fish enough to affect signal strength. If the signal strength was not altered by the splash test, I removed the fish from analysis and assumed that the fish was dead. I assumed all detections determined to be in a live fish were in originally tagged *O. mykiss* rather than in the stomach of a predator. I qualitatively investigated each observation history of each fish to ensure that movement patterns did not suddenly change to indicate predation (Melnychuk et al. 2007; Melnychuk and Christensen 2009; Perry et al. 2010).

In 2007 I conducted 17 standardized surveys from February 26 to November 20. In 2008 I conducted 22 standardized surveys between February 26, 2008 and February 12, 2009. I subsampled these 39 surveys so that there were even intervals both among years and among seasons each year. This resulted in approximately monthly intervals (including between tagging and the first subsampled survey) for each year. Each monthly survey was generally within days of the equivalent survey for the other year. Although battery life of the tags is stated to last approximately 283 days, detection dropped dramatically around 218 days after tagging each year. Therefore, only data within 218 days of tagging for each fish were used. This resulted in eight subsampled surveys in 2007 beginning February 26 and continuing monthly

until the last survey on September 26 (Figure 2). In 2008, I used eleven monthly surveys from February 26 to December 23 (Figures 2 and 3). To maintain consistency among sampling methodologies, I used data from one location logged from the network of stationary readers downstream of WIDD for fish that emigrated out of the survey area coincident with each standardized stream survey date. I entered time and location for each fish observation into a GIS database and calculated the distance moved (km) between observations as both the raw distance, either positive indicating an upstream movement, or negative indicating a downstream movement, and the absolute value of each movement.

I analyzed detection data with certain assumptions. While tagged individuals may have emigrated and returned prior to tagging, or done so after the tag battery died, movement patterns observed in this study, including emigration, are derived from a sample of equal time among individuals, spanning the expected period of emigration (Northcote 1992; Mellina et al. 2005; Sogard et al. 2012). By conducting splash tests I removed any data from tags on the river bottom from tag shedding or predation from analyses. Therefore, I assumed mortality for any fish that was observed at least once but never observed again after a given survey. No fish was able to leave in an upstream direction due to Camanche Dam. It is also highly unlikely that a fish could leave the study area downstream to the ocean and not be detected, since it would have to swim by at least 20 of the over 150 downstream receivers. Given that the detection probability of each of these receivers is extremely high (and in some cases were estimated to be 1.0; Perry et al. 2010), the probability of a fish emigrating

from the rearing section and not being detected by one of the receivers downstream rapidly approaches zero (Williams et al. 2002; Perry et al. 2010). Finally, measurements of resident survival are based on assumptions that observed mortality was not simply a lack of detection, including due to battery failure. Truncating my data to 218 days to avoid apparent mortality due to battery failure, and a mean detection probability of 94% lend credence to survival measures of resident fish reported here (Williams et al. 2002).

What are the proportions of and correlates with anadromy in *O. mykiss*?

To determine the proportion of tagged fish that emigrated from the river, I calculated the number of fish that passed by the WIDD receiver in each year. I used logistic regressions to test for differences in the binary response of anadromous emigration (or not) in response to individual FL and season, or FL and streamflow. For this and all other statistical analyses I used R statistical software (R Development Core Team 2008).

What are the correlates with movement in resident *O. mykiss?*

To test the hypotheses that movement is related to individual or environmental characteristics, I tested for differences in a standardized movement metric with respect to fish size (FL) and an individual's home reach. I used general linear models, separately for each year, with FL and home reach, and the interaction between FL and home reach each as fixed factors. I calculated standardized

movement *(MVT)* for each fish for each year by summing the absolute value of each distance (km) moved per sampling interval (i.e. period between observations) and then dividing this by the number of intervals that individual was observed, or:

$$MVT = \frac{\Sigma\,|distance\ moved|}{intervals\ observed}. \quad (1)$$

The "intervals observed" was determined by subtracting the first survey observed from the last survey the individual was observed and adding one to include the interval between tagging and the first observation. In this regard movement was standardized by interval to account for missed observations and mortality so that all individuals regardless of recapture history and duration of survival were comparable using a single metric of movement. *MVT* data were log transformed to meet assumptions of normality and homoscedasticity of variance for analysis. I determined the "home reach" or the reach in which an individual *O. mykiss* spent most of its time (calculated as median reach number) for each fish, each year. In 2007, the number of fish in each reach were, Reach 6 (n=24), Reach 5 (n=4), Reach 4 (n=3), and in 2008 were Reach 6 (n=26), Reach 5 (n=10), Reach 4 (n=6).

To determine the extent to which MVT resulted in net displacement of individuals within the river I calculated the "home range" as the most upstream river kilometer minus the most downstream river kilometer at which each individual was observed. I then calculated the proportion of home ranges within each of three scales: < 0.5km, 0.5 – 8 km, and > 8km.

What are the seasonal movement patterns of resident *O. mykiss?*

To test the hypotheses that movement varied as a function of FL, home reach and season I used linear mixed effects models to investigate a metric of seasonal standardized movement in relation to each of these independent variables, and each of their interactions as fixed factors, and individual as a random factor (Zuur et al. 2009; Bolker et al. 2009). I considered observations between February 26 and May 24 to be spring, between May 25 and August 26 to be summer, between August 27 and November 24 to be fall, and between November 25 and December 23 to be winter. This resulted in four intervals in spring and three intervals in summer for both years, one interval for fall of 2007, and three intervals for fall of 2008, and one interval for winter of 2008. As a standardized seasonal movement metric I calculated the mean absolute value of kilometers moved per season (mvt_s) for each individual. Like MVT, mvt_s also standardizes movement by survey accounting for missed observations and mortality. Standardized seasonal movement (mvt_s) was also log transformed to meet assumptions of normality and homoscedasticity of variance. In 2007, I observed 26, 25 and 18 fish in the spring, summer and fall seasons, respectively. Due to relatively early tagging and by truncating my data to only use observations within 218 days of tagging I did not use any of my surveys conducted in the winter of 2007. In 2008, I observed 20, 35, 25 and 12 fish in the spring, summer, fall and winter seasons, respectively.

How is survival associated with size, movement or location?

To determine if survival was related FL, home reach or movement I separately tested the hypotheses that binary survival and a standardized metric of survival varied as a function of each of these independent variables. I calculated standardized survival for each individual *O. mykiss* as the survey number on which the fish was last observed divided by the total possible number of surveys for that fish. Due to variation in tagging date, the date of final survey also varied among individuals, but did not exceed 218 days since tagging. Thus, standardized survival is a continuous relative index comparable among all individuals among both years. Standardized survival was ArcsinSquareroot transformed to meet assumptions of normality and homoscedasticity of variance for analyses. For each year I performed two separate analyses of standardized survival using general linear models. The first tested for effects of MVT, home reach, FL, and all interactions on standardized survival. MVT, home reach, and FL were treated as fixed factors. The second tested for effects of the percent of observations moved, home reach, FL, and all interactions as fixed factors on standardized survival. I calculated the percent observations moved as the number of times a tagged fish was observed in a new location relative to the total number of observations for that fish. To meet assumptions of normality and homoscedasticity of variance I transformed percent observations moved using an ArcsinSquareroot transformation.

I also measured survival as a binary metric of whether or not the individual survived the entire 218 day sample period. I used logistic regressions to investigate

binary survival in response to home reach, FL, and degree of movement (as either MVT or percent observations moved), and each two-way interaction as fixed factors.

RESULTS

Anadromy in wild *O. mykiss*

Of the 31 fish observed in 2007 only one emigrated from the rearing section. This individual (225 mm FL) emigrated 48.5 km into the estuary in the spring, and then returned to Reach 4 in the summer, where it remained for the remainder of the study. The other 30 fish remained in the study reaches over the duration of the study, or until they were no longer detected and assumed to have died.

In 2008, none of the 42 fish captured by electrofishing emigrated from the rearing habitat. All 5 fish captured by screw trap below WIDD continued their emigration further downstream. The largest individual (411 mm) entered the Pacific Ocean in late April, 14 days after it was tagged. This successful 170 km anadromous migration coincided with the peak streamflow for 2008 and the only period of streamflow greater than 12 $m^3 s^{-1}$ for both years (Figure 3). All five emigrants exhibited directional downstream movement focused in the spring (Figure 3). One of the five emigrated into the Delta in spring and then returned to WIDD in the summer (Figure 3). The other three emigrants moved down into the estuary where they either died or remained between stationary readers until the batteries in their tags died. In 2008, I did not detect a relationship between FL and whether fish emigrated (logistic

regression: $p = 0.18$), but emigration was significantly higher in the spring and with higher streamflows ($p < 0.001$ and $p = 0.03$, respectively; Figure 3).

Resident *O. mykiss* movement relative to individual size and habitat

There was very little movement of resident *O. mykiss* in both years (Figure 2; Appendix Figure A3). Most individuals had MVT values less than 0.5 km /mo each year and nearly all fish moved less than 1 km/mo (Appendix Figure A3). In 2007 there were three individuals with MVT values between 1 km/mo and 7.5 km/mo, and in 2008 there were five individuals with MVT values between 1 km/mo and 5.5 km/mo (Appendix Figure A3). MVT values were directly related to individual net displacement or "home ranges." Specifically, mean home range was 2.9 km in 2007 and 1.7 km in 2008, and the distribution of home range sizes among fish was similar to the distribution of MVT (Appendix Figure A3). A large proportion of individuals had home ranges less than 0.5 km (43% in 2007 and 67% in 2008). Many fish had home ranges within the scale of a reach i.e. 0.6 - 8 km (47% in 2007 and 26% in 2008). Few fish had larger home ranges. In 2007, 10% of fish had home ranges from 8.1 to 22 km; in 2008 7% of fish had home ranges from 8.1 to 16 km.

In 2007, MVT of resident wild *O. mykiss* was not related to FL, home reach, or an interaction between FL and home reach ($p = 0.16$, $p = 0.21$, and $p = 0.14$, respectively). By contrast, in 2008 there was a significant relationship between MVT and FL, such that smaller fish moved more (df = 1; F = 5.24; $p = 0.03$; Figure 4).

Home reach and the interaction between home reach and FL were not significant in 2008 (p = 0.84 and p = 0.98, respectively).

Seasonal patterns of resident *O. mykiss* movement relative to size and habitat

Standardized seasonal movement *(mvt_s)* differed among seasons, and the patterns differed between the two years (Figure 2; Appendix Figure A4). In 2007, resident wild *O. mykiss* moved most in the spring, followed by the summer, and then the fall (df = 2; F = 4.27; p = 0.02; Figure 2; Appendix Figure A4, no data available for winter). There was a significant interaction effect between FL and home reach on mvt_s in 2007 (df = 2; F = 3.42; p = 0.049), such that mvt_s increased with FL in Reach 6, movement increased dramatically with FL in Reach 5, and there was no movement of any FL in Reach 4 (Figure 5). In 2007, seasonal movement was not related to home reach, FL or the interaction between FL and season (p = 0.23, p = 0.13, and p = 0.84, respectively).

In 2008 resident *O. mykiss* moved the most in the summer, less in spring, very little in fall and not at all in winter (df = 3; F = 5.65; p = 0.002; Figure 2; Appendix Figure A4). Smaller fish had higher mvt_s (df = 1; F = 4.88; p = 0.03). Unlike 2007, there was also a significant interaction effect between season and FL on mvt_s in 2008 (df = 3; F = 3.12; p = 0.04; Figure 6), such that smaller fish moved more than larger fish in the summer and spring, with no relationship in the fall due to limited movement across all sizes, and no movement by fish of any size in the winter (Figure

6). In 2008, seasonal movement of resident *O. mykiss* was not related to home reach or the interaction between home reach and FL ($p = 0.75$ and $p = 0.99$, respectively).

Effects of size, movement and habitat on resident *O. mykiss* survival

In 2007 19 of 31 resident *O. mykiss* (60%) survived the entire 218 day study. Standardized survival, a continuous metric of survival based upon the duration of time survived, ranged from 14% to 100% of the 218 day study with a mean of 77% (SD = 31%). In 2008, 19 of 42 residents (45%) survived the entire 218 day study. In 2008 standardized survival ranged from 14% to 100% over the 218 day study with a mean of 72% (SD = 33%). I did not detect any effect of movement, home reach, or FL on binary survival (surviving the entire 218 day study) in either year (logistic regression: $\alpha = 0.05$). Nor did I detect any effect of movement, home reach, or FL on standardized survival in 2007 ($\alpha = 0.05$). However, in 2008, individuals that exhibited a higher percent observations of movement had lower standardized survival (full model: df = 1; F = 10.18; $p = 0.003$; percent observations of movement vs. standardized survival: df = 1; F = 10.74; $p = 0.002$; Figure 7). There were no significant effects of home reach or FL on standardized survival in 2008 ($p = 0.57$, and $p = 0.50$, respectively).

DISCUSSION

I observed a "richness" (i.e. number) of life histories based on movement patterns within post-yearling wild *O. mykiss* of the lower Mokelumne River. Though

in low proportions, I did observe anadromous individuals. I also observed individuals that emigrated to the estuary only to return to the freshwater rearing section of the lower Mokelumne River each year. Furthermore, I observed a range of movement patterns within the rearing section, with home ranges at multiple scales < 0.5km, 0.5 – 8 km, and 8 – 22 km. However, this "richness" of movement patterns was distributed disproportionately across the population, as the vast majority of individuals had home ranges less than 0.5 km.

In 2008, individuals that moved more (i.e. with a higher percent observations of movement) had lower standardized survival (Figure 7). The fact that this relationship was significant with the relative number of moves but not the relative distance moved implies that movement, regardless of distance, increases the likelihood of mortality. Except for anglers, most predators in this system are visual predators including river otter *(Lontra canadensis)*, Osprey *(Pandion haliaetus)*, Sacramento pike minnow *(Ptychocheilus grandis)*, and striped bass *(Morone saxatilis)*. Movement of prey has long been known to increase vulnerability of prey to visual predators in several ways (Lidicker 1975; Yoder et al. 2004). By moving, prey leave protective cover, they can become more apparent relative to their background, and they increase the likelihood of encounter with predators. All three mechanisms, and others, are likely to contribute to the overall increased vulnerability and mortality of prey with increased movement frequency or distance.

Patterns of size-dependent movement differed between 2007 and 2008. In 2007, I observed greater movement of larger fishes, but only in Reaches 5 and 6

relative to Reach 4 (i.e. a reach by size interaction; Figure 5). This interaction between the effects of FL and home reach on movement appears to be driven by fish of fork lengths ranging from 300 to 450 mm, for which there was a higher proportional representation in 2007 than 2008 (Appendix Figure A2). Reaches 5 and 6 have high growth potential (Merz 2002; Sogard et al. 2012), presumably due to habitats dominated by gravels leading to high invertebrate production (Merz and Ochikubo Chan 2005) and delivery as drift (Sogard et al. 2012) via higher relative stream velocities. The complexity of habitats in Reaches 5 and 6 means that the short distance movement observed here (from less than 0.5 to 8 km) can expose *O. mykiss* to an assortment of habitats ranging in prey composition, abundance and availability. Thus, with minimal energy expended, larger fish can move about to determine the feeding station of optimal growth and, due to their size, have a high potential of displacing smaller, subordinate fish (Gowan and Fausch 2002; Gowan 2007). By contrast, Reach 4 is a series of long, deep, wide habitats of slow moving water with limited morphological complexity. Thus, short distance movements would likely not expose *O. mykiss* to differences in habitat quality. Consequently, there may be little benefit to short scale movements in Reach 4, and considering the potential for movement related mortality, the costs may outweigh the benefits to any movement other than emigration in this reach.

In contrast to the previous year, smaller fish moved more than larger fish in the spring and summer of 2008 (Figure 6). The increased sample size of smaller fish in 2008 (Appendix Figure A2) may explain why I was able to detect this interaction

between size and season on movement in 2008 and not in 2007. Although both years were considered low flow for the lower Mokelumne River (Figure 2 relative to Appendix Figure A1; Pasternack et al. 2004), 2008 still showed more variability in discharge, with much higher peaks in flow. Thus, the lower predictability in streamflow, coupled with movement related mortality may cause larger more dominant fish to move less, forcing movement of smaller subordinate fish in seasons of higher flow (Northcote 1992; Keeley 2001). Higher movement of smaller fish relative to larger fish has been detected by previous studies on the lower Mokelumne River (Merz 2002). In the Mokelumne River, like other snow fed Central Valley rivers, peak flows are naturally elevated in the spring and summer months. Therefore, increased movement among smaller individuals in the spring and summer may be the result of evolutionary history selecting for increased dispersal and emigration coincident with peak streamflows (Figs. 2 and 3; Quinn 2004; Mellina et al. 2005). Indeed, all anadromous emigrations observed in this study were during months of typical peak flows (Appendix Figure A1), and corresponded with spikes in discharge from Camanche Dam (Figure 3). For instance, all 14 days of the single successful 170 km anadromous migration were within the highest peak flows observed for either year (Figure 3). Thus, while there may be evolutionary or ontogenetic influences, discharge from Camanche Dam may also be important to movement and anadromous emigration of wild *O. mykiss* in the lower Mokelumne River. The increased movement observed during seasons of typical high flows, and the strong correlation

of anadromous emigration with peak streamflows (Figures 2 and 3) point to the importance of peak stream flows to movement and anadromy.

As hypothesized by others, I found a negligible proportion of tagged fish that exhibited anadromy in the lower Mokelumne River. While the tagging methods used here limited inferences to post-yearling *O. mykiss*, the high proportions of larger fish captured within the rearing section and low numbers of smaller emigrants captured with rotary screw traps are further evidence of a high proportion of residency in the lower Mokelumne River. In another dammed Central Valley river, the lower Feather River, Kurth (2012) also found low proportions of anadromous *O. mykiss* using acoustic telemetry and, similar to patterns previously observed in the lower Mokelumne River (Del Real et al. 2012), Kurth (2012) also found lower proportions of anadromy in wild fish than hatchery fish. By contrast, nearly all *O. mykiss* of the lower American River, another dammed Central Valley river, are anadromous (Sogard et al. 2012). Previous studies hypothesed that the low proportion of anadromy in both the lower Mokelumne River (Sogard et al. 2012) and the lower Feather River (Kurth (2012) result from elevated stream growth potential due to discharge practices from upstream dams, coupled with increased mortality during anadromous migrations. Indeed, I found evidence of movement related mortality (Figure 7), creating a cost of movement of any degree from small scale resident movements to large scale anadromous migration. Hopefully the empirically derived mortality from this study (40% in 2007, 55% in 2008) will inform models used to explain variation in anadromy among these watersheds. Unfortunately, there are no

undammed rivers within the Central Valley to compare with the Mokelumne to assess the effects of dams on anadromy in *O. mykiss*.

While it is unclear whether the habitat loss associated with dams diminished the diversity of movement-related life histories in this watershed, the results of this study indicate that a "richness" of life histories still exists for future restoration and management efforts to maintain and build on. With recent recognition of the importance of life history diversity to the persistence of populations in the face of unpredictable variation or directional change in environmental conditions (i.e. the portfolio effect), management to maintain or increase life history diversity of individuals within and among populations may be crucial for the persistence of Central Valley salmonids (Schindler et al. 2010; Greene et al. 2010; Moore et al. 2010; Carlson and Satterthwaite 2011).

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LITERATURE CITED

Augerot X, Foley DN (2005) Atlas of Pacific salmon: the first map-based status assessment of salmon in the North Pacific. University of California Press

Bolker BM, Brooks ME, Clark CJ, Geange SW, Poulsen JR, Stevens MHH, White J-SS (2009) Generalized linear mixed models: a practical guide for ecology and evolution. Trends in ecology & evolution 24:127–35

Carlson SM, Satterthwaite WH (2011) Weakened portfolio effect in a collapsed salmon population complex. Canadian Journal of Fisheries and Aquatic Sciences 68:1579–1589

Conomos TJ (1980) San Francisco Bay: The urbanized estuary. American Association for the Advancement of Science, San Francisco, CA

Cooke SJ, Hinch SG, Farrell AP, Patterson DA, Miller-Saunders K, Welch DW, Donaldson MR, Hanson KC, Crossin GT, Mathes MT, Lotto AG, Hruska KA, Olsson IC, Wagner GN, Thomson R, Hourston R, English KK, Larsson S, Shrimpton JM, Van der Kraak G (2008) Developing a mechanistic understanding of fish migrations by linking telemetry with physiology, behavior, genomics and experimental biology: an interdisciplinary case study on adult Fraser River sockey salmon. Fisheries 33:321–339

Dingle H, Drake VA (2007) What is migration? BioScience 57:113–121

Gerking SD (1959) The restricted movement of fish populations. Biological Reviews 34:221–242

Gowan C (2007) Short-term cues used by foraging trout in a California stream. Environmental Biology of Fishes 78:317–331

Gowan C, Fausch KD (2002) Why do foraging stream salmonids move during summer? Environmental Biology of Fishes 64:139–153

Gowan C, Young MK, Fausch KD, Riley SC (1994) Restricted movement in resident stream salmonids: a paradigm lost? Canadian Journal of Fisheries and Aquatic Sciences 51:2626–2637

Greene CM, Hall JE, Guilbault KR, Quinn TP (2010) Improved viability of populations with diverse life-history portfolios. Biology letters 6:382–386

Gross MR, Coleman RM, Mcdowall RM (1988) Aquatic productivity and the evolution of diadromous fish migration. Science 239:1291–1293

Hollibaugh JT (ed.). (1996) San Francisco Bay: the ecosystem. In: Proceedings of the San Francisco Bay Symposium, 75th Annual Meeting, Pacific Division of the American Association for the Advancement of Science, 20 to 24 June 1994. Pacific Division, American Association for the Advancement of Science, San Francisco, CA

Holyoak M, Leibold MA, Holt RD (2005) Metacommunities: Spatial dynamics and ecological communities. University of Chicago Press, Chicago

Höjesjö J, Økland F, Sundström LF, Pettersson J, Johnsson JI (2007) Movement and home range in relation to dominance; a telemetry study on brown trout Salmo trutta. Journal of Fish Biology 70:257–268

Kahler TH, Roni P, Quinn TP (2001) Summer movement and growth of juvenile anadromous salmonids in small western Washington streams. Canadian Journal of Fisheries and Aquatic Sciences 58:1947–1956

Keeley ER (2001) Demographic responses to food and space competition by juvenile steelhead trout. Ecology 82:1247–1259

Kondolf GM, Boulton AJ, Daniel SO, Poole GC, Rahel FJ, Stanley EH, Wohl E, Bång A, Carlstrom J, Cristoni C, Huber H (2006) Process-based ecological river restoration: Visualizing three-dimensional connectivity and dynamic vectors to recover lost linkages. Ecology and Society 11:5

Kurth R (2012) Migratory patterns of lower Feather River natural and hatchery-origin *Oncorhynchus mykiss*. Environmental Biology of Fishes

Lidicker WZ (1975) The role of dispersal in the demography of small mammals. In: Petrusewicz K, Goley EB, Ryszkowski L (eds) Small mammals: their productivity and population dynamics. Cambridge University Press, Cambridge, pp 103–128

Lindley ST, Schick RS, Agrawal A, Goslin M, Pearson TE, Mora E, Anderson JJ, May B, Greene S, Hanson C, Low A, Mcewan D, Macfarlane RB, Swanson C, Williams JG (2006) Historical population structure of Central Valley steelhead and its alteration by dams. San Francisco Estuary & Watershed Science 4:1–19

Lindley ST, Schick RS, Mora E, Adams PB, Anderson JJ, Greene S, Hanson C, May BP, McEwan DR, MacFarlane RB, Swanson C, Williams JG (2007) Framework for assessing viability of threatened and endangered Chinook salmon and

steelhead in the Sacramento-San Joaquin Basin. San Francisco Estuary & Watershed Science 5:1–26

Mangel M, Satterthwaite WH (2008) Combining proximate and ultimate approaches to understand life history variation in salmonids with application to fisheries, conservation, and aquaculture. Bulletin of Marine Science 83:107–130

McEwan DR (2001) Central Valley steelhead. In: Brown RL (ed) Contributions to the Biology of Central Valley Salmonids, Fish Bulletin 179(1). Scripps Institutions of Oceanography Library, San Diego, CA, pp 1–44

Meador MR, Cuffney T, Gurtz M (1993) Methods for sampling fish communities as part of the National Water-Quality Assessment Program. US Geological Survey Raleigh, North Carolina Open File Report:93–104

Mellina E, Hinch SG, MacKenzie KD, Pearson G (2005) Seasonal movement patterns of stream-dwelling rainbow trout in North-Central British Columbia, Canada. Transactions of the American Fisheries Society 134:1021–1037

Melnychuk MC, Christensen V (2009) Methods for estimating detection efficiency and tracking acoustic tags with mobile transect surveys. Journal of fish biology 75:1773–1794

Melnychuk MC, Hausch SJ (2011) Method for estimating detection probabilities of nonmigrant tagged fish: Applications for quantifying residualization rates. Transactions of the American Fisheries Society 140:1613–1628

Melnychuk MC, Welch DW, Walters CJ, Christensen V (2007) Riverine and early ocean migration and mortality patterns of juvenile steelhead trout *(Oncorhynchus mykiss)* from the Cheakamus River, British Columbia. Hydrobiologia 582:55–65

Merz JE (2001) Association of fall-run Chinook salmon redds with woody debris in the lower Mokelumne River, California. California Fish and Game 87:51–60

Merz JE (2002) Seasonal feeding habits, growth, and movement of steelhead trout in the Lower Mokelumne River, California. California Fish and Game 88:95–111

Merz JE, Moyle PB (2006) Salmon, wildlife, and wine: marine-derived nutrients in human-dominated ecosystems of central California. Ecological applications 16:999–1009

Merz JE, Ochikubo Chan LK (2005) Effects of gravel augmentation on macroinvertebrate assemblages in a regulated California River. River Research and Applications 21:61–74

Merz JE, Pasternack GB, Wheaton JM (2006) Sediment budget for salmonid spawning habitat rehabilitation in a regulated river. Geomorphology 76:207–228

Merz JE, Setka JD, Pasternack GB, Wheaton JM (2004) Predicting benefits of spawning-habitat rehabilitation to salmonid *(Oncorhynchus spp.)* fry production in a regulated California river. Canadian Journal of Fisheries and Aquatic Sciences 61:1433–1446

Moore JW, McClure M, Rogers LA, Schindler DE (2010) Synchronization and portfolio performance of threatened salmon. Conservation Letters 3:340–348

Moyle PB (2002) Inland fishes of California. University of California Press, Berkeley

Myrick CA, Cech JJ (2004) Temperature effects on juvenile anadromous salmonids in California's central valley: what don't we know? Reviews in Fish Biology and Fisheries:113–123

NMFS NMFS, NOAA NO and AA (2006) Endangered and threatened species: Final listing determinations for 10 distinct population segments of West coast steelhead; Final rule. Federal Registrar 71:1–30

Northcote TG (1992) Migration and residency in stream salmonids - some ecological consideration and evolutionary consequences. Nordic Journal of Freshwater Research 67:5–17

Pasternack GB, Wang CL, Merz JE (2004) Application of a 2D hydrodynamic model to design of reach-scale spawning gravel replenishment on the Mokelumne River, California. River Research and Applications 20:205–225

Perry RW, Skalski JR, Brandes PL, Sandstrom T, Klimley AP, Ammann A (2010) Estimating survival and migration route probabilities of juvenile Chinook salmon in the Sacramento – San Joaquin River Delta. North American Journal of Fisheries Management 30:142–156

Quinn TP (2004) The behavior and ecology of Pacific salmon and trout. University of Washington Press, Seattle

R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Del Real SC, Workman M, Merz J (2012) Migration characteristics of hatchery and natural-origin *Oncorhynchus mykiss* from the lower Mokelumne River, California. Environmental Biology of Fishes 94:363–375

Satterthwaite WH, Beakes MP, Collins EM, Swank DR, Merz JE, Titus RG, Sogard SM, Mangel M (2010) State-dependent life history models in a changing (and regulated) environment: steelhead in the California Central Valley. Evolutionary Applications 3:221–243

Schindler DE, Hilborn R, Chasco B, Boatright CP, Quinn TP, Rogers LA, Webster MS (2010) Population diversity and the portfolio effect in an exploited species. Nature 465:609–612

Semmens BX (2008) Acoustically derived fine-scale behaviors of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) associated with intertidal benthic habitats in an estuary. Canadian Journal of Fisheries and Aquatic Sciences 65:2053–2062

Shapovalov L, Taft AC (1954) The life histories of the steelhead rainbow trout *(Salmo gairdneri)* and silver salmon *(Oncorhynchus kisutch)* with special reference to Waddell Creek, California and recommendations regarding their management. California Department of Fish and Game Fish Bulletin 98:373p

Skalski JR, Lady J, Townsend R, Giorgi AE, Stevenson JR, Peven CM, McDonald RD (2001) Estimating in-river survival of migrating salmonid smolts using radiotelemetry. Canadian Journal of Fisheries and Aquatic Sciences 58:1987–1997

Sogard SM, Merz JE, Satterthwaite WH, Beakes MP, Swank DR, Collins EM, Titus RG, Mangel M (2012) Contrasts in habitat characteristics and life history patterns of *Oncorhynchus mykiss* in California's Central Coast and Central Valley. Transactions of the American Fisheries Society 141:747–760

Turchin P (1998) Quantitative Analysis of Movement. Sinauer Associates, Sunderland

Welch DW, Rechisky EL, Melnychuk MC, Porter AD, Walters CJ, Clements S, Clemens BJ, McKinley RS, Schreck C (2008) Survival of migrating salmon smolts in large rivers with and without dams. PLoS biology 6:2101–2108

Williams BK, Nichols JD, Conroy MJ (2002) Analysis and Management of Animal Populations, 1st edn. Academic Press, San Diego

Yoder JM, Marschall EA, Swanson DA (2004) The cost of dispersal: predation as a function of movement and site familiarity in ruffed grouse. Behavioral Ecology 15:469–476

Zuur AF, Ieno EN, Walker NJ, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R (M Gail, K Krickeberg, JM Samet, A Tsiatis, and W Wong, Eds.). Springer, New York

FIGURES

Figure 1 Map of the lower Mokelumne River between Camanche Dam and Woodbridge Irrigation District Dam. Stationary acoustic receivers were placed at each reach boundary (arrows). The three study reaches are numbered between each receiver. Inset shows the Sacramento River, San Joaquin River and the San Francisco Estuary. Map adapted with permission from (Merz 2002).

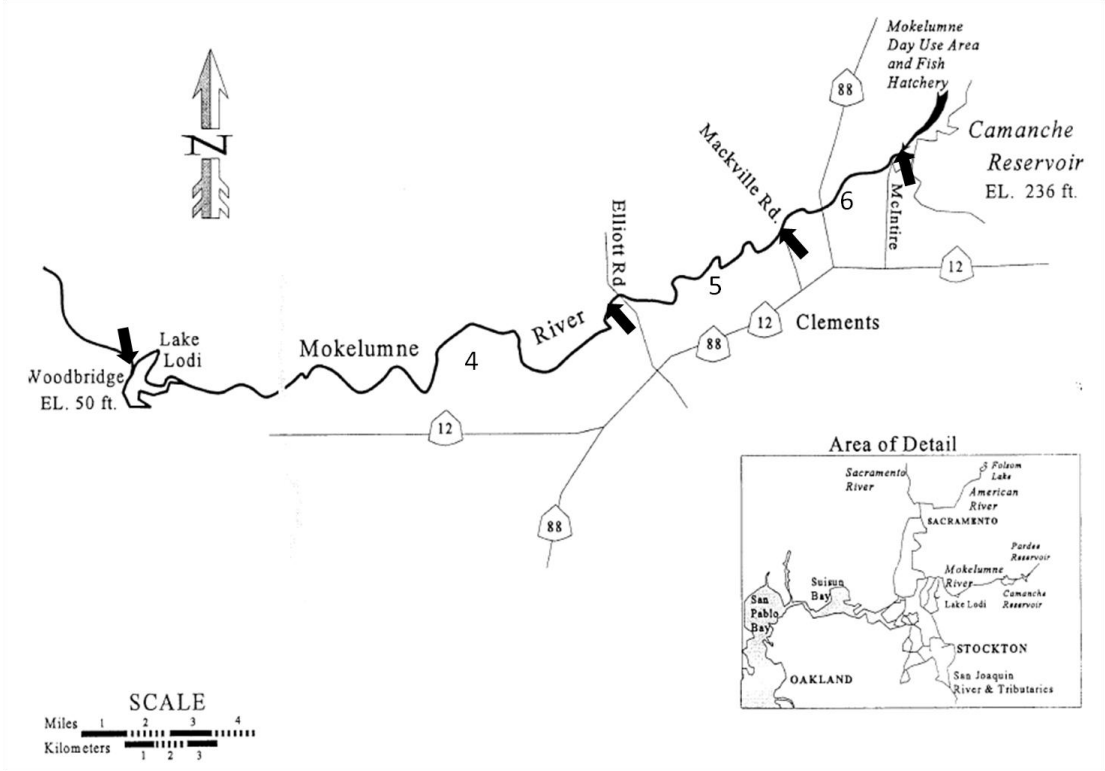


Figure 2 Movement of individual acoustically tagged resident *O. mykiss* (left y-axis; km/mo) from 2007 (star, left panel) and 2008 (circle, right panel), with positive values indicating upstream movement and negative values indicating downstream movement relative to no observed movement between surveys (horizontal line). Lines are daily discharge in cubic meters per second ($m^3 s^{-1}$; right axis) from Camanche Dam for each year. Sample size are below each survey (n=).

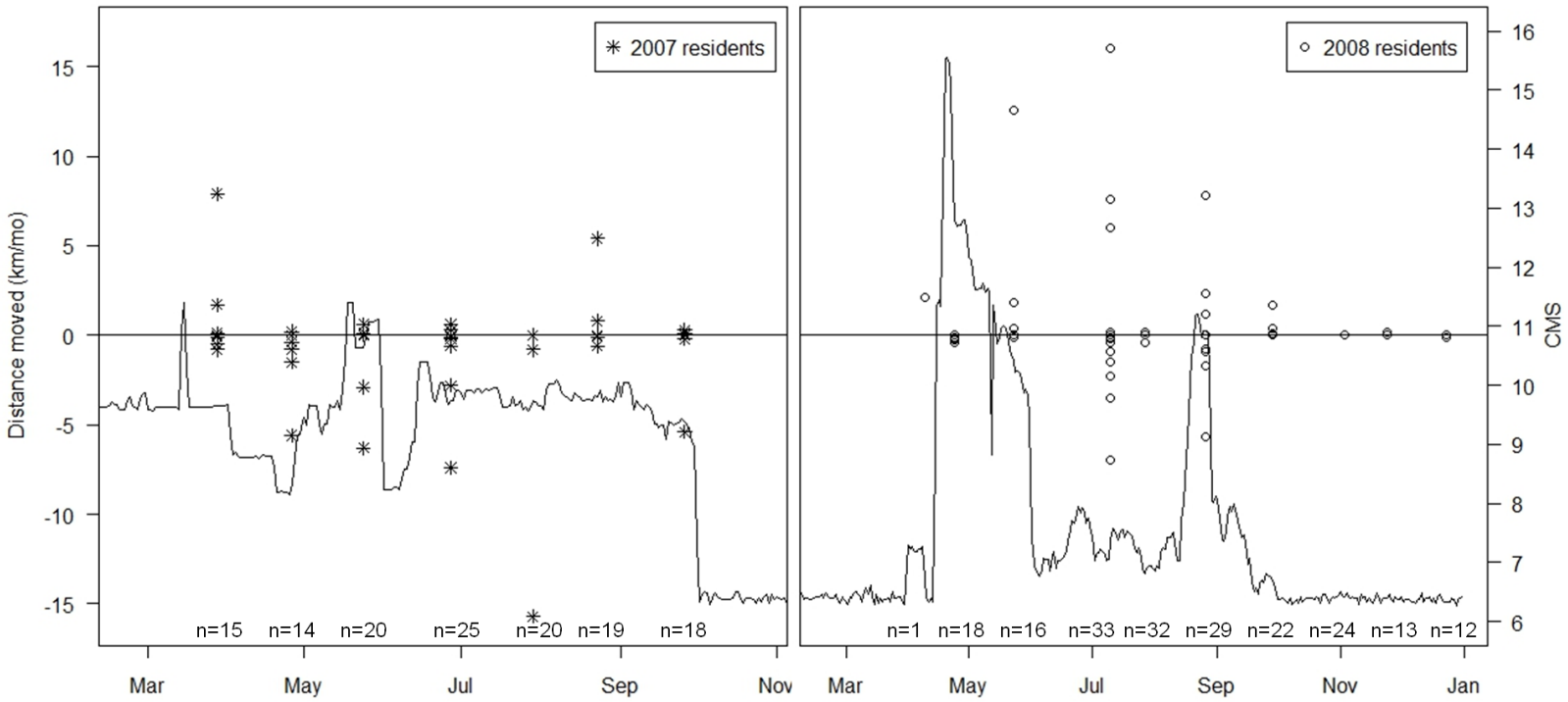


Figure 3 Movement of individual acoustically tagged emigrant *O. mykiss* (left y-axis; km/mo) from 2008 (filled circle), with positive values indicating upstream movement and negative values indicating downstream movement relative to no observed movement between sampling (horizontal line). Line is daily discharge in cubic meters per second ($m^3 s^{-1}$; right axis) from Camanche Dam for each year.

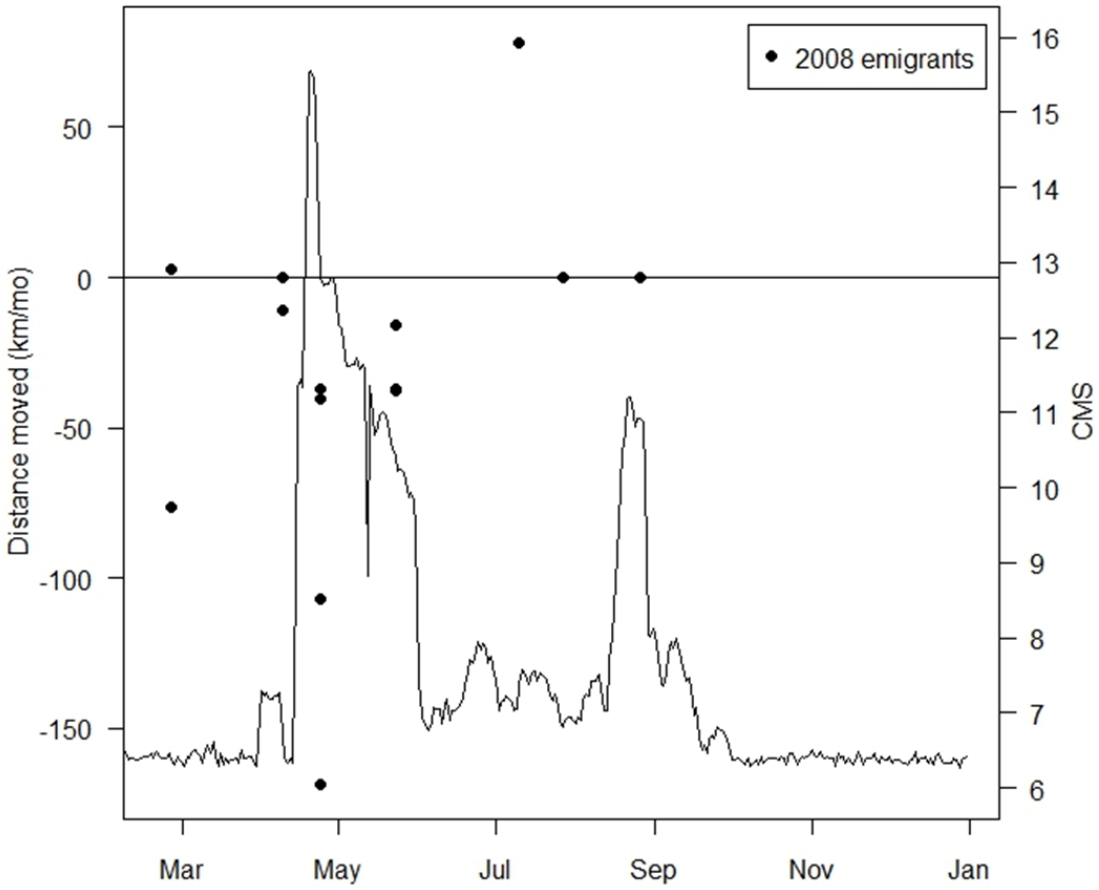


Figure 4 Standardized movement MVT (km/mo), for individual resident *O. mykiss* tagged in 2007 (stars) and 2008 (circles). The line represents the best fit relationship between size and movement, which was significant for 2008 only.

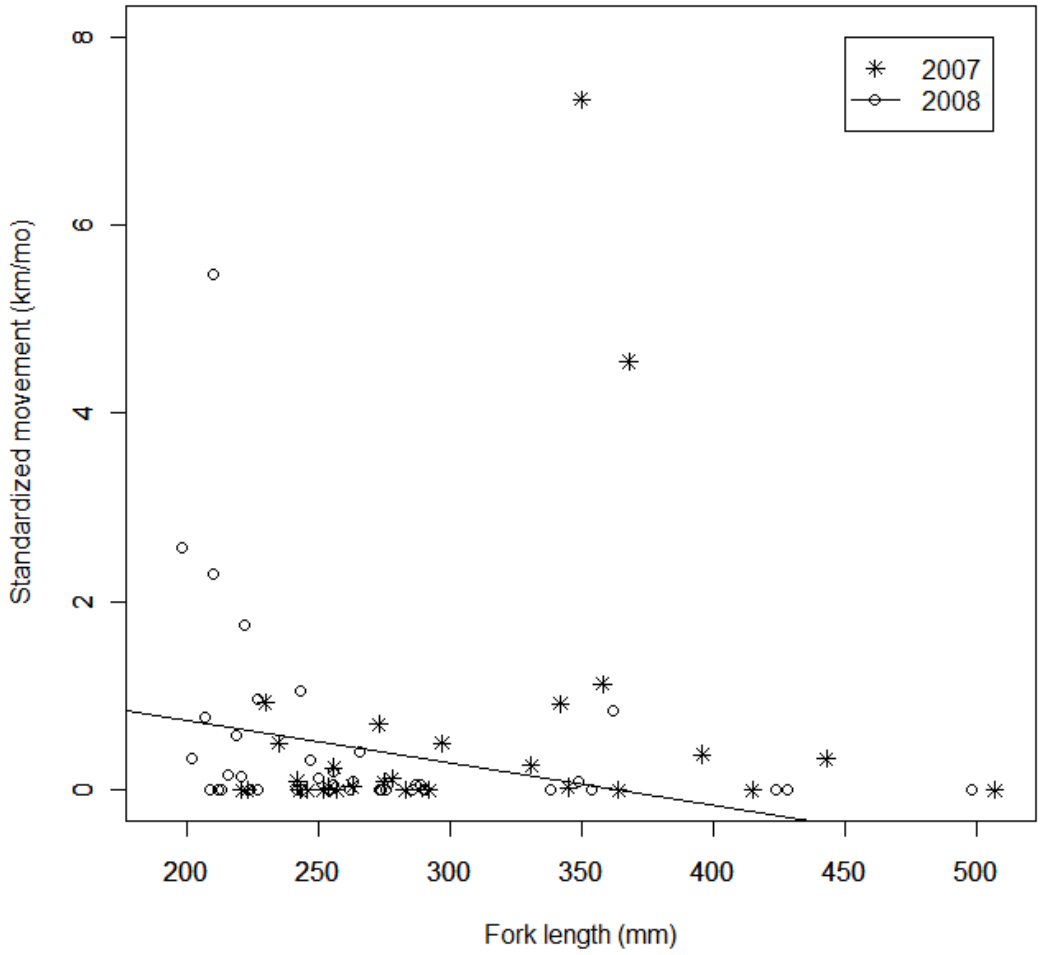


Figure 5 Standardized seasonal movement (mvts km/mo) relative to fork length (mm) and home reach for individually tagged fish in 2007. Points are individual fish coded by their home reach number. Lines are model fits for Reach 6 (solid green), Reach 5 (large dashed red), and Reach 4 (small dashed orange). Lines were plotted using the size range common to all three reaches with the upper limit at 375 mm FL (vertical dotted line).

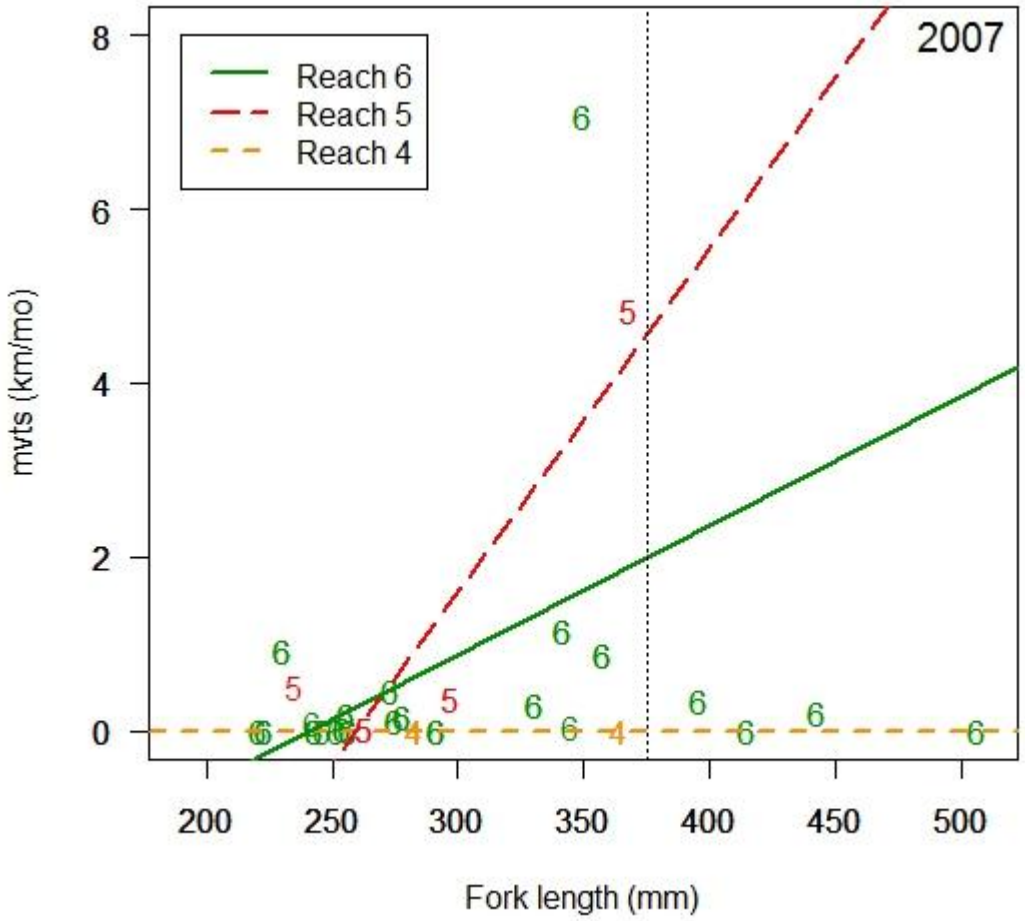


Figure 6 Standardized seasonal movement (km/mo) relative to fork length (mm) and season for individually tagged fish in 2008. Points are individual fish in spring (upward triangles), summer (circles), fall (downward triangles), and winter (stars). Lines are model fits for spring (small mixed dashed green), summer (solid red), fall (medium mixed dashed brown), and winter (large dashed blue). The season x FL interaction significantly affected seasonal standardized movement (df = 3; F = 3.12; *p* = 0.04).

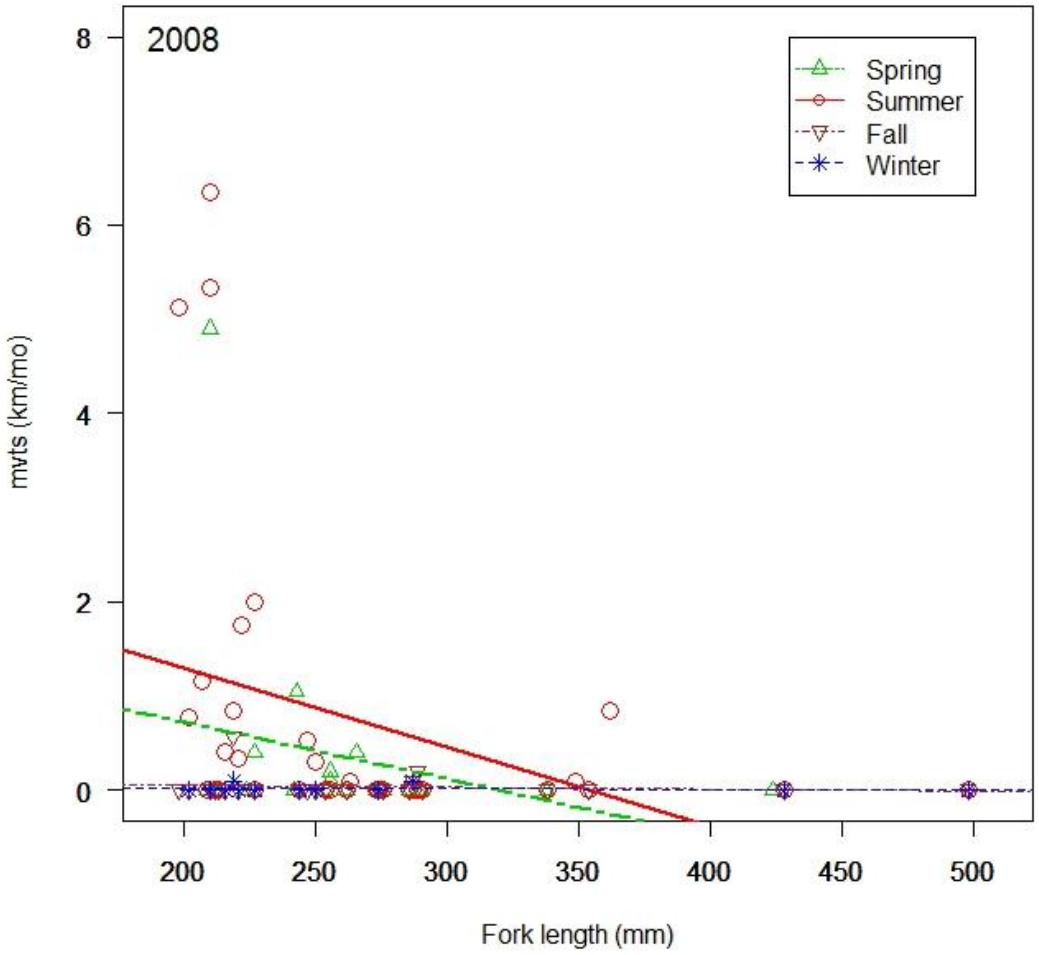
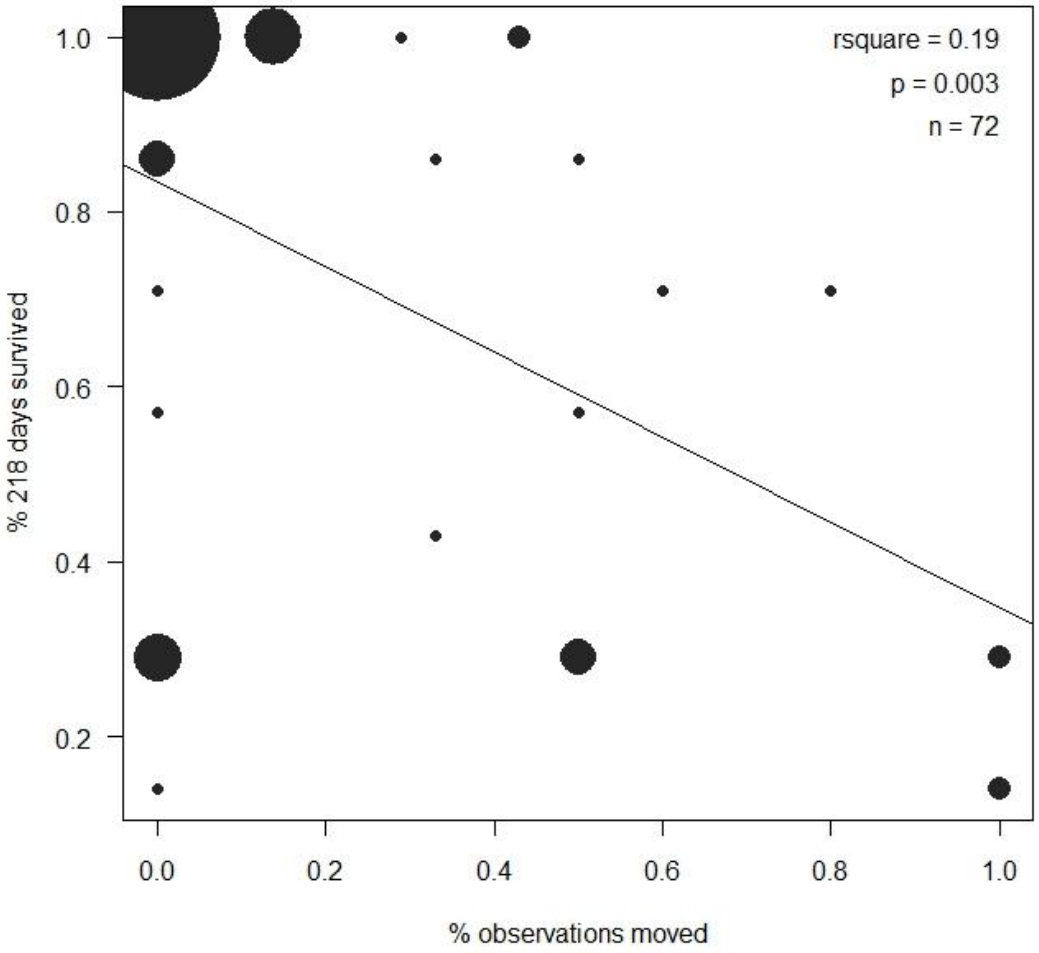


Figure 7 Standardized survival in relation to percent observations moved for tagged resident *O. mykiss* observed in 2008. Bubble size is proportional to the number of individuals for that coordinate.



CHAPTER 2

Population differences in temperature-dependent growth of coastal rainbow trout *(Oncorhynchus mykiss)*

INTRODUCTION

We are witnessing unprecedented levels of anthropogenic alteration of natural ecosystems possibly exceeding the ability of species to respond, with potential for dramatic effects on entire communities and ecosystems (Chevin et al. 2010; Steffen et al. 2011; Barnosky et al. 2012). Variation among individuals allows populations to persist through environmental change in space and time (Darwin 1859; Bolnick et al. 2011). Similarly, trait variation among populations may help species persist by dampening the greater variability of individual populations (i.e. the portfolio effect, Schindler et al. 2010; Moore et al. 2010). Thus knowledge of the magnitude and spatial patterns of variation among populations is important to understanding how species as collections of populations will respond to anthropogenic habitat alteration and climate change.

Anthropogenic alterations and climate change are particularly relevant to poikilothermic organisms within aquatic systems (Deutsch et al. 2008; Woodward 2009). Anthropogenic impacts dramatically alter nutrient dynamics, trophic interactions, and fundamental environmental qualities such as water temperature in aquatic ecosystems (Woodward 2009). Population and community dynamics are all influenced by basic temperature-dependent physiological processes such as growth

and survival for poikilothermic organisms (Deutsch et al. 2008; Reed et al. 2011). Salmonids, such as coastal rainbow trout (steelhead; *Oncorhynchus mykiss*), are faced with potentially strong selective forces from environmental variation in coming years due to climate change (Quinn 2004; Beer and Anderson 2011). Temperature effects on growth may be more important for steelhead that rear in warmer waters of summer and fall than for other salmon that may emigrate prior to these seasonally elevated temperatures (McEwan 2001). At the southern end of their range (California), steelhead populations are already at reduced numbers due to anthropogenic alterations and reductions of upwards of 80% of original freshwater habitat (McEwan 2001; Lindley et al. 2006), and are facing further expected increases in water temperatures of 2-8^{o}C, coupled with possible declines in flows in the next 100 years (Lindley et al. 2007). However, alteration in environmental conditions from climate change are predicted to vary across the heterogeneous landscape of this region (Lindley et al. 2007; Cayan et al. 2008; Null et al. 2010). Therefore, knowledge of the range of variation in temperature-dependent growth among populations will allow predictions of how populations and the species as a whole are likely to respond to changing water temperatures. Such information has valuable applications for spatial approaches of management for the recovery of imperiled populations.

Strong spawning site fidelity coupled with local scale habitat variability creates the potential for local adaptation among *O. mykiss* populations (Nielsen 1999; Quinn 2004). Currently *O. mykiss* populations with access to the ocean within the United States are managed by NOAA Fisheries under the Endangered Species Act

(ESA) as a collection of Distinct Population Segments (DPS, Figure 1; USFWS and NMFS 1996), where each DPS is discrete and significant to the overall species. Unfortunately, important population specific parameters such as temperature-dependent growth are lacking (Myrick and Cech 2004). What little information that exists comes from more northerly populations that likely encounter colder temperatures than those of more southerly populations (Myrick and Cech 2004). Currently, eleven of the fifteen *O. mykiss* DPSs are listed as either threatened or endangered under the ESA, creating a further urgency for investigating population differences in temperature-dependent growth. Therefore, information specific to each DPS could be critical to recovery and management efforts (Myrick and Cech 2004).

How temperature affects growth of juvenile *O. mykiss* is important to population dynamics for several reasons. First, there is increasing evidence that growth rates of juveniles determine life history trajectories in *O. mykiss*, i.e. whether individuals complete their life cycle in freshwater as "resident rainbow trout," or instead after rearing in freshwater undergo "anadromous" migrations to sea to attain larger adult sizes before returning to their natal freshwaters to spawn. Faster growth rates may allow individuals to adopt an anadromous life history earlier, thereby avoiding prolonged freshwater mortality risk, while individuals that experience extremely high or depressed growth rates are more likely to adopt a resident life history (Mangel and Satterthwaite 2008; Satterthwaite et al. 2009, 2010). Furthermore, several studies have shown that the size of individual steelhead upon ocean entry determines the probability of survival at sea and returning to spawn

(Ward et al. 1989; Hayes et al. 2008, 2011; Bond et al. 2008). Thus, growth of juvenile steelhead prior to ocean entry confers adult survival and the likelihood to reproduce (Hayes et al. 2011). Finally, like other fishes, *O. mykiss* exhibit size-based fecundity with larger fish having higher fecundity (Shapovalov and Taft 1954; Bulkley 1967; Bromage et al. 1990). Therefore, how juvenile growth is affected by temperature may have both ecological and fitness consequences (Deutsch et al. 2008; Reed, et al. 2011).

It is unclear whether the history of exposure to different water temperatures through time affects temperature-dependent growth and the associated consequences. For a species like *O. mykiss* with complex life histories there are several ways an individual may experience different water temperatures through its lifetime. Resident individuals may experience temperature differences among habitats within their home range. *O. mykiss* may also experience varying water temperatures within one habitat through time, especially if that habitat becomes isolated due to stream reaches drying in low flow scenarios, an event that is more common for southerly populations (Boughton et al. 2007; Bell et al. 2011). In contrast, anadromous individuals may experience different water temperatures as they migrate from their headwater rearing habitats through higher order stream reaches and estuary habitats en route to the ocean (MacFarlane and Norton 2002; Myrick and Cech 2004).

In this study, I investigate two major questions: 1) how do temperature-dependent growth and survival vary among populations of *O. mykiss*; and 2) do temperatures previously experienced by individuals affect their growth and survival at

current temperatures, and does this effect vary among populations? To answer these questions, I conducted controlled laboratory experiments using *O. mykiss* from three different populations among two different DPSs. I then compared my results with previous studies from two more populations, including one from an additional more northerly DPS, to further examine within and among DPS population differences in temperature-dependent growth across a latitudinal gradient for *O. mykiss*. This study thereby: i) tested the hypothesis that more southerly populations had higher mean growth rates at higher temperatures, and ii) compared growth rates among populations at higher temperatures to better understand potential variation in population responses to future increases in water temperature due to climate change.

METHODS

Source populations

I used three different populations in my experiments. Young-of-the-year *O. mykiss* fry were obtained from (1) Battle Creek, a tributary to the Sacramento River, within the Central Valley, California DPS, and (2) Scott Creek and (3) the San Lorenzo River, both of which flow directly into the Monterey Bay within the Central California Coast DPS (Figure 1). The Battle Creek population was provided by the Coleman National Fish Hatchery, and the Scott Creek and San Lorenzo River populations were from wild parents of these streams provided by the Monterey Bay Salmon and Trout Project Conservation Hatchery (for more information on these hatcheries and populations see Beakes et al. 2010). Battle Creek is a snow fed,

regulated watershed representative of other northerly watersheds in California's Central Valley. While water temperatures are typically 14^{o}C, steelhead from this stream must migrate through the Sacramento River and San Francisco Estuary both of which may have water temperatures up to 21^{o}C (MacFarlane and Norton 2002; Myrick and Cech 2004; Lindley et al. 2007). Scott Creek and the San Lorenzo River are representative of rain fed coastal California watersheds with variable episodic flows and water temperatures that vary through space and time (7-24^{o}C). The San Lorenzo River watershed has several small dams and diversions and is large for a coastal watershed, whereas Scott Creek is smaller, relatively unregulated and also has a long history of *O. mykiss* research (e.g. Shapovalov and Taft 1954; Bond et al. 2007, 2008; Hayes, Bond, Hanson, Freund, Ellen, et al. 2008; Hayes et al. 2011; Beakes et al. 2010; Moore et al. 2011; Sogard et al. 2012). Thus, results from a common garden experiment using these watersheds can provide insight into local adaptation of different populations to dissimilar environmental conditions (Garland Jr. and Adolph 1991; Beakes et al. 2010; Primmer 2011).

Experimental Design

All experiments were conducted at the NOAA Southwest Fisheries Science Center, Santa Cruz, California. The experimental facility consisted of three separate banks of tanks, each with its own water reservoir to maintain constant water temperatures. Within each of these three banks were six cylindrical black tanks (43cm diameter, 110L) that were continually fed recirculating oxygenated water from their specific reservoir. This allowed me to test two populations simultaneously with three

replicate tanks per population per temperature bank. Thus, all experiments were run in two trials (May-August 2008; August-October 2008) due to available facilities and the timing of available fry. I used a "semi-random" nested design in which six individuals from each population were randomly allocated to each of three tanks within each temperature level (14°C, 20°C, and 24°C). Fish were then sorted to achieve similar distributions of individual wet weights among all tanks (Table 1). Populations were maintained in separate tanks to avoid artifacts such as inter-population aggression or dominance that may have otherwise confounded population specific temperature-dependent effects on growth (Imre et al. 2004). Each fish was individually marked with an elastomer tag (Pacific Northwest Technology) to track individual growth in response to experimental manipulations. Tanks were cleaned and water quality, including temperature and oxygen levels, was checked daily.

The facilities available for this study allowed the testing of three treatment temperatures, 14°C, 20°C, and 24°C. It has historically been thought that 14°C was an intermediate or optimal temperature for growth (Wurtsbaugh and Davis 1977; McEwan 2001), and that 20°C was an elevated temperature, at or beyond the apex of the temperature-growth curve for *O. mykiss* (Wurtsbaugh and Davis 1977; McEwan 2001; Myrick and Cech 2004). Therefore treatment temperatures used in this study test for differences among populations in the mean growth rate at an "intermediate" (14°C), an "elevated temperature" (20°C), and a temperature higher than has previously been tested (24°C). Each of these three treatment temperatures are within the range of temperatures that the three source populations naturally experience

(Lindley et al. 2007; Herbert et al. 2009; Hayes et al. 2011). Each experiment was conducted for approximately two weeks. Two weeks has been shown to be sufficient time for salmonid populations to show differences in temperature-dependent growth rates (Geist et al. 2011).

It is understood that for poikilothermic organisms such as *O. mykiss,* growth rate will increase with temperature to a thermal-growth optimum and then drop precipitously, but that increased growth depends upon ample food to sustain elevated metabolic rates at warmer temperatures (Brett et al. 1969; Wurtsbaugh and Davis 1977; Hoar et al. 1979; Sumpter 1992). Wurtsbaugh and Davis (1977) found this relationship to be true for *O. mykiss* under *ad libitum* feeding, but that under lower rations growth rate was lower at elevated temperatures. By feeding fish *ad libitum* this study focuses on the *potential* maximum growth rates at different temperatures among *O. mykiss* populations.

Experimental tests

To test for population differences in *O. mykiss* temperature-dependent growth, and to investigate if there was any effect of previous temperatures experienced on these patterns, I used the same individuals in a series of three different phases of experiments, which I will refer to as "Experiments 1, 2 and 3." Experiment 1 was designed to test for population differences in temperature-dependent rates of growth and survival. Experiment 2 examined how the effects of temperature-dependent growth experienced in Experiment 1 (14^{o}C, 20^{o}C, and 24^{o}C) affected subsequent growth at a "benign" temperature (14^{o}C; Sumpter 1992; Myrick and Cech 2004).

Experiment 3 tested for effects of previous temperature levels experienced in Experiment 1 (14^oC, 20^oC, and 24^oC) on juvenile *O. mykiss* growth and survival at three different treatment temperatures (14^oC, 20^oC, and 24^oC).

Experiment 1: Population differences in temperature-dependent rates of growth and survival

To test for population differences in temperature-dependent growth I measured growth and survival of individual *O. mykiss* exposed to one of three temperatures (14^oC, 20^oC, and 24^oC) for each population. Fish from Battle Creek and the San Lorenzo River were transported to the lab on the fourth week of May 2008, and acclimated to continual flowing oxygenated water at approximately 14^oC for 14 days. I then raised water temperatures in treatment blocks $1^oC\ day^{-1}$ (Myrick and Cech 2005). Once all treatment temperatures (14^oC, 20^oC, and 24^oC) were reached, I measured the initial wet weight of all fish (Table 1). Fish were reared under treatment temperatures for 16 days and then measured again in early July at the end of the experiment. Scott Creek *O. mykiss* fry were available in mid-August 2008 and were subjected to the same procedures described above, with initial measurements taken in early September (Table 1) and final wet weight and survival measures recorded 16 days later.

I tested population and temperature effects on survival using generalized linear models with an assumed underlying Poisson distribution and logit link function (JMP statistical software). I examined *Survival* as a function of *Population* and *Temperature* and the interaction between *Population* and *Temperature*.

Population and *Temperature* were modeled as fixed factors and tanks were used as replicates (n= 3) for each *Temperature* and *Population* combination. Data were tested for normality and homogeneity of variance before all statistical analyses.

The growth metric used in poikilothermic organisms may affect the results of growth experiments (Sigourney et al. 2008). Because growth in salmonids has been shown to be size-dependent (Elliott et al. 1995; Elliott and Hurley 1995, 1997; Vøllestad et al. 2002; Sigourney et al. 2008; Sogard et al. 2009), I used standardized mass-specific growth rate (Ostrovsky 1995):

$$Growth\ rate = \frac{(w_t^b - w_0^b)}{bt},$$

where *w* is mass at times *t* and 0, *t* is the duration of the growth experiment and *b* is the allometric growth rate exponent (Elliott and Hurley 1995; Sigourney et al. 2008)**.** The allometric growth rate exponent can be estimated empirically by performing linear regressions of *ln(Growth)* and *ln(W)* across a range of sizes and estimating the slope coefficient (Elliott and Hurley 1995; Sigourney et al. 2008). Several studies have shown the allometric growth rate exponent to be approximately 0.31 for salmonids (Elliott et al. 1995; Elliott and Hurley 1995, 1997; Sigourney et al. 2008) and so like other studies (Vøllestad et al. 2002; Quinn et al. 2004; Grader and Letcher 2006), I used a value of $b = 0.31$. The use of standardized mass-specific growth rate also allowed the comparison of my results to previous studies of temperature-dependent growth in *O. mykiss,* which presented growth in weight (Wurtsbaugh and Davis 1977; Myrick and Cech 2005).

Population and temperature effects on growth were analyzed only on survivors of the 16-day experiments using general linear models (Systat Software). There were no differences in mean initial wet weights detected between temperature levels and populations (df = 4, F = 0.25, $p = 0.9$), or by temperature treatment alone (df = 2, F = 1.58, $p = 0.2$), but, mean initial wet weight did vary among populations (df = 2, F = 8.5, $p = 0.0002$; Table 1). However, standardized mass-specific growth provides a growth rate independent of initial wet weight, by explicitly accounting for the change in growth rate with mass (Elliott and Hurley 1995; Sigourney et al. 2008). Using a general linear model, I investigated standardized mass-specific growth rate (here after referred to as growth) as a function of *Population* and *Temperature* as fixed factors, the interaction of *Population* x *Temperature*, and tank nested within the interaction of *Population* x *Temperature* (Systat Software). The tank effect was not significant ($p = 0.50$) allowing me to remove tank and re-run the reduced model using individuals as replicates (Quinn and Keough 2002) and facilitating the resorting of individuals for Experiment 3. I further investigated population specific patterns of temperature-dependent growth rates using post-hoc specified hypothesis tests (Systat Software). I tested for differences in mean growth rate among populations at each temperature level, as well as differences in mean growth rates among the temperature levels for each population, resulting in 18 specific hypothesis tests. Thus, to control for family-wise Type I error I used a Bonferroni corrected alpha (0.05 / 18) for these specific post-hoc hypothesis tests of $\alpha = 0.0028$ (Quinn and Keough 2002).

To further investigate population differences in temperature-dependent growth I compared my results with those from previous studies of juvenile *O. mykiss* from the Santiam River, OR, Upper Willamette River DPS (Wurtsbaugh and Davis 1977) and Nimbus State Fish Hatchery on the American River, CA, Central Valley DPS (Myrick and Cech 2005). All three studies had similar starting distributions of individual *O. mykiss* wet weights (Table 1), and only fish fed ad libitum were used for the comparison. I calculated standardized mass-specific growth rates using mean starting mass, mean ending mass and duration of growth published in each report (Wurtsbaugh and Davis 1977; Myrick and Cech 2005). I then plotted standardized mass-specific growth in response to temperature levels for all five populations for visual comparison.

Experiment 2: Effects of previous temperature exposure on growth and survival at 14^{o}C

I tested for effects of temperatures previously experienced (Experiment 1; 14^{o}C, 20^{o}C, and 24^{o}C) on growth and survival of *O. mykiss* reared at the benign temperature of 14^{o}C (Sumpter 1992; Myrick and Cech 2004). At the termination of Experiment 1, I decreased water temperature in all tanks to 14^{o}C. Temperatures remained at 14^{o}C for 14 days after which I measured all fish again. Similar to the analyses of Experiment 1, I used generalized linear models with an assumed underlying Poisson distribution and logit link function in (JMP statistical software) to test for the effects of previous temperature experienced on survival. *Survival* was analyzed in response to *Population*, and $Temperature_1$ (temperature in Experiment 1:

14^{o}C, 20^{o}C, or 24^{o}C) as fixed factors, and their interaction (i.e. *Population x Temperature$_1$*).

To test for effects of previous temperature on growth, I used full factorial general linear models investigating *Growth* in response to *Population and Temperature$_1$* as fixed factors, and their interaction *Population x Temperature$_1$* (Systat Software).

For each analysis I used an $\alpha = 0.25$ to independently drop non-significant interaction terms and re-run the reduced model with pooled sources (Quinn and Keough 2002).

Experiment 3: Effects of previous temperature exposure on temperature-dependent rates of growth and survival

I expanded upon Experiment 2 to investigate the effects of thermal histories on survival and growth rates across the full range of treatment temperatures. At the end of Experiment 2, in which all individuals had acclimated to 14^{o}C, I randomly reassigned each fish into a new tank so that each tank contained two fish from each temperature treatment from Experiment 1: 14^{o}C, 20^{o}C, 24^{o}C. Again populations were maintained in separate tanks and fish were sorted to attain as similar distributions of wet weights among tanks and treatments as possible (Table 2). I then raised bank temperatures in the new treatment blocks 1^{o}Cday^{-1} (Myrick and Cech 2005) to achieve treatment temperatures of 14^{o}C, 20^{o}C, and 24^{o}C. This resulted in three tanks per population per temperature level, each with two fish per previous temperature for a total of six fish per tank (actual numbers potentially varied due to previous

mortality, however, efforts were made to maximize consistency among treatments; Table 2). Fish were maintained at the treatment temperature for 14 days and then measured. The experiment was conducted in July/August for Battle Creek and San Lorenzo River *O. mykiss* and in October for Scott Creek fish. To test for the effects of previous temperature experience on survival, I assumed an underlying Poisson distribution and used a logit link function in generalized linear models (JMP statistical software). I tested *Survival* in response to *Population*, $Temperature_3$ (temperature in Experiment 3: 14°C, 20°C, or 24°C), and $Temperature_1$ (temperature in Experiment 1: 14°C, 20°C, or 24°C) as fixed factors, and all interactions.

I used full factorial general linear models to investigate *Growth* in response to *Population*, $Temperature_3$ (14°C, 20°C, or 24°C) and $Temperature_1$ (14°C, 20°C, or 24°C) as fixed factors, all two-way and three way interactions (Systat Software).

Again, for each analysis I independently dropped non-significant interaction terms ($\alpha = 0.25$) consecutively and re-ran each reduced model with pooled sources (Quinn and Keough 2002).

Comparison of Experiment 1 to Experiment 3: Population level temperature-dependent growth rates

To compare temperature-dependent growth rates between Experiment 1 and 3 I used a general linear model to investigate growth response at each treatment temperature 14°C, 20°C, or 24°C, with each population–by-experiment combination and the interaction with temperature as fixed factors. I used post-hoc specified hypothesis tests to compare temperature-specific mean growth rates for each

population from Experiment 3. Further, I used post-hoc specified hypothesis tests to compare temperature-specific mean growth rates for each population between Experiments 1 and 3. This resulted in 15 specific hypothesis tests for an adjusted Bonferroni alpha (0.05/15) of $\alpha = 0.0033$ to control for family-wise Type I error (Quinn and Keough 2002).

RESULTS

Experiment 1: Population differences in temperature-dependent rates of survival

All fish from Battle Creek and the San Lorenzo River survived Experiment 1. Of Scott Creek fish, one died at 14^{o}C, and two died at 24^{o}C. I did not detect an effect of *Population* or *Temperature* or any interaction between *Population* and *Temperature* on *Survival* of *O. mykiss* in Experiment 1 ($\alpha = 0.05$).

Experiment 1: Population differences in temperature-dependent rates of growth

A significant *Population* x *Temperature* interaction (df = 4, F = 6.05, p = 0.0002) indicated population differences in temperature-dependent growth patterns (Figure 2). I then decomposed this interaction using post-hoc specified hypothesis tests with a Bonferroni corrected $\alpha = 0.0028$ (Quinn and Keough 2002).

Growth rate varied across the three temperature levels and this relationship varied among populations (Figure 2). At 14^{o}C Battle Creek fish had the highest mean

growth rate of 0.77 ± 0.04 g · day^{-1}, which was significantly higher than either San Lorenzo River (0.45 ± 0.04 g · day^{-1}) or Scott Creek fish growth rates (0.32 ± 0.04 g · day^{-1}), which were not significantly different from each other (p = 0.025 vs. Bonferroni corrected α = 0.0028). At 20^{o}C, none of the three populations differed in mean growth rate (p > 0.10 for each comparison): Battle Creek = 0.67 +/- 0.04 g · day^{-1}, San Lorenzo River = 0.57 +/- 0.04 g · day^{-1}, and Scott Creek = 0.57 +/- 0.05 g · day^{-1}. Similarly, mean growth rates did not differ among populations at 24^{o}C (p > 0.03 vs. Bonferroni corrected α = 0.0028 for each comparison): Battle Creek = 0.37 ± 0.04 g · day^{-1}, Scott Creek = 0.28 +/- 0.05 g · day^{-1}, and San Lorenzo River = 0.25 +/- 0.04 g · day^{-1}.

The temperature level at which each population experienced highest mean growth rate differed among populations (Figure 2). For example, Battle Creek growth rates at 14^{o}C (0.77 ± 0.04 g · day^{-1}) and 20 ^{o}C (0.67 +/- 0.04 g · day^{-1}) were not different from each other (p = 0.06 vs. Bonferroni corrected α = 0.0028), but both were markedly higher than the mean growth rate at 24 ^{o}C (0.37 ± 0.04 g · day^{-1}; p < 0.0001 each). Similarly, the mean growth rates of San Lorenzo River fish were not different (p = 0.0037 vs. Bonferroni corrected α = 0.0028) between 14^{o}C (0.45 ± 0.04 g · day^{-1}) and 20^{o}C (0.57 ± 0.04 g · day^{-1}) but were both higher than the mean growth rate at 24^{o}C (0.25 ± 0.04 g · day^{-1}; p < 0.0005 each). In contrast, the highest mean growth rate for Scott Creek fish at 20^{o}C (0.56 +/- 0.05 g · day^{-1}), was significantly higher than that at 14^{o}C (0.32 ± 0.04 g · day^{-1}) and 24^{o}C (0.28 ± 0.05 g · day^{-1}; p < 0.0002 each), which did not differ from each other (p = 0.62).

A visual comparison of these results to those of previous studies shows further evidence of population differences in temperature-dependent growth rates (Figure 2). Wurtsbaugh and Davis (1977) found the growth rate of Santiam River *O. mykiss* to increase with increasing water temperatures from 9.4^{o}C to 13.3^{o}C, after which growth rates decreased with increasing water temperatures to 22.5^{o}C (Figure 2). Myrick and Cech (2005) measured increased growth rate in American River *O. mykiss* from 11^{o}C to 19^{o}C temperatures (Figure 2). By contrast, growth rates were high at 14^{o} and 20^{o}C and declined towards 24^{o}C for Battle Creek (this study), providing evidence for differences in temperature-dependent growth between populations within the same DPS (Figure 2). When comparing all five populations, a general pattern emerges of higher mean growth rates at colder temperatures for more northerly populations and at warmer temperatures for more southerly populations (Figures 1 and 2: Santiam River versus San Lorenzo and Scott Creek and Battle Creek versus American River). However, the general pattern is not always consistent (American River versus San Lorenzo and Scott Creek) and possibly obscured by the lack of intervals of temperatures to characterize each population's full relationship between temperature and growth.

Experiment 2: Effects of previous temperature exposure on rates of survival at 14^{o}C

In Experiment 2 all San Lorenzo River fish survived, one Battle Creek fish died from the 14^{o}C treatment of Experiment 1, and four Scott Creek fish died from the 14^{o}C treatment of Experiment 1. No significant relationships were detected

between *Survival* and *Population*, *Temperature*$_1$, or *Population x Temperature*$_1$ (α = 0.05).

Experiment 2: Effects of previous temperature exposure on rates of growth at 14^{o}C

I found no effect of previous temperature exposure on the mean growth rate of individuals at 14^{o}C (p = 0.68). At 14^{o}C, mean growth rates ± SE of fish that had previously experienced 14^{o}C, 20^{o}C, and 24^{o}C were 0.52 ± 0.06 g · day^{-1}, 0.52 ± 0.06 g · day^{-1}, and 0.48 ± 0.06 g · day^{-1}, respectively for the Battle Creek population, 0.32 ± 0.07 g · day^{-1}, 0.44 ± 0.06 g · day^{-1}, and 0.38 ± 0.07 g · day^{-1} respectively for the Scott Creek population, and 0.21 ± 0.06 g · day^{-1}, 0.21 ± 0.06 g · day^{-1}, and 0.18 ± 0.06 g · day^{-1} for the San Lorenzo River population. Although the mean of growth rates at 14^{o}C from all three previous temperatures differed among the three populations (Battle Creek = 0.51 ± 0.03 g · day^{-1}, Scott Creek = 0.38 ± 0.04 g · day^{-1}, and San Lorenzo = 0.20 ± 0.03 g · day^{-1}; df = 2, F = 2,402.3, p < 0.0001), there was no interaction (p = 0.90) between *Population and Temperature*$_1$ on *Growth* at 14^{o}C.

Experiment 3: Effects of previous temperature exposure on temperature-dependent rates of survival across the range of treatment temperatures

During Experiment 3, fish from Battle Creek suffered no mortality. Two San Lorenzo River fish died at *Temperature*$_3$ of 24^{o}C: one was from *Temperature*$_1$ of 20^{o}C, and another from *Temperature*$_1$ of 24^{o}C. Four Scott Creek fish died. Of two

that died at $Temperature_3$ of 24°C, one was from $Temperature_1$ of 14°C, and the other was from $Temperature_1$ of 20°C. The third Scott Creek mortality was at 14°C in Experiment 1 and 3, and the fourth was at 20°C in Experiment 1 and 3. Scott Creek had very poor replication among treatments because of the high mortality experienced both during and between all experiments (Table 2). Therefore, I analyzed *Survival* data from Experiment 3 for Battle Creek and the San Lorenzo River combined and Scott Creek alone. I found no effect ($\alpha = 0.05$) of *Population*, $Temperature_3$, $Temperature_1$, or any interactions on *Survival* in Battle Creek and San Lorenzo River fish together or Scott Creek fish alone.

Experiment 3: Effects of previous temperature exposure on temperature-dependent rates of growth across the range of treatment temperatures

Due to significant mortality of fish from Scott Creek among all treatments, I tested for the cumulative effects of temperature exposure in Experiments 1 and 2 on growth performance across the range of temperatures in Experiment 3 (14°C, 20°C, 24°C) using only *O. mykiss* from Battle Creek and the San Lorenzo River.

I first tested for significant interactions between populations and temperatures in Experiments 1 and 3 (i.e. *Population x* $Temperature_3$ *x* $Temperature_1$, *Population x* $Temperature_3$, *Population x* $Temperature_1$, and $Temperature_3$ *x* $Temperature_1$; $\alpha = 0.05$), dropped each non-significant interaction independently ($\alpha = 0.25$; Quinn and Keough 2002) and consecutively re-analyzed the model. The final model (*Growth = Population* + $Temperature_1$ + $Temperature_3$ + *Population x* $Temperature_3$ +

Temperature$_3$ x Temperature$_1$) did not detect any effect of prior history of temperature experience on growth performance across the range of temperatures tested (14^{o}C, 20^{o}C, 24^{o}C; $p = 0.24$; Table 3). The interaction between current temperature and previous temperature was not significant ($p = 0.07$). Similar to results from Experiment 1, effects of population ($p < 0.0001$), current water temperature ($p < 0.0001$) and their interaction ($p = 0.009$) each significantly affected growth rate (Table 3, Figure 3).

Comparison of Experiment 1 to Experiment 3: Consistent patterns of population level temperature-dependent growth rates

With no significant effects of previous temperature experienced on temperature-dependent growth rates, for each population I pooled growth rates of individuals from each previous temperature to compare mean growth rates of each temperature in Experiment 3 to mean growth rates at those same temperatures in Experiment 1 (Figure 3). I used specified post-hoc hypothesis tests and a Bonferroni corrected $\alpha = 0.0033$ to test for differences in mean growth rates across the three temperature levels between Experiments 1 and 3 for each of the two populations. Overall patterns of temperature-dependent growth were consistent between Experiment 1 and Experiment 3 for both Battle Creek and San Lorenzo River fish. For example, Battle Creek fish in Experiment 3 exhibited the same pattern as observed in Experiment 1, with mean growth rates that were not significantly different between 14^{o}C (0.79 ± 0.04 g $\cdot$ day^{-1}) and 20^{o}C (0.95 ± 0.04 g $\cdot$ day^{-1}; $p = 0.007$ vs. Bonferroni corrected $\alpha = 0.0033$), but each of these growth rates were

significantly higher than growth rates at 24°C (0.25 ± 0.04 g · day^{-1}; $p < 0.0001$ for each). Temperature-dependent growth rates of San Lorenzo River fish were also similar in Experiment 3 to those of Experiment 1. In Experiment 3, mean growth rates for San Lorenzo River fish did not differ between 14°C (0.41 ± 0.04 g · day^{-1}) and 20°C (0.46 ± 0.04 g · day^{-1}; $p = 0.45$), but each of these rates were higher than the mean growth rate at 24°C (0.06 ± 0.05 g · day^{-1}; $p < 0.0001$ for each). Mean growth rates at 14°C were the same within each population between Experiments 1 and 3, but significantly higher in Battle Creek than the San Lorenzo population ($\alpha = 0.0033$). At 20°C, the mean growth rate of San Lorenzo River fish did not differ between Experiments 1 and 3 ($p = 0.06$), but the mean growth rate of Battle Creek fish in Experiment 3 was higher than in Experiment 1 at that temperature ($p = 0.000001$). There were no significant differences in mean growth rates among populations or experiments at 24°C ($\alpha = 0.0033$).

DISCUSSION

Differences in temperature-dependent growth among populations

Using a series of three controlled laboratory experiments, I found differences in the relationship between temperature and growth among populations of *O. mykiss*. Although the complete temperature-growth curve still remains unclear for each population, the complex interaction between population and temperature-dependent growth illuminates how populations vary in their growth at different ambient water temperatures. For example, the relationship between mean growth rate at 14°C and

20°C varied among the three populations tested in my experiments. While there was no difference in growth rates between 14°C and 20°C for either Battle Creek or the San Lorenzo River fish, mean growth rates for Scott Creek *O. mykiss* were significantly highest at 20°C, but statistically indifferent between 14°C and 24°C (Figure 2). However, mean growth rates at 20°C were statistically indistinguishable among the three populations tested in Experiment 1 (Figure 2). This indicates that relative to the other two temperature levels tested, all three populations have the potential for moderate growth at 20°C, previously considered at or beyond upper limits (Wurtsbaugh and Davis 1977; McEwan 2001; Myrick and Cech 2004) given sufficient food supply. Furthermore, mean growth rates declined by a similar degree from 20°C to 24°C for all three populations tested here (Figure 2). However, all three populations expressed positive growth, with no increased mortality at 24°C. Thus, California *O. mykiss* have the capacity to grow and survive at warmer water temperatures. In fact, I found no temperature related mortality, but the variation in temperature dependent growth observed here highlights the importance of sub-lethal effects of temperature (Myrick and Cech 2004).

I found some evidence of a latitudinal gradient in temperature-dependent growth between Oregon and California populations. Growth rates of the Santiam River, Or population declined from 14°C to 20°C, in contrast, growth rates of the Battle Creek and San Lorenzo populations were maintained and the Scott Creek and American River populations actually increased in growth rate across that temperature range. However, within California, the most northerly population, Battle Creek had

similar temperature-dependent growth among the three treatment temperatures as the San Lorenzo River, the most southerly population. I did not see differences in temperature-dependent growth among treatment temperatures between central valley, California and central California coast populations. In fact, Battle Creek and San Lorenzo River populations showed the most similar patterns and the American River and Scott Creek populations showed similar patterns to one another. Notably, these patterns of similarities and differences do not correspond with existing DPS designations.

The spatial scale of the source populations used in these experiments suggests very local scale processes shaping this phenotypic variability. With strong spawning site fidelity across heterogeneous environments, natural *O. mykiss* populations should show such strong local adaptation, with the potential for rapid evolution (Nielsen 1999; Quinn 2004; Pearse et al. 2011). For example, while there was no difference in average growth rate between 14^{o}C and 20^{o}C for the San Lorenzo River population, growth was significantly higher at 20^{o}C for the Scott Creek population; these adjacent watersheds are both within the Central California Coast DPS (Fig. 2). Differences in temperature-dependent growth reported here reflect the environmental conditions of each population's natal watershed such as water temperature and estuary conditions (see Lindley et al. 2007; Hayes et al. 2008; Herbert et al. 2009). For example, a lack of significant difference in average growth rates between 14^{o}C and 20^{o}C for *O. mykiss* from Battle Creek, which generally experience water temperatures of approximately 14^{o}C, may be in response to these steelhead migrating through the

Sacramento River and San Francisco Estuary, both of which regularly experience temperatures above 21^{o}C (MacFarlane and Norton 2002; Myrick and Cech 2004; Lindley et al. 2007). Similarly, Scott Creek flows through a seasonally closed coastal lagoon before entering the ocean. This lagoon has elevated water temperatures relative to upstream habitats (including temperatures over 24^{o}C), with ample food supply, thusly providing elevated growth potential for Scott Creek *O. mykiss* (Hayes et al. 2008, 2011). Increased survival to reproduce confers a selective advantage to rearing under these conditions (Hayes et al. 2011; Reed et al. 2011). However, it is unclear whether fish that utilize the highly altered San Lorenzo River estuary share this advantage. Thus, local scale watershed differences may shape population differences in *O. mykiss* temperature-dependent growth even within a DPS. Noted population differences in temperature-dependent growth at such local scales is surprising given a history of hatchery out-planting and mixing of *O. mykiss* stocks throughout the state (Moyle 2002; Pearse et al. 2011).

As climate change is predicted to have varying effects across the heterogeneous landscape described here (Lindley et al. 2007; Cayan et al. 2008; Null et al. 2010), the degree of plasticity and/or local adaptation may be crucial to a population's persistence (Reed et al. 2011). Furthermore, variation in temperature-dependent growth within a population provides the basis for a its ability to adapt to shifting water temperatures due to climate change (Melack et al. 1997; Bolnick et al. 2003). However, predicting population responses to climate change is challenged by the interplay of ecological interactions with environmental changes (e.g. Sanford

1999; and see Mcewan 2001; Reed et al. 2011). For example, patterns of temperature-dependent growth presented here depend upon food availability being sufficient to facilitate elevated growth at warmer temperatures (Brett et al. 1969; Wurtsbaugh and Davis 1977). *O. mykiss* prey are also poikilothermic and their response to climate change might not be sufficient to fulfill the increased consumption demands of *O. mykiss* with rising water temperatures (Wurtsbaugh and Davis 1977; Myrick and Cech 2005; Deutsch et al. 2008). Not only might their productivity be insufficient, changes in temperatures may drive phenological and ontogenetic changes giving rise to mismatches between prey availability and increasing demands by predators due to elevated temperatures (Reed et al. 2011). While the details of ecological changes from climate effects are hard to predict, the relationships hinge upon our understanding of basic physiological responses such as temperature-dependent growth.

Effects of prior thermal experience on temperature-dependent growth

I was surprised to find no evidence that previously-experienced thermal environments affect how current temperatures influence growth or survival. The lack of a significant effect of previous temperatures experienced on current growth rate has important methodological and ecological implications. A major assumption of laboratory experiments is the lack of influence from previous conditions (i.e. differences between source hatchery and laboratory conditions). Thus, results presented here lend credence to laboratory experiments like this and others

(Wurtsbaugh and Davis 1977; Myrick and Cech 2005; Beakes et al. 2010). Myrick and Cech (2005) found evidence that acclimation temperature affected upper incipient lethal temperatures. However, I did not find any acclimation effect on growth at any temperature, highlighting the importance of juvenile *O. mykiss* ability to grow at different temperatures. While juvenile growth strongly influences demographics (Ward et al. 1989; Mangel and Satterthwaite 2008; Hayes et al. 2011), other important life history stages may not share this flexibility (e.g. egg survival; Myrick and Cech 2004). Furthermore, regardless of whether an individual is resident or anadromous, *O. mykiss* will likely encounter varying water temperatures within their life, yet associated benefits of juvenile growth rates (Satterthwaite et al. 2009, 2010; Hayes et al. 2011) may still be attained even after experiencing water temperatures elevated beyond growth optimums. This result is also important considering the extent to which freshwater habitats are altered in the southern portion of the range of *O. mykiss* (McEwan 2001; Lindley et al. 2006), and given the potential for future effects related to climate change (Lindley et al. 2007; Cayan et al. 2008).

Management implications

Given that growth rates for juvenile *O. mykiss* determine life history (Satterthwaite et al. 2009, 2010) and confer survival to reproduction (Hayes et al. 2011), the temperature-dependent growth responses of individual *O. mykiss* identified in this study have critical implications for the conservation and management of steelhead. First, temperature of freshwater rearing habitat is an important determinant of juvenile steelhead growth, and thus is likely an important variable to target for

management and restoration. Second, my experiment suggests that there is important local adaptation to stream temperature. Hatchery propagation, especially out-planting, could homogenize populations and swamp these local adaptations (Pearse et al. 2011).

Evidence of population specific temperature-dependent growth in *O. mykiss* suggests the need for local scale management. The fact that many imperiled populations of *O. mykiss* are found in stream systems with dams and diversions (McEwan and Jackson 1996; Lindley et al. 2006; Gustafson et al. 2007) poses obvious challenges to the recovery of the species, but could also be used advantageously to recover threatened and endangered populations. For example, by using knowledge of temperature-dependent growth from local populations, water releases could be managed strategically to match the temperature range best suited for populations of that basin.

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LITERATURE CITED

Barnosky AD, Hadly EA, Bascompte J, Berlow EL, Brown JH, Fortelius M, Getz WM, Harte J, Hastings A, Marquet PA, Martinez ND, Mooers A, Roopnarine P, Vermeij G, Williams JW, Gillespie R, Kitzes J, Marshall C, Matzke N, Mindell DP, Revilla E, Smith AB (2012) Approaching a state shift in Earth's biosphere. Nature 486:52–58

Beakes MP, Satterthwaite WH, Collins EM, Swank DR, Merz JE, Titus RG, Sogard SM, Mangel M (2010) Smolt transformation in two California steelhead populations: effects of temporal variability in growth. Transactions of the American Fisheries Society 139:1263–1275

Beer WN, Anderson JJ (2011) Sensitivity of juvenile salmonid growth to future climate trends. River Research and Applications 27:663–669

Bell E, Albers SM, Krug JM, Dagit R (2011) Juvenile growth in a population of southern California steelhead (Oncorhynchus mykiss). California Fish and Game 97:25–35

Bolnick DI, Amarasekare P, Araújo MS, Bürger R, Levine JM, Novak M, Rudolf VHW, Schreiber SJ, Urban MC, Vasseur DA (2011) Why intraspecific trait variation matters in community ecology. Trends in ecology & evolution 26:183–192

Bolnick DI, Svanbäck R, Fordyce J a, Yang LH, Davis JM, Hulsey CD, Forister ML (2003) The ecology of individuals: incidence and implications of individual specialization. The American naturalist 161:1–28

Bond M, Hanson C, Baertsch R, Hayes S, MacFarlane RB (2007) A New Low-Cost Instream Antenna System for Tracking Passive Integrated Transponder (PIT)-Tagged Fish in Small Streams. Transactions of the American Fisheries Society 136:562–566

Bond MH, Hayes SA, Hanson CV, MacFarlane RB (2008) Marine survival of steelhead (Oncorhynchus mykiss) enhanced by a seasonally closed estuary. Canadian Journal of Fisheries and Aquatic Sciences 65:2242–2252

Boughton DA, Gibson M, Yedor R, Kelley E (2007) Stream temperature and the potential growth and survival of juvenile Oncorhynchus mykiss in a southern California creek. Freshwater Biology 52:1353–1364

Brett JR, Shelbourn JE, Shoop CT (1969) Growth rate and body composition of fingerling sockeye salmon, Oncorhynchus nerka in relation to temperature and ration size. Journal of the Fisheries Research Board of Canada 26:2363–2394

Bromage N, Hardiman P, Jones J, Springate J, Bye V (1990) Fecundity , egg size and total egg volume differences in 12 stocks of rainbow trout , Oncorhynchus mykiss Richardson. Aquaculture and Fisheries Management 21:269–284

Bulkley RV (1967) Fecundity of steelhead trout , Salmo gairdneri , from Alsea River , Oregon. Journal of Fisheries Research Board of Canada 24:917–926

Cayan DR, Maurer EP, Dettinger MD, Tyree M, Hayhoe K (2008) Climate change scenarios for the California region. Climatic Change 87:21–42

Chevin L-M, Lande R, Mace GM (2010) Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. PLoS biology 8:e1000357

Darwin C (1859) On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life, 1st edn. John Murray, London.

Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR (2008) Impacts of climate warming on terrestrial ectotherms across latitude. Proceedings of the National Academy of Sciences 105:6668–6672

Elliott JM, Hurley M a. (1995) The functional relationship between body size and growth rate in fish. Functional Ecology 9:625–627

Elliott JM, Hurley MA (1997) A functional model for maximum growth of Atlantic salmon parr, Salmo salar, from two populations in Northwest England. Functional Ecology 11:592–603

Elliott JM, Hurley MA, Fryer RJ (1995) A new, improved growth model for brown trout, Salmo trutta. Functional Ecology 9:290–298

Garland Jr. T, Adolph SC (1991) Physiological differentiation of vertebrate populations. Annual Review of Ecology and Systematics 22:193–228

Geist DR, Deng Z, Mueller RP, Cullinan V, Brink S, Chandler JA (2011) Theeffect of fluctuating temperatures and ration levels on the growth of juvenile Snake River fall Chinook salmon. Transactions of the American Fisheries Society 140:190–200

Grader M, Letcher BH (2006) Diel and seasonal variation in food habits of Atlantic salmon parr in a small stream. Journal of Freshwater Ecology 21:503–517

Gustafson RG, Waples RS, Myers JM, Weitkamp LA, Bryant GJ, Johnson OW, Hard JJ (2007) Pacific salmon extinctions: quantifying lost and remaining diversity. Conservation biology 21:1009–1020

Hayes SA, Bond MH, Hanson CV, Freund, Ellen V, Smith JJ, Anderson EC, Ammann AJ, MacFarlane RB (2008) Steelhead growth in a small central California watershed : Upstream and estuarine rearing patterns. Transactions of the American Fisheries Society 137:114–128

Hayes SA, Bond MH, Hanson CV, Jones AW, Ammann AJ, Harding JA, Collins AL, Perez J, Macfarlane RB (2011) Down , up , down and "smolting" twice? Seasonal movement patterns by juvenile steelhead (Oncorhynchus mykiss) in a coastal watershed with a bar closing estuary. Canadian Journal of Fisheries and Aquatic Sciences 68:1341–1350

Herbert B, Heady WN, Alley D, Johnson NM, McPherson F, Menzies R (2009) San Lorenzo Valley Water District watershed management plan Part I: Existing conditions report. Boulder Creek

Hoar WS, Randall DJ, Brett JR (Eds) (1979) Fish physiology: Bioenergetics and growth, Volume VIII. Academic Press, New York

Imre I, Grant JW a, Keeley ER (2004) The effect of food abundance on territory size and population density of juvenile steelhead trout (Oncorhynchus mykiss). Oecologia 138:371–8

JMP statistical software Version 7. SAS Institute Inc, Cary, NC

Lindley ST, Schick RS, Agrawal A, Goslin M, Pearson TE, Mora E, Anderson JJ, May B, Greene S, Hanson C, Low A, Mcewan D, Macfarlane RB, Swanson C, Williams JG (2006) Historical population structure of Central Valley steelhead and its alteration by dams. San Francisco Estuary & Watershed Science 4:1–19

Lindley ST, Schick RS, Mora E, Adams PB, Anderson JJ, Greene S, Hanson C, May BP, McEwan DR, MacFarlane RB, Swanson C, Williams JG (2007) Framework for assessing viability of threatened and endangered Chinook salmon and steelhead in the Sacramento-San Joaquin Basin. San Francisco Estuary & Watershed Science 5:1–26

MacFarlane RB, Norton EC (2002) Physiological ecology of juvenile chinook salmon (Oncorhynchus tshawytscha) at the southern end of their distribution , the San Francisco Estuary and Gulf of the Farallones , California. Fishery Bulletin 100:244–257

Mangel M, Satterthwaite WH (2008) Combining proximate and ultimate approaches to understand life history variation in salmonids with application to fisheries, conservation, and aquaculture. Bulletin of Marine Science 83:107–130

McEwan DR (2001) Central Valley steelhead. In: Brown RL (ed) Contributions to the Biology of Central Valley Salmonids, Fish Bulletin 179(1). Scripps Institutions of Oceanography Library, San Diego, CA, pp 1–44

McEwan D, Jackson TA (1996) Steelhead restoration and management plan for California. California Department of Fish and Game

Melack JM, Dozier J, Goldman CR, Greenland D, Milner AM, Naiman RJ (1997) Effects of climate change on inland waters of the Pacific coastal mountains and western great basin of North America. Hydrological Processes 11:971–992

Moore JW, Hayes SA, Duffy W, Gallagher S, Michel CJ, Wright D (2011) Nutrient fluxes and the recent collapse of coastal California salmon populations. Canadian Journal of Fisheries and Aquatic Sciences 68:1161–1170

Moore JW, McClure M, Rogers LA, Schindler DE (2010) Synchronization and portfolio performance of threatened salmon. Conservation Letters 3:340–348

Moyle PB (2002) Inland fishes of California. University of California Press, Berkeley

Myrick CA, Cech JJ (2004) Temperature effects on juvenile anadromous salmonids in California's central valley: what don't we know? Reviews in Fish Biology and Fisheries:113–123

Myrick CA, Cech JJ (2005) Effects of temperature on the growth, food consumption, and thermal tolerance of age-0 Nimbus-strain steelhead. North American Journal of Aquaculture 67:324–330

Nielsen JL (1999) The evolutionary history of steelhead (Oncorhynchus mykiss) along the US Pacific Coast: developing a conservation strategy using genetic diversity. ICES Journal of Marine Science 56:449–458

Null SE, Viers JH, Mount JF (2010) Hydrologic response and watershed sensitivity to climate warming in California's Sierra Nevada. PloS one 5:1–16

Ostrovsky I (1995) The parabolic pattern of animal growth: determination of equation parameters and their temperature dependencies. Freshwater Biology 33:357–371

Pearse DE, Martinez E, Garza JC (2011) Disruption of historical patterns of isolation by distance in coastal steelhead. Conservation Genetics 12:691–700

Primmer CR (2011) Genetics of local adaptation in salmonid fishes. Heredity 106:401–403

Quinn TP (2004) The behavior and ecology of Pacific salmon and trout. University of Washington Press, Seattle

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists. Cambridge University Press

Quinn TP, Vøllestad LA, Peterson J, Gallucci V (2004) Influences of freshwater and marine growth on the egg size–egg number tradeoff in coho and Chinook salmon. Transactions of the American Fisheries Society 133:55–65

Reed TE, Schindler DE, Waples RS (2011) Interacting effects of phenotypic plasticity and evolution on population persistence in a changing climate. Conservation Biology 25:56–63

Sanford E (1999) Regulation of keystone predation by small changes in ocean temperature. Science 283:2095–2097

Satterthwaite WH, Beakes MP, Collins EM, Swank DR, Merz JE, Titus RG, Sogard SM, Mangel M (2009) Steelhead life history on California's central coast: insights from a state-dependent model. Transactions of the American Fisheries Society 138:532–548

Satterthwaite WH, Beakes MP, Collins EM, Swank DR, Merz JE, Titus RG, Sogard SM, Mangel M (2010) State-dependent life history models in a changing (and regulated) environment: steelhead in the California Central Valley. Evolutionary Applications 3:221–243

Schindler DE, Hilborn R, Chasco B, Boatright CP, Quinn TP, Rogers LA, Webster MS (2010) Population diversity and the portfolio effect in an exploited species. Nature 465:609–612

Shapovalov L, Taft AC (1954) The life histories of the steelhead rainbow trout (Salmo gairdneri) and silver salmon (Oncorhynchus kisutch) with special reference to Waddell Creek, California and recommendations regarding their management. California Department of Fish and Game Fish Bulletin 98:373p

Sigourney DB, Letcher BH, Obedzinski M, Cunjak RA (2008) Size-independent growth in fishes: patterns, models and metrics. Journal of Fish Biology 72:2435–2455

Sogard SM, Merz JE, Satterthwaite WH, Beakes MP, Swank DR, Collins EM, Titus RG, Mangel M (2012) Contrasts in Habitat Characteristics and Life History

Patterns of Oncorhynchus mykiss in California's Central Coast and Central Valley. Transactions of the American Fisheries Society 141:747–760

Sogard SM, Williams TH, Fish H (2009) Seasonal patterns of abundance, growth, and site fidelity of juvenile steelhead in a small coastal California stream. Transactions of the American Fisheries Society 138:549–563

Steffen W, Persson Å, Deutsch L, Zalasiewicz J, Williams M, Richardson K, Crumley C, Crutzen P, Folke C, Gordon L, Molina M, Ramanathan V, Rockström J, Scheffer M, Schellnhuber HJ, Svedin U (2011) The Anthropocene: From global change to planetary stewardship. Ambio 40:739–761

Sumpter JP (1992) Control of growth of rainbow trout (Oncorhynchus mykiss). Aquaculture 100:299–320

Systat Software Systat 13.

USFWS, NMFS (1996) Policy regarding the recognition of distinct vertebrate population segments under the Endangered Species Act. Federal Register (7 February 1996)61(26):4722-4725

Vøllestad LA, Olsen EM, Forseth T (2002) Growth-rate variation in brown trout in small neighbouring streams: evidence for density-dependence? Journal of Fish Biology 61:1513–1527

Ward B, Slaney P, Facchin A, Land R (1989) Size-based survival in stealhead trout (Oncorhynchus mykiss): Back-calculated lengths from adults' scales compared to migrating smolts at the Keogh River, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 46:1853–1858

Woodward G (2009) Biodiversity, ecosystem functioning and food webs in fresh waters: assembling the jigsaw puzzle. Freshwater Biology 54:2171–2187

Wurtsbaugh WA, Davis GE (1977) Effects of temperature and ration level on the growth and food conversion efficiency of Salmo gairdneri , Richardson. Journal of Fish Biology 11:87–98

TABLES

Table 1. Initial mean wet weight in grams (SD) of *O. mykiss* from each population and temperature treatment for Experiment 1 (this study) and for other studies from the Santiam River, OR (Wurtsbaugh and Davis 1977) and American River, CA (Myrick and Cech 2005). n= initial number of individual fish. ND = no data presented.

Population	Temperature$_1$ (^{o}C)	n	Wet weight (g)
Battle Creek	14	18	1.44 (0.33)
	20	18	1.55 (0.60)
	24	18	1.41 (0.34)
San Lorenzo	14	18	1.18 (0.42)
	20	17	1.37 (0.42)
	24	18	1.13 (0.36)
Scott Creek	14	15	1.60 (0.85)
	20	13	1.93 (1.08)
	24	12	1.80 (1.00)
American	11	10	3.1 (0.09)
River	15	10	2.4 (0.11)
	19	10	3.0 (0.18)
Santiam	9.4	ND	2.23
River	10	ND	1
	13.3	ND	0.97
	16.4	ND	0.92
	19.5	ND	1.18
	22.5	ND	1.16

Table 2. Initial mean wet weight in grams (SD) of *O. mykiss* from each population and temperature treatment in Experiment 3. *Temperature*$_1$ is the previous temperature exposure from Experiment 1, and *Temperature*$_3$ is the block temperature treatment in Experiment 3. Two fish from each of the three treatments from *Temperature*$_1$ were held in each of the three replicate tanks for *Temperature*$_3$. Sample sizes presented here are pooled across all three replicate tanks of Experiment 3.

***Temperature*$_3$**	**14°C**			**20°C**			**24°C**		
***Temperature*$_1$**	**14°C**	**20°C**	**24°C**	**14°C**	**20°C**	**24°C**	**14°C**	**20°C**	**24°C**
Battle Creek	6.4	7.3	4.9	6.9	7.1	4.0	6.7	5.4	3.9
	(1.8)	(4.7)	(1.3)	(2.3)	(3.7)	(1.2)	(2.9)	(2.9)	(0.9)
	n=6	n=5	n=7	n=5	n=6	n=6	n=6	n=7	n=5
San Lorenzo River	2.7	4.4	2.4	3.8	3.4	2.2	3.3	3.3	2.0
	(0.9)	(1.4)	(0.8)	(1.8)	(0.8)	(0.6)	(1.7)	(1.3)	(0.4)
	n=6	n=5	n=6	n=6	n=6	n=5	n=4	n=4	n=6
Scott Creek	6.0	8.2	5.3	7.4	5.5	3.6	3.5	4.8	6.8
	--	5.6	(2.5)	(2.6)	--	(2.2)	(3.0)	(3.9)	(5.3)
	n=1	n=3	n=2	n=4	n=1	n=2	n=3	n=3	n=3

Table 3. Results from a general linear model of standardized mass-specific growth rate at 14°C, 20°C and 24°C for Experiment 3 in response to the fixed factors of *Population*, *Temperature*$_1$ (temperature previously experienced in Experiment 1) *and* *Temperature*$_3$ (temperature currently experienced in Experiment 3) and their interaction effects. Because there was substantial mortality in the Scott Creek population, results are presented for Battle Creek and San Lorenzo River *O. mykiss* only.

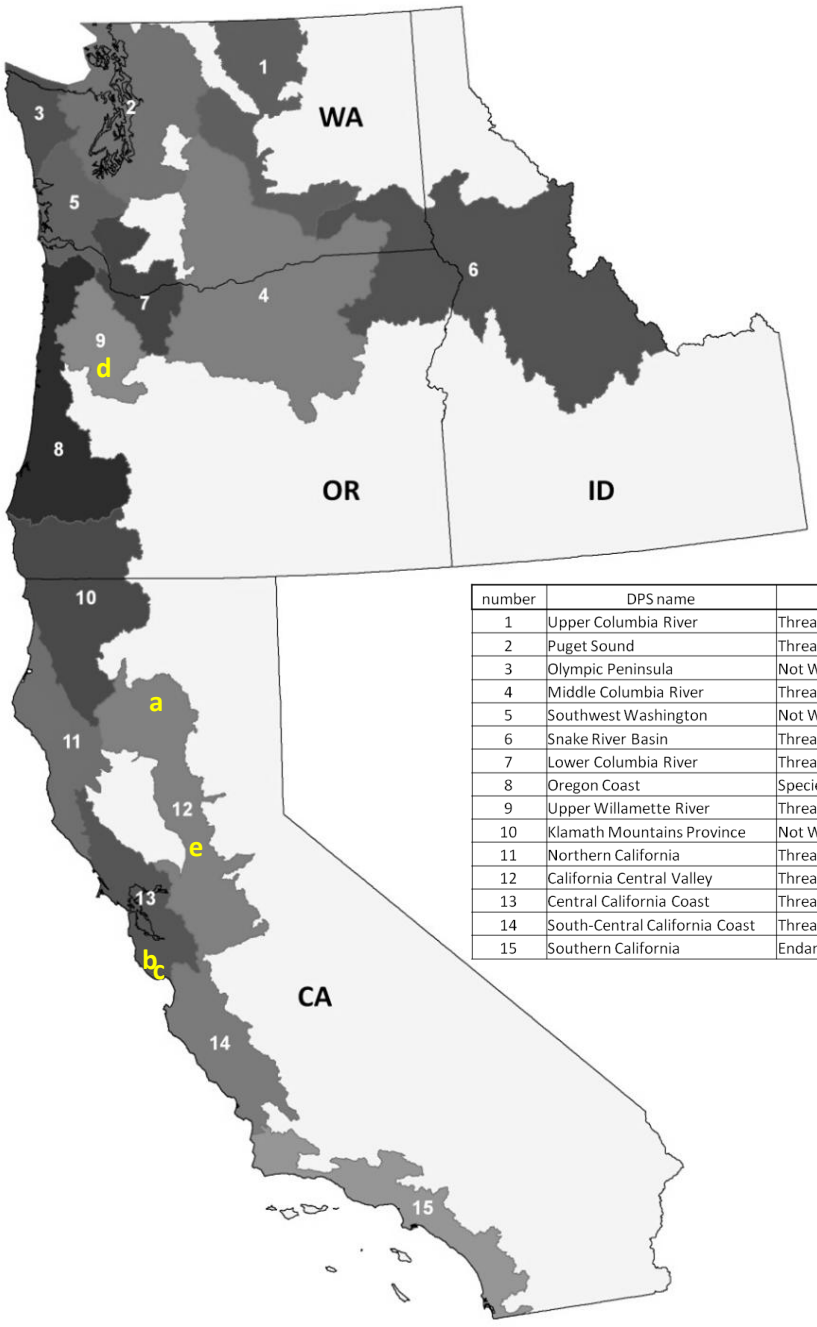
Source	**df**	**MSE***	**F**	***p***
Population	1	3.14	7,921.5	0.000
Temperature$_3$	2	2.75	6,933.8	0.000
Temperature$_1$	2	0.06	1.45	0.24
Population x Temperature$_3$	2	0.20	5.00	0.009
Temperature$_3$ *x Temperature*$_1$	4	0.09	2.25	0.07
Error*	89	0.04		

*MSE, mean square error

** *Population x Temperature 3 x Temperature 1, Population x Temperature 1* were each not significant and thus removed from the model individually, successively (α = 0.25; Quinn and Keough 2002).

FIGURES

Figure 1. Geographic distribution of Oncorhynchus mykiss Distinct Population Segments (DPS; delineated by different shades of grey). Populations used in this experiment were from a) Battle Creek, b) Scott Creek, and c) the San Lorenzo River; compared to previous studies from d) the Santiam River (Wurtsbaugh and Davis 1977), and e) the American River (Myrick and Cech 2005). The inset table lists each DPS name and status under the Endangered Species Act.



number	DPS name	status
1	Upper Columbia River	Threatened
2	Puget Sound	Threatened
3	Olympic Peninsula	Not Warranted
4	Middle Columbia River	Threatened
5	Southwest Washington	Not Warranted
6	Snake River Basin	Threatened
7	Lower Columbia River	Threatened
8	Oregon Coast	Species of Concern
9	Upper Willamette River	Threatened
10	Klamath Mountains Province	Not Warranted
11	Northern California	Threatened
12	California Central Valley	Threatened
13	Central California Coast	Threatened
14	South-Central California Coast	Threatened
15	Southern California	Endangered

Figure 2. The results of Experiment 1, depicting the relationship between standardized mass-specific growth rate (g · day-1) and temperature (°C) for juvenile *O. mykiss* from Battle Creek, CA (large dash), the San Lorenzo River, CA (solid line), and Scott Creek, CA (medium dash). Error bars are standard error (this study). Relationships between standardized mass-specific growth rate and temperature for the Santiam River, OR (Wurtsbaugh and Davis 1977) (dotted), and the American River, CA (Myrick and Cech 2005) (small dash) are plotted for comparison. DPS are represented by color, with Upper Willamette River in green, Central Valley, California in blue, and Central California Coast in red.

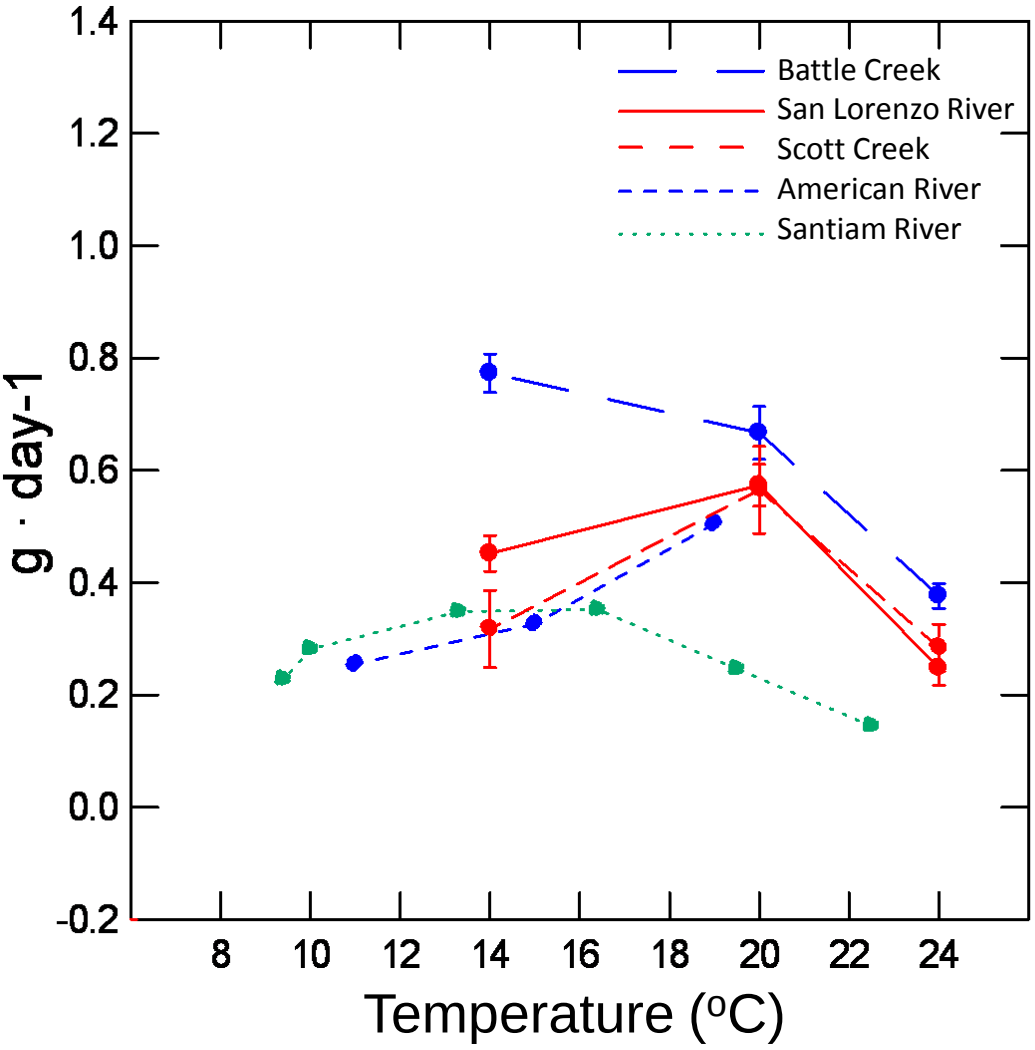
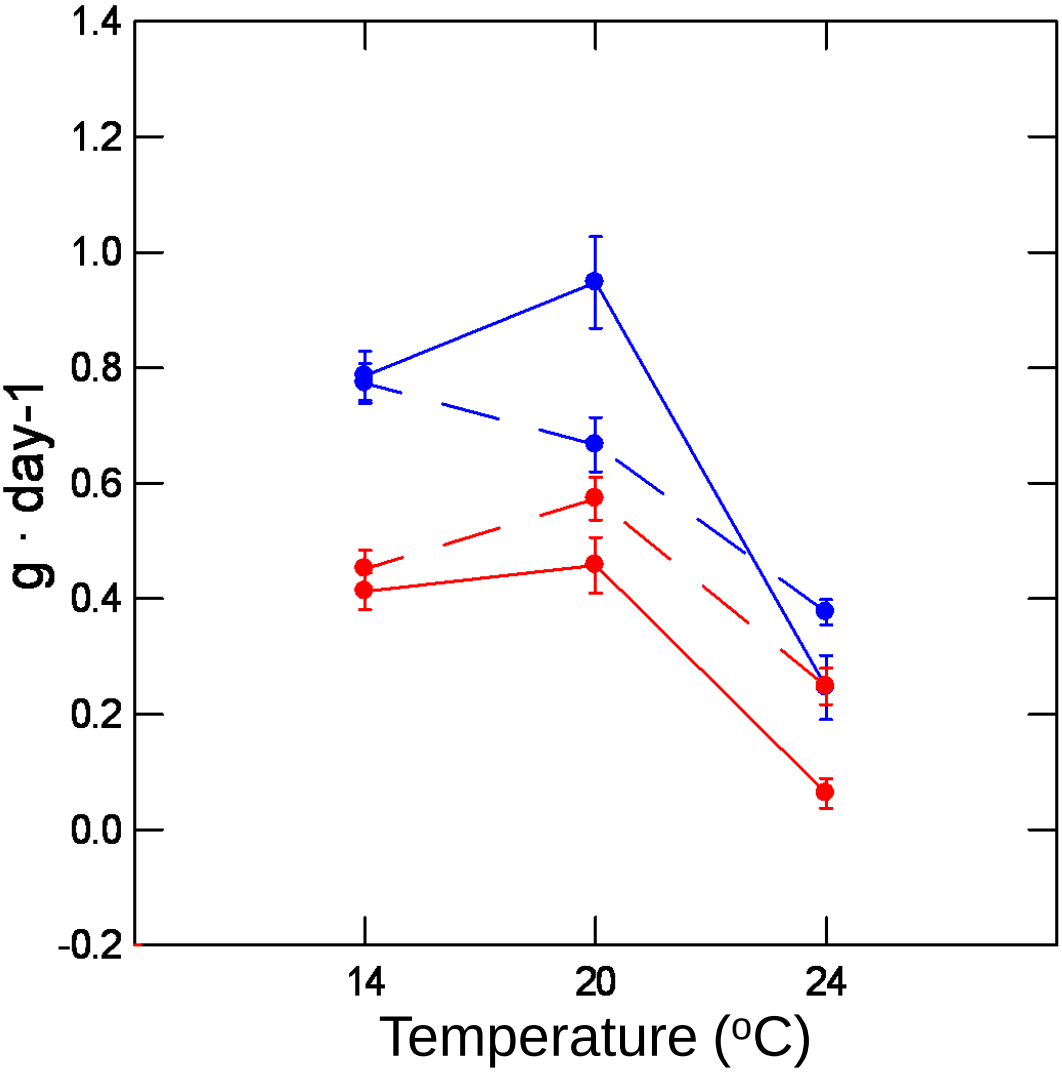


Figure 3. Comparison of standardized mass specific growth rate (g · day-1) and temperature (°C) between Experiment 1 (dashed lines) and Experiment 3 (solid lines) for Battle Creek (blue) and the San Lorenzo River (red).



CHAPTER 3

Tissue turnover and stable isotope clocks to quantify resource shifts in anadromous rainbow trout

INTRODUCTION

Stable isotope analysis can provide insight into ecological processes that would otherwise be difficult or impossible to detect (e.g., Koch et al. 1994; Studds et al. 2008; Newsome et al. 2010). However, experimental validation of parameters like tissue turnover and discrimination factors is important to sound ecological inference of windows of resource use and trophic interactions (Deniro and Epstein 1981; Gannes et al. 1997; Martínez del Rio and Wolf 2005). Further, many stable isotope model frameworks rest on assumptions that, if not tested, provide an uncertain foundation for field application (Martínez del Rio and Wolf 2005; Martínez del Rio and Anderson-Sprecher 2008; Boecklen et al. 2011).

Stable isotope values of an organism provide a dynamic window into its assimilated food (Deniro and Epstein 1981; Gannes et al. 1998; Carleton and Martínez del Rio 2010). After a diet-switch, different tissues take different amounts of time to turnover to the novel diet isotopic signature due to tissue-specific rates of macromolecular synthesis and catabolism (Martínez del Rio and Wolf 2005; Carleton et al. 2008). For example, splanchnic tissues have been shown to have faster turnover rates than structural tissues (Tieszen et al. 1983; Carleton et al. 2008; Bauchinger and McWilliams 2009; Buchheister and Latour 2010). Studies of fresh-water fishes have

also found that turnover rates can vary across tissues (e.g., McIntyre and Flecker 2006; Church et al. 2009; Carleton and Martínez del Rio 2010). By using known turnover rates with measured isotopic values from tissues and the environment, clocks can be developed to estimate the timing of resource switches (Phillips and Eldridge 2006; Klaassen et al. 2010; Buchheister and Latour 2010), for a variety of applications such as estimating timing of migration (e.g., Oppel and Powell 2010) or settlement (e.g., Herzka et al. 2001).

How tissue turnover is modeled can affect many ecological applications of stable isotopes, including the estimation of turnover rate and discrimination factors (Martínez del Rio and Wolf 2005; Martínez del Rio and Anderson-Sprecher 2008; Kurle 2009), and may affect the estimation of the timing of resource shifts. In certain tissues turnover may be better represented by modeling multiple compartments, i.e. multiple turnover rates within a tissue, each with its own proportional contribution to the tissue's turnover (Cerling et al. 2007; Carleton et al. 2008; Martínez del Rio and Anderson-Sprecher 2008). Tissue turnover models also can differ in terms of how they incorporate end-members. For instance, data may necessitate using diet values and a discrimination factor rather than equilibrium tissue values (Martínez del Rio and Wolf 2005; Martínez del Rio and Anderson-Sprecher 2008). Alternatively, due to differences in protein content, different diet items may show diet-specific discrimination factors (McCutchan et al. 2003; Pearson et al. 2003; Caut et al. 2009). Despite these different alternative formulations for isotopic turnover, to date isotopic clocks have assumed one-compartment and used equilibrium tissue values (Phillips

and Eldridge 2006; Klaassen et al. 2010). It remains unknown how model formulation affect estimates of time since diet-switch.

Stable isotopes may be especially useful to examine organisms with complex life histories and trophic roles, e.g., anadromous rainbow trout (steelhead; *Oncorhynchus mykiss). O. mykiss* are found in cold waters around the world (Moyle 2002) and, depending upon location, can be either an important invasive species (e.g., Cambray 2003) or an imperiled native species (e.g., Gustafson et al. 2007). Coastal estuaries play a nursery role for *O. mykiss* by providing a far greater growth potential than upper watershed rearing habitats, thereby increasing marine survival (Hayes et al. 2011). In fact, *O. mykiss* can migrate multiple times between freshwater, estuarine and marine habitats (e.g., Hayes et al. 2011). Thus, *O mykiss* lend themselves as a model system where the application of stable isotope clocks may answer important ecological questions, while simultaneously serving as a system to examine the general theory and application of stable isotopes.

In this study I developed and applied stable isotope clocks for *O. mykiss*. Previously, Church et al. (2009) quantified tissue turnover for two tissues (mucus and muscle) of *O. mykiss*. In our study, I used a controlled diet-switch experiment to investigate isotopic turnover in seven different tissues of *O. mykiss*. I also compared how the number of compartments and isotopic end-member inputs affected tissue turnover model fit and parameter estimates. Using bootstrapped resampling, I investigated how tissue turnover estimation and clock frameworks affect estimates of

the time since resource switch. This paper thus examines the strengths, and limitations of using stable isotope clocks to track resource shifts.

MATERIALS AND METHODS

Diet switch experiment

On May 6, 2008, 128 *O. mykiss* fry with a mean mass of 0.48g were transported from Coleman National Hatchery, California to aquaria at NOAA Southwest Fisheries Science Center, Santa Cruz, California. Fish were held in two cylindrical tanks (1.6 m diameter, 1500 L) with continuous flow of oxygenated 14^{o}C fresh water for the duration of the experiment. Fish were fed at an "ad libitum" rate throughout the experiment.

I chose two hatchery feeds that had different nitrogen stable isotope values. To minimize nutritional stress upon switching (Hobson and Clark 1992) the first and second diets had the same crude protein (50%), fat (20 and 22% respectively), and fiber (1%) derived from similar mixed food sources of fish meal, fish oil, wheat, and a vitamin mix, but the first diet also contained poultry meals, and corn meal whereas the second diet instead contained krill meal. For approximately 180 days prior to switch, I fed all fish diet-1 (Bio-Oregon Bio Olympic Fry, δ^{15}N of food = 7.9 ± 0.2; mean ± SD) to equilibrate tissues to that diet. On September 23, 2008 (Day 0), I placed eight fish representative of the experiment's size range in a separate tank to act as a control group and continued to feed them diet-1 for the duration of the experiment. I then switched the remaining fish to diet-2 (Bio-Oregon Bio Vita Fry,

$\delta^{15}N$ of food = 13.9 ± 0.1, mean ± SD). Fourteen days prior to and immediately prior to the switch on day 0, fish were sampled to establish initial $\delta^{15}N$ values for all tissues. Fish were sampled 1, 3, 7, 14, 28, 56, 121, and 210 days after switching to diet-2 to track $\delta^{15}N$ changes in tissues through time. At each sampling interval, eight fish were selected across the range of observed sizes.

Sample collection and preparation

From each of the eight fish per sample I collected plasma, liver, fin, mucus, red blood cells (RBC), muscle, and scales to be analyzed for stable isotope composition. Fish were euthanized using tricaine methanesulfonate (MS-222). The fork length and weight of each fish was measured. I took blood directly from the caudal vein. Blood samples were refrigerated immediately after collection (2 - 4^{o}C), then immediately centrifuged for ten minutes at 3000 rpm to separate RBC from plasma, and the plasma was pipetted into a new vial. The caudal fin was removed and later subsampled (see below). An approximately 1x1x2cm cube of muscle was cut from just below the dorsal fin, the fish was dissected and the liver was removed. Scales scraped from just below the dorsal fin were washed with high pressure deionized water in a 425 micron sieve, and then lightly agitated in the sieve under running deionized water. Fin, liver, and muscle were rinsed with de-ionized water. All samples stored in 1.5 ml centrifuge tubes and each fish stored in individual bags were frozen. Mucus was taken from frozen fish using methodologies adapted from Church et al. (2009). Specifically, after thawing fish for five minutes I scraped clean mucus from the dorsal area into 30 ml scintillation vials. Using deionized water

mucus was diluted and filtered through a 212 micron sieve into 50 ml scintillation vials to remove foreign particles. I gave the sieve a final rinse, resulting in approximately 25 ml of clean diluted mucus samples that were then frozen.

Stable isotope analysis

All samples were freeze dried for 48 hours. Plasma, mucus, and RBC were homogenized into a fine powder in the vial and liver and muscle were homogenized with mortar and pestle. Dried powder, pieces of dried caudal fin trimmed from the distal edge, or whole dried scales were placed into 5x9mm tin capsules until target mass was attained (0.7mg +/- 0.05mg). The $\delta^{15}N$ values and elemental composition of tissues and food were measured at the University of California, Santa Cruz on a Carlo Erba 1108 elemental analyzer coupled to a ThermoFinnigan Delta Plus XP isotope ratio mass spectrometer. Repeated samples of internal PUGel (n = 221) and acetanilide (n = 77) standards were used for calibration and quality control for our tissue and diet samples (n = 814). The international standard is atmospheric nitrogen, with precision better than 0.2 ‰ for $\delta^{15}N$ values.

Statistical approach

Modeling tissue turnover

I compared how different tissue turnover models fit our data from the laboratory diet-switch experiment (Table 1). Specifically, I examined model formulations with different approaches to end member data by estimating either: equilibrium tissue values, single-, or diet-specific tissue discrimination factors.

Further, I considered multiple-compartment frameworks for each approach of end member data. All of these differences in isotopic incorporation can be represented in two general types of models:

Equilibrium tissue model:

$$\boldsymbol{\delta X_t = \delta X_{Post} - (\delta X_{Post} - \delta X_{Pre})\left(p\boldsymbol{e}^{-\frac{t}{\tau_1}} + (1-p)\boldsymbol{e}^{-\frac{t}{\tau_2}}\right)}, \text{ and} \qquad (1)$$

Discrimination factor model:

$$\boldsymbol{\delta X_t = \left(\delta X_{diet_2} + \Delta\right) - \left(\delta X_{diet_2} - \delta X_{diet_1}\right)\left(p\boldsymbol{e}^{-\frac{t}{\tau_1}} + (1-p)\boldsymbol{e}^{-\frac{t}{\tau_2}}\right)}. \qquad (2)$$

For both equations δX_t is the measured isotopic value of a given element (in this case nitrogen) for a tissue at time *t.* I estimated turnover rate as the average residence time (τ) or the reciprocal of the fractional incorporation rate $\tau = 1/\lambda$; with half-lives calculated as $t_{1/2} = \tau ln(2) = ln(2)/\lambda$ for one compartment models (Carleton et al. 2008; Martínez del Rio and Anderson-Sprecher 2008). For each equation I modeled tissue specific isotopic turnover with both one- and two-compartment models. In essence, two compartment models allow for the change in isotope signatures to be described by two different rates—perhaps tissue materials are being cycled through a fast and a slow pathway (e.g. the dashed line of mucus shows a faster turnover rate between day 0 and 3, and a slower turnover rate after day 3 relative to the constant turnover rate of the one-compartment solid line, Fig. S1, ESM). For two-compartment models I simultaneously estimated each compartment's average residence time, τ_1 and τ_2, and *p*, the proportional contribution of τ_1 to the overall turnover within a tissue at time *t* ($\Sigma p_i = 1$; Martínez del Rio and Anderson-Sprecher 2008). For one-compartment models *p*

= 1. In Eq. 1 I estimated the isotopic ratio of the tissue in equilibrium with the pre-switch and post-switch diets as δX_{Pre} and δX_{Post} respectively. I refer to Eq. 1 as the 'equilibrium tissue model.' Alternatively in Eq. 2 I used measured isotopic values of the pre- and post-switch diets (as opposed to the tissue), and estimated a common discrimination factor Δ that accounts for the difference between diet and tissue. I refer to Eq. 2 as the 'discrimination factor model.' I also separately modeled tissue turnover using diet-specific discrimination factors (Pearson et al. 2003; Caut et al. 2009), combined with measured isotopic values of the pre- and post-switch diets. Interestingly, this approach returned identical estimates and standard error of τ for single compartment and p, τ_1, τ_2 for two compartment models, as well as identical model fit and model error to modeling tissue turnover using equilibrium tissue values (Eq. 1). In this regard, both one- and two-compartment model approaches of modeling tissue turnover using single- or diet-specific discrimination factors, as well as modeling equilibrium tissue values are all represented using Eqs. 1 and 2. Considering one- and two-compartment versions of Eqs. 1 and 2 I will compare four competing models (Table 1): a one-compartment equilibrium tissue model (ET 1), a two-compartment equilibrium tissue model (ET 2), a one-compartment discrimination factor model (DF 1), and a two-compartment discrimination factor model (DF 2).

I used non-linear least squares to estimate each model's parameter point estimates and associated standard error. I calculated AICc (Akaike Information Criteria corrected for small sample sizes) scores to evaluate the relative support for each model and refer to parameter standard errors for how well each model described

the data (Burnham and Anderson 2010). I performed all analyses is R (R Development Core Team 2008).

Isotopic tissue turnover can reflect rates of growth and catabolic tissue replacement (Carleton et al. 2008; Carleton and Martínez del Rio 2010). I modeled growth of our experimental diet-switch fish to investigate if variation in turnover rate could be attributed to variation in growth rate and used individual calculated growth rates $k = (ln(W/W_o)/t)$ to determine the relative contribution of growth *(k)* and estimated catabolic tissue replacement *(m)* to isotopic tissues turnover (ESM; $1/\tau = \lambda = k+m$; Hesslein et al. 1993).

Using tissue turnover to estimate the timing of a diet switch

I present clocks derived from both tissue turnover models (Eqs. 1 and 2) because these models have different end member data inputs and thus could be applicable for different data scenarios (Table 1). Specifically, the application of clocks derived from Eq. 1 requires measured equilibrium tissue values from individuals known to have resided in each of the two specific environments *(Pre* and *Post)* for a period longer than the turnover time of the focal tissue. In contrast, the application of clocks derived from Eq. 2 requires knowledge of the isotopic diet values from each of the two environments, as well as established tissue-specific discrimination factors. I examined clocks derived from one- and two-compartment versions of Eqs. 1 and 2; however, for all tissues the two-compartment clocks were biased and less precise than the analogous one-compartment versions (Fig. S2, ESM). Therefore, I only present one-compartment clocks here. I also anticipate different

clock frameworks will be better suited to different field applications and therefore examine single-, two-, and averaged clocks derived from both Eq. 1 and Eq. 2 (Table 1).

Single-Tissue Clocks

For single-tissue clocks I first solved each tissue turnover model for the time since diet switch *(t_{est})* in a similar manner to Klaassen et al. (2010), thereby deriving single-tissue clocks for each model. Then the model's best estimate of tissue-specific turnover rates *(τ)* and discrimination factors *(Δ)* can be used in combination with known end members (δX_{Pre} and δX_{Post}) or (δX_{diet1} and δX_{diet2}) and a measured isotopic tissue value at time *t* (δX_t), to calculate t_{est}.

Single-tissue clock using equilibrium tissue values:

$$\boldsymbol{t_{est} = -\tau * ln\left(\frac{\delta X_{Post} - \delta X_t}{\delta X_{Post} - \delta X_{Pre}}\right)}. \quad (3)$$

Single-tissue clock using a single discrimination factor:

$$\boldsymbol{t_{est} = -\tau * ln\left(\frac{\left(\delta X_{diet_2} + \Delta\right) - \delta X_t}{\left(\delta X_{diet_2} - \delta X_{diet_1}\right)}\right)}. \quad (4)$$

Algebraic Two-Tissue Clocks

If two tissues with different turnover rates have been collected and analyzed for isotopic composition then algebraic two-tissue clocks can be used. For algebraic two-tissue clocks I first solved for *(δX_{Post} - δX_{Pre})* for $Tissue_1$ and $Tissue_2$. Like Phillips and Eldridge (2006) and Klaassen et al. (2010), by assuming that these differences were equal among tissues within an individual, I set the remaining

equation for $Tissue_1$ equal to the remaining equation for $Tissue_2$ and solved for the common time since diet switch (t_{est}). Using data from our diet switch experiment I examined this assumption and found it to generally be true within error of parameter estimation (see results, Table 2).

Algebraic two-tissue clock using equilibrium tissue values:

For a two-tissue clock from Eq. 1 with $Tissue_1$ and $Tissue_2$:

$$\boldsymbol{t_{est} = \frac{ln\left(\frac{\delta X_{Post_1} - \delta X_{t_1}}{\delta X_{Post_2} - \delta X_{t_2}}\right)}{\left(\frac{1}{\tau_1} - \frac{1}{\tau_2}\right)}}. \quad (5)$$

Algebraic two-tissue clock using a single discrimination factor:

For a two-tissue clock from Eq. 2 with $Tissue_1$ and $Tissue_2$:

$$\boldsymbol{t_{est} = \frac{ln\left(\frac{\left(\delta X_{diet_2} + \Delta\right) - \delta X_{t_1}}{\left(\delta X_{diet_2} + \Delta\right) - \delta X_{t_2}}\right)}{\left(\frac{1}{\tau_1} - \frac{1}{\tau_2}\right)}}. \quad (6)$$

Averaged Clocks

I also propose the approach of averaging independent estimates of t_{est} from multiple tissues. I refer to this approach as using 'averaged clocks.' In this approach, I simply calculate two (or more) single-tissue clocks independently using Eqs. 3 or 4 and take the mean value of t_{est}. I hypothesize that this approach should provide more precise values of t_{est} by averaging across potential tissue specific biases.

Boot-strapped resampling

I used a bootstrapping routine to investigate how parameter uncertainty affects error in calculating t_{est}. To do this I took simultaneous random draws of δX_{Pre} and δX_{Post}, and τ from the estimated multivariate normal defined by the parametric variance-covariance matrix of each model fit. Associated values of δX_t were drawn from a normal distribution produced from our model fitting. I iterated this 10,000 times for each true time (t_{true}) to produce sets of parameter estimates to be used for each different clock's calculation. Thus, I produced distributions (n=10,000) of t_{est} for each clock derived from the same parameter sets at each t_{true} and therefore each clock is comparable at each time step. I produced the median and the 95% prediction intervals from each clock's resampled distributions of t_{est}. Prediction intervals delineate the area within which 95% of all future calculated t_{est} will occur considering variability from measurement, environmental conditions, or individual physiology (Ott 1993). Thus, more precise clocks will have narrower prediction intervals and more accurate clocks will have median t_{est} values closer to the 1:1 line of observed to expected values.

RESULTS

Following the diet switch, δ^{15}N tissue values changed towards that of the second diet, asymptotically approaching equilibrium levels of the second diet (Fig. 1). At each sampling period, data are clustered tightly around the model fit, indicating low variation among individuals in tissue turnover. Furthermore, tissue turnover data fall evenly and tightly along the 1:1 line of observed vs. expected indicating a good

model fit (inset, Fig. 1). Differences between δX_{Post} and δX_{Pre} among tissues were within ranges of error for most tissues but were slightly different than the difference in δX_{diet2} and δX_{diet1} suggesting that different diets were associated with different discrimination factors (Table 2). I also had control fish that did not undergo a diet switch - differences in mean $\delta^{15}N$ tissue values between control fish and pre-switch fish were within individual variability (Deniro and Epstein 1981) ranging from 0.12 to 0.65 ‰. Further, all tissues appeared to reach equilibrium with the second diet by the end of the experiment (Fig. 1).

Growth for our experimental fish was best described by a specialized von Bertalanffy growth model, also known as the Richards model (Fig. 2 and ESM, $Mass_t = 557*(1- 0.78\ exp(-0.00595*Day))^{2.27}$) (Richards 1959; Essington et al. 2001). I found relationships between variability of individual growth rate and individual turnover rate in muscle, fin, mucus, and liver (ESM). Calculated growth rates $(k = (ln(W/W_o)/t))$ varied among individuals from 0.014 to 0.034 with a mean of 0.024 * d^{-1}. When I used measured growth (k) and estimated catabolic tissue turnover (m) to model isotopic turnover ($\lambda = 1/\tau$; $\lambda = k+m$; Hesslein et al. 1993) I found that catabolism contributed more to turnover for faster turnover tissues. Specifically, the estimated percent contributions of catabolism to isotopic tissue turnover were: fin (68%), plasma (68.3%), liver (65.7%), mucus (32%), RBC (6.6%), muscle (6.1%), and scale (0.7%) (Table S1, ESM).

Turnover models

Isotope turnover in different tissues was best described by different models (Table 2). In particular, different tissues were supported by one- versus two-compartment models. Specifically, fin, RBC, muscle, and scale tissues were better supported by a one-compartment equilibrium tissue model (Eq. 1; Table 2). In contrast, AICc scores supported two-compartment models for plasma, liver, and mucus. Tissue isotopes generally were modeled best with the use of equilibrium tissues rather than a general discrimination factor. For instance, plasma and liver were best represented by the two-compartment equilibrium tissue model (Eq. 1; Table 2). Mucus was best represented by a two-compartment discrimination factor model (Eq. 2) with the two-compartment equilibrium tissue model (Eq. 1) having a near identical AICc score (Table 2). Aside from mucus' virtually equal support for two-compartment versions of both Eq. 1 and 2, all tissues were best supported by either a one- or two-compartment equilibrium tissue model (Eq. 1) rather than models using discrimination factor (Table 2). The resulting implication is that different diet items had different discrimination factors (Table 2). While the data supported different models, there was tight coherence of estimated tissue-specific parameters across models (Table 2, Fig. 3). For all tissues I found a linear relationship between average residence times of one- vs. two-compartment versions of the equilibrium tissue value model (Eq. 1; $\tau_{2\text{-comp}} = 2.8 + 0.98*\tau_{1\text{-comp}}$, $r^2 = 0.97$). Both within and among model variation in turnover rates were low for fast turnover tissues and increased slightly for longer turnover tissues (Table 2, Fig. 3).

Parameter estimates

Model estimates revealed that different tissues were characterized by dramatically different turnover rates (Table 2, Fig. 3). Fast turnover tissues with their AICc supported average residence times are fin (12.9 days), plasma (14.1 days), and liver (16.1 days) (Table 2, Fig. 3). Slower turnover tissues and their AICc supported average residence times include mucus (35.7 days), RBC (37.6 days), muscle (39.0 days), and scale (40.0 days) (Table 2, Fig. 3). Best supported $\delta^{15}N$ diet-tissue discrimination factors were fin (1.6‰), plasma (2.1‰), liver (1.5‰) for fast turnover tissues, and mucus (1.3‰), RBC (1.7‰), muscle (3.4‰), and scale (2.2‰) for slower turnover tissues.

Stable isotope clocks

Bootstrapped prediction intervals allowed the quantification of when different tissues yielded high precision (narrow prediction intervals) and accurate (closest to true value) values of t_{est}. In general, values of t_{est} were more accurate immediately after the switch. As time since diet switch increased, prediction intervals widened, representing decreasing precision in predicting t_{est}. Through time, as values of δX_t approached values of δX_{Post}, models had increasing chances of returning values of log(0) in the numerator of Eqs. 3-6, and thereby began to fail to provide t_{est} (dashed lines; Figs. 4 and 5). For all tissue clocks, median t_{est} began to underestimate t_{true} after leaving this reliable period of t_{est} (defined as the range of t_{est} for which no values of log(0) were returned in 10,000 iterations) (solid lines; Fig. 4). This bias increased with time. Furthermore, outside of this reliable range of t_{est} the prediction intervals

drifted from the 1:1 or the median t_{est} and were skewed wider towards over-estimating t_{est} for each tissue (Fig. 4).

Model selection influenced the accuracy, precision, and range of inference of single-tissue clocks. One-compartment equilibrium tissue clocks (Eq. 3) consistently returned the most precise and accurate values of t_{est} over the longest period of inference (Fig. S2, ESM). For each tissue, median t_{est} from this clock was within 0.5 days of actual time since diet switch within the period of reliable estimates of t_{est} (solid lines, Fig. 4) and prediction intervals remained narrower than other model frameworks (Fig. S2, ESM). One-compartment discrimination factor clocks (Eq. 4) performed better than any two-compartment clock and only slightly worse than one-compartment equilibrium tissue clocks (Eq. 3, Fig. S2, ESM). For all tissues, two-compartment clocks provided approximately the same duration of reliable estimates of t_{est} as one-compartment clocks, but prediction intervals were wider throughout. Median t_{est} for two-compartment clocks tended to over-estimate t_{true} soon after the switch and then under-estimate t_{true} later (Fig. S2, ESM). Due to the bias and inaccuracies of t_{est} using two-compartment clocks I compare the results from single-tissue, algebraic two-tissue, and averaged clocks derived from the one-compartment equilibrium tissue turnover model (Eq. 1) in the following paragraphs.

Single-tissue clocks

Single-tissue clock performance varied across tissues with different turnover rates. For the faster turnover tissues of plasma, liver, and fin, Eq. 3 provided reliable t_{est} for approximately the same duration as the half-life of the element (for example,

approximately 8.2, 8.6, and 9 days respectively for these fast turnover tissues; Fig. 4). During this reliable period, median t_{est} was within 0.1 days of t_{true} for fast turnover tissues. Mucus, a medium turnover rate tissue, also returned reliable t_{est} approximately the same duration as its half-life (18.8 days), with median t_{est} within 0.2 days of t_{true} (Fig. 4). The longer turnover tissues of RBC, muscle, and scale returned reliable t_{est}, with the median value of t_{est} within 0.5 days of t_{true} for approximately twice their half-life (ca. 52, 50, and 55 days respectively for RBC, muscle, and scale; Fig. 4). Faster turnover tissues returned the narrowest prediction intervals for a duration post switch equal to their half-lives; however, longer turnover tissues were more precise from that point on (Fig. 4).

Algebraic two-tissue clocks

Algebraic two-tissue clocks were also more precise soon after the diet switch, but prediction intervals widened through time. For all algebraic two-tissue clocks the reliable period of t_{est} was the same duration as that of the shortest single-tissue clock of the tissue combination, and prediction intervals widened dramatically outside of this reliable prediction period (Fig. 5a). In general, prediction intervals were wider than those of either single-tissue clocks of the pair, but were narrower than the longer turnover clocks soon after switch. Within the reliable period of t_{est}, algebraic two-tissue clocks over-estimated t_{true} by approximately 2 days, yet eventually underestimated t_{true} (Fig. 5a). Of the algebraic two-tissue clocks, the fin-scale clock was the most precise returning the narrowest prediction intervals for the longest period of reliable inference (11 days, solid lines; Fig. 5a).

Averaged clocks

Of all approaches, averaged clocks provided the most precise values of t_{est} over the longest period of time. Using multiple tissues potentially increased the reliable range of t_{est} in that if one tissue returned a value of log(0) another tissue may not. Prediction intervals were at least as narrow as the most precise tissue in the combination for at least as long as the tissue with the longest period of reliable t_{est}. All tissue combinations presented here returned median t_{est} within one day of t_{true} for the same duration as the shortest turnover clock of the pair (Fig. 5b). After this time, median t_{est} began to underestimate t_{true} even within the period of reliable estimates, apparently pulled down by the shorter turnover tissue. Nevertheless, averaged clocks provided reliable values of t_{est} for up to 64 days post switch, with median t_{est} within 6 days of t_{true} at this time, and the narrowest prediction intervals throughout (e.g., fin-mucus-scale; Fig. 5b).

DISCUSSION

In this study, I performed a laboratory diet-switch experiment on juvenile *O. mykiss* to illuminate patterns of isotopic turnover. This data allowed accurate estimations of discrimination factors and turnover rates which varied among tissues for a wide range of use in *O. mykiss* ecology. Using an information theoretic approach I compared how six competing tissue turnover models represented this diet-switch for each of seven tissues. I found consistent support for using equilibrium tissue value, but support for one- or two-compartments differed among tissues. Furthermore, by

comparing several different isotope clocks, I revealed that the number of compartments affected values of τ_{mean} and t_{est} more than whether equilibrium tissue values or diet and a discrimination factor were used (Table 2 and ESM). One-compartment equilibrium tissue clocks (Eqs. 3 and 5) consistently returned the most precise and accurate values of t_{est} (ESM) supporting previous assumptions of turnover modeling and clocks (Martínez del Rio and Wolf 2005; Phillips and Eldridge 2006; Klaassen et al. 2010; Buchheister and Latour 2010). Slow turnover tissues like scale quantified resource switches over a longer window of time, while faster turnover tissues like fin provided more precise estimates of the timing of a recent switch. Like previous studies I found single-tissue clocks provided the most accurate and precise values of t_{est} relative to algebraic two-tissue clocks (Phillips and Eldridge 2006; Klaassen et al. 2010) but that averaged clocks provided more precise t_{est} over an extended range of inference.

The remaining discussion is comprised of two major sections. In the first section I discuss patterns of isotopic tissue turnover and how physiology might influence those dynamics. In the second section I discuss the advantages, disadvantages, and limitations of different tissues and clocks in estimating the timing of resource switches.

Isotope dynamics and physiology

Different tissues often exhibit different diet-tissue discrimination values and turnover rates (e.g., McCutchan et al. 2003; Bauchinger and McWilliams 2009). Our data allowed precise estimation of $\delta^{15}N$ discrimination factors which varied among *O.*

mykiss tissues from 1.3‰ for mucus to 3.4‰ for muscle (Table 2, Fig. 2). Our estimated discrimination factors were within range of values previously described for *O. mykiss* tissues of muscle, liver, and mucus (Pinnegar and Polunin 1999; McCutchan et al. 2003; Church et al. 2009). I also found that tissues such as plasma, liver, and fin had faster turnover rates than did tissues such as RBC and muscle. This relative ranking of tissue turnover rate is similar to results from studies of other fish, birds, and mammals (Tieszen et al. 1983; MacAvoy et al. 2005; Podlesak et al. 2005; McIntyre and Flecker 2006; Guelinckx et al. 2007; Carleton et al. 2008; Bauchinger and McWilliams 2009; Kurle 2009; Buchheister and Latour 2010). Intriguingly, our estimates of turnover rates were approximately 71% faster for muscle and 48% faster for mucus (Table 2) than estimates from a previous *O. mykiss* isotope diet-switch study (Church et al. 2009). These differences in turnover estimates could be driven by extrinsic factors such as environmental parameters or experimental design (Martínez del Rio and Wolf 2005; Logan et al. 2006; McIntyre and Flecker 2006) or intrinsic population-level differences in rates of growth and catabolic tissue replacement (Carleton and Martínez del Rio 2010).

Rates of growth and catabolism have each been shown to influence isotopic tissue turnover (Carleton and Martínez del Rio 2010). Using methods from Hesslein et al. (1993) I found growth to be the primary determinant of isotopic turnover rate in more structural tissues, a pattern common to growing ectotherms (Martínez del Rio et al. 2009). However, our results from the remaining tissues add to a growing literature showing the importance of catabolism to turnover in growing poikilothermic fishes

(Herzka et al. 2001; Logan et al. 2006; McIntyre and Flecker 2006; Carleton and Martínez del Rio 2010). Given that growth may influence turnover rates, it is tempting to try to correct for growth when comparing studies or applying laboratory based parameters. However, Carleton and Martínez del Rio (2010) found complex relationships between tissue, ration, and resulting proportional contributions of growth and catabolism to isotopic turnover. Therefore, predicting or correcting for differences in turnover is not simple (Boecklen et al. 2011), and I reiterate Carleton and Martínez del Rio's (2010) suggestion to use caution when applying lab based estimates to wild populations. For example, prediction intervals around our estimates of time since diet-switch were based on bootstrapped individual variability; however, this uncertainty does not include potential population or environmental differences.

Our study found that different tissues had apparently different fundamental patterns of turnover. Two-compartment turnover models were best supported for *O. mykiss* plasma, liver, and mucus, while one-compartment models were more supported in fin, RBC, muscle, and scale. Other studies have found support for the number of compartments to vary among tissues in birds and mammals (Carleton et al. 2008; Bauchinger and McWilliams 2009; Kurle 2009). While there is growing mathematical support for multiple compartments, there is still limited understanding of the physiological mechanisms underlying this phenomenon (Martínez del Rio and Anderson-Sprecher 2008; Boecklen et al. 2011).

Estimates of turnover and discrimination factors were relatively consistent among different model formulations (Table 2; Fig. 3). For example, I found a linear

relationship between turnover rates estimated from one- and two-compartment versions of an equilibrium tissue model (τ2-compartment = 2.8 + 0.98* τ1-compartment, r2 = 0.97), similar to that found by Carleton et al. (2008) for birds (τ2-compartment = 3.54 + 0.96 τ1-compartment, r2 = 0.98). In other words, at least for fish and birds, two-compartment models generally provide average residence times that are approximately 3 days longer than one-compartment models (Table 2). Estimated discrimination factors were relatively insensitive to the number of compartments used in turnover modeling, supporting the generality of these estimates.

Isotopic clocks in practice

Turnover model selection somewhat affected the accuracy, precision and range of inference of single-tissue clocks. For example, two-compartment models provided tissue turnover rates approximately 3 days longer than one-compartment models, a similar degree to which two-compartment clocks over-estimated t_{true} (Fig. 2, ESM). While two-compartment clocks best described isotopic turnover for some tissues, I found that one-compartment clocks still provided relatively precise and accurate estimates of the timing of resource switches. Thus, while there is clear evidence of complexities in isotopic turnover, the more simple model still provides adequate predictions of resource switches (Martínez del Rio and Wolf 2005). Furthermore, whether equilibrium tissue values or diet values and a discrimination factor were used had minimum effect on τ and resulting t_{est} (ESM), implying a versatility in clocks to available end-member data.

I found algebraic two-tissue clocks to be less precise and accurate than single-tissue clocks from either tissue of the pair, similar to previous studies (Phillips and Eldridge 2006; Klaassen et al. 2010). The reduced precision of algebraic two-tissue clocks relative to other clocks is likely because more parameters and their associated error are necessary to calculate t_{est}. Furthermore, the assumption that the difference in end members $(\delta X_{Post} - \delta X_{Pre})$ is equal among tissues (Phillips and Eldridge 2006; Klaassen et al. 2010) is only loosely met (Table 2), thereby creating additional error around t_{est}. Regardless, algebraic two-tissue clocks may be of particular use for some applications because they use the relative rather than absolute turnover rates of two tissues, and because the *Pre* switch end member is not necessary to generate t_{est}.

Isotopic clocks from tissues had different strengths and limitations (Fig. 4 and 5). Longer turnover tissues such as scale provide the most useful single-tissue clocks by reliably providing the most precise and accurate t_{est} over the largest window of inference (up to approximately 55 days post switch). Using faster turnover tissues like fin can complement these results by providing more precise t_{est} if the switch was more recent, for example, up to approximately 13 days post switch for fin. These quantifications of the effective time windows of different tissues inform the design of a field sampling regime to pinpoint the timing of resource switches. For example, by using an averaged fin-mucus-scale clock, samples would only need to be collected roughly every nine weeks rather than the analogous eight weeks if using a clock derived from scale alone (Figs. 4 and 5).

In this study I parameterized and formulated stable isotope clocks to estimate the timing of resource shifts of *O. mykiss.* The parameters generated in this paper will hopefully provide a strong base for future stable isotope studies of this common fish (see Deniro and Epstein 1981; Gannes et al. 1997; Martínez del Rio and Wolf 2005). More generally, through model fitting and an information theoretic approach, this study provides a glimpse into the physiological underpinnings of isotope dynamics. Furthermore, through exploring the strengths and limitations of different clock formulations, this study illustrates how estimates of resource switch timing are affected by clock formulations as well as the tissue turnover models used to derive them. Stable isotope studies will be the most effective when they can link laboratory, theory, and field-based insights (Martínez del Rio et al. 2009; Layman et al. 2012).

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LITERATURE CITED

Bauchinger U, McWilliams S (2009) Carbon turnover in tissues of a passerine bird: allometry, isotopic clocks, and phenotypic flexibility in organ size. Physiological and Biochemical Zoology 82:787-97

Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the use of stable isotopes in trophic ecology. Annual Review of Ecology, Evolution, and Systematics 42:411-440

Buchheister A, Latour RJ (2010) Turnover and fractionation of carbon and nitrogen stable isotopes in tissues of a migratory coastal predator, summer flounder *(Paralichthys dentatus)*. Canadian Journal of Fisheries and Aquatic Sciences 67:445-461

Burnham KP, Anderson DR (2010) Model selection and multi-model inference: a practical information-theoretic approach. Springer

Cambray JA (2003) Impact on indigenous species biodiversity caused by the globalisation of alien recreational freshwater fisheries. Hydrobiologia 500:217-230

Carleton SA, Kelly L, Anderson-Sprecher R, Martínez del Rio C (2008) Should I use one-, or multi-compartment models to describe 13C incorporation into animal tissues? Rapid Communications in Mass Spectrometry 22:3008-3014

Carleton SA, Martínez del Rio C (2010) Growth and catabolism in isotopic incorporation: a new formulation and experimental data. Functional Ecology 24:805-812

Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors (Δ15N and Δ13C): the effect of diet isotopic values and applications for diet reconstruction. Journal of Applied Ecology 46:443-453

Cerling TE, Ayliffe LK, Dearing MD, Ehleringer JR, Passey BH, Podlesak DW, Torregrossa A-M, West AG (2007) Determining biological tissue turnover using stable isotopes: the reaction progress variable. Oecologia 151:175-189

Church MR, Ebersole JL, Rensmeyer KM, Couture RB, Barrows FT, Noakes DLG (2009) Mucus: a new tissue fraction for rapid determination of fish diet switching using stable isotope analysis. Canadian Journal of Fisheries and Aquatic Sciences 66:1-5

Deniro MJ, Epstein S (1981) Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Acta 45:341-351

Essington TE, Kitchell JF, Walters CJ (2001) The von Bertalanffy growth function, bioenergetics, and the consumption rates of fish. Canadian Journal of Fisheries and Aquatic Sciences 58:2129-2138

Gannes LZ, Martínez del Rio C, Koch P (1998) Natural abundance variations in stable isotopes and their potential uses in animal physiological ecology. Comparative Biochemistry and Physiology 119:725-737

Gannes LZ, O'Brien DM, Martínez del Rio C (1997) Stable isotopes in animal ecology: assumptions, caveats and a call for more laboratory experiments. Ecology 78:1271-1276

Guelinckx J, Maes J, Van Den Driessche P, Geysen B, Dehairs F, Ollevier F (2007) Changes in δ13C and δ15N in different tissues of juvenile sand goby *Pomatoschistus minutus*: a laboratory diet-switch experiment. Marine Ecology Progress Series 341:205-215

Gustafson RG, Waples RS, Myers JM, Weitkamp LA, Bryant GJ, Johnson OW, Hard JJ (2007) Pacific salmon extinctions: quantifying lost and remaining diversity. Conservation biology 21:1009-1020

Hayes SA, Bond MH, Hanson CV, Jones AW, Ammann AJ, Harding JA, Collins AL, Perez J, Macfarlane RB (2011) Down , up , down and "smolting" twice? Seasonal movement patterns by juvenile steelhead *(Oncorhynchus mykiss)* in a coastal watershed with a bar closing estuary. Canadian Journal of Fisheries and Aquatic Sciences 68:1341-1350

Herzka SZ, Holt SA, Holt GJ (2001) Documenting the settlement history of individual fish larvae using stable isotope ratios : model development and validation. Journal of Experimental Marine Biology and Ecology 265:49-74

Hesslein R, Hallard K, Ramlal P (1993) Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish *(Coregonus nasus)* in response to a change in diet traced by δ34S, δ13C, and δ15N. Canadian Journal of Fisheries and Aquatic Sciences 50:2071–2076

Hobson KA, Clark RG (1992) Assessing avian diets using stable isotopes I: turnover of 13C in tissues. The Condor 94:181-188

Klaassen M, Piersma T, Korthals H, Dekinga A, Dietz MW (2010) Single-point isotope measurements in blood cells and plasma to estimate the time since diet switches. Functional Ecology 24:796-804

Koch PL, Fogel ML, Tuross N (1994) Tracing the diets of fossil animals using stable isotopes. In: Lajtha K, B. M (eds) Stable Isotopes in Ecology and Environmental Science. Blackwell Scientific Publication, Boston, pp 63-92

Kurle CM (2009) Interpreting temporal variation in omnivore foraging ecology via stable isotope modelling. Functional Ecology 23:733-744

Layman CA, Araujo MS, Boucek R, Hammerschlag-Peyer CM, Harrison E, Jud ZR, Matich P, Rosenblatt AE, Vaudo JJ, Yeager LA, Post DM, Bearhop S (2012) Applying stable isotopes to examine food-web structure: an overview of analytical tools. Biological Reviews:doi: 10.1111/j.1469-185X.2011.00208.x

Logan J, Haas H, Deegan L, Gaines E (2006) Turnover rates of nitrogen stable isotopes in the salt marsh mummichog, *Fundulus heteroclitus*, following a laboratory diet switch. Oecologia 147:391-395

MacAvoy SE, Macko SA, Arneson LS (2005) Growth versus metabolic tissue replacement in mouse tissues determined by stable carbon and nitrogen isotope analysis. Canadian Journal of Zoology 83:631-641

Martínez del Rio C, Anderson-Sprecher R (2008) Beyond the reaction progress variable: the meaning and significance of isotopic incorporation data. Oecologia 156:765-772

Martínez del Rio C, Wolf BO (2005) Mass-balance models for animal isotopic ecology. In: Starck MJ, Wang T (eds) Physiological and ecological adaptations to feeding in vertebrates. Science Publishers, Enfield

Martínez del Rio C, Wolf N, Carleton SA, Gannes LZ (2009) Isotopic ecology ten years after a call for more laboratory experiments. Biological Reviews 84:91-111

McCutchan JH, Lewis WM, Kendall C, McGrath CC (2003) Variation in trophic shift or stable isotope ratios of carbon, nitrogen and sulphur. Oikos 102:378-390

McIntyre PB, Flecker AS (2006) Rapid turnover of tissue nitrogen of primary consumers in tropical freshwaters. Oecologia 148:12-21

Moyle PB (2002) Inland fishes of California. University of California Press, Berkeley

Newsome SD, Clementz MT, Koch PL (2010) Using stable isotope biogeochemistry to study marine mammal ecology. Marine Mammal Science 26:509-572

Oppel S, Powell AN (2010) Carbon isotope turnover in blood as a measure of arrival time in migratory birds using isotopically distinct environments. Journal of Ornithology 151:123-131

Ott RL (1993)An Introduction to Statistical Methods and Data Analysis, Fourth Edi. Duxbury Publishers, Belmont

Pearson SF, Levey DJ, Greenberg CH, Martínez del Rio C (2003) Effects of elemental composition on the incorporation of dietary nitrogen and carbon isotopic signatures in an omnivorous songbird. Oecologia 135:516-523

Phillips DL, Eldridge PM (2006) Estimating the timing of diet shifts using stable isotopes. Oecologia 147:195-203

Pinnegar JK, Polunin NVC (1999) Differential fractionation of δ13C and δ15N among fish tissues: implications for the study of trophic interactions. Functional Ecology 13:225-231

Podlesak DW, Mcwilliams SR, Hatch KA (2005) Stable isotopes in breath, blood, feces and feathers can indicate intra-individual changes in the diet of migratory songbirds. Oecologia 142:501-510

R Development Core Team (2008) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Richards FJ (1959) A flexible growth function for empirical use. Journal of Experimental Botany 10:290-300

Shapovalov L, Taft AC (1954) The life histories of the steelhead rainbow trout *(Salmo gairdneri)* and silver salmon *(Oncorhynchus kisutch)* with special reference to Waddell Creek, California and recommendations regarding their management. California Department of Fish and Game Fish Bulletin 98:373p

Studds CE, Kyser TK, Marra PP (2008) Natal dispersal driven by environmental conditions interacting across the annual cycle of a migratory songbird. Proceedings of the National Academy of Sciences 105:2929-33

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: implications for δ13C analysis of diet. Oecologia 57:32-37

TABLES

Table 1 Overview of tissue turnover models compared in model selection and used to generate three different isotopic clocks. These four models were created by varying two factors. First, models differed by how they dealt with tissue isotope signatures: equilibrium tissue models did not deal with prey isotope signatures and instead modeled tissue isotope signature; in contrast, discrimination factor models used prey isotope signatures and discrimination factors. Second, models differed by the number of turnover compartments: one compartment models use a single pool through which isotopes turn over at a single rate; in constrast, two compartment versions allowed for the turnover to be described by two different turnover rates with different proportional contributions to the overall tissue turnover. Parameter estimates from each of the above described tissue turnover models may be used in combination with field measurements in one of three clocks. Single-tissue clocks back calculate the timing of resource switch using information from one tissue, whereas algebraic two-tissue clocks perform a single algebraic calculation of the timing of resource switch using data from two different tissues. Averaged clocks take the mean of two or more independently calculated single-tissue clocks.

Model name	**Abbreviation**	**Equation(s)**
Equilibrium tissue—one compartment	ET 1	(1)
Discrimination factor—one compartment	DF 1	(2)
Equilibrium tissue—two compartment	ET 2	(1)
Discrimination factor—two compartment	DF 2	(2)
Clock name		
Single-tissue clock		(3 and 4)
Algebraic two-tissue clock		(5 and 6)
Averaged clocks		(3 and 4)

Table 2 Parameter estimates for seven *O. mykiss* tissues using either a one- or two-compartment equilibrium tissue model, or one- or two-compartment single discrimination factor model (respectively as; ET 1 and ET 2, Eq. 1; and DF 1 and DF 2, Eq. 2). Estimates ± SE of the fractional size of compartment one *(p)*, with $p = 1$ for one-compartment models, average residence time for each compartment (τ_1 and τ_2)(days), equilibrium tissue values (δX_{Pre} and δX_{Post})(‰), discrimination factor (Δ)(‰) and τ_{mean}, where $\tau_{mean} = p\,\tau_1 + (1-p)\,\tau_2$ (Carleton et al. 2008). Tissues are ordered from fastest turnover to slowest, and models are ranked by AICc score within each tissue, with lowest AICc scores being the best supported by the data.

Tissue	Model	p in τ_1	τ_1	τ_2	δX_{Pre}	δX_{Post}	Δ	τ_{mean}	AICc
Fin	**ET 1**	1	12.9±1.1	--	10.6±0.1	15±0.1	--	12.9±1.1	82.9
Fin	**ET 2**	0.9±0.2	14.3±3.4	2.5±6.6	10.5±0.1	15.1±0.1	--	13.3	86.6
Fin	**DF 2**	0.7±0.1	19.4±3	0.8±0.4	--	--	1.6±0.1	14.5	133.2
Fin	**DF 1**	1	12.4±1.3	--	--	--	1.8±0.1	12.4±1.3	160.1
Plasma	**ET 2**	0.7±0.1	20±5.2	2.6±1.4	10.4±0.2	15.6±0.1	--	14.1	103.5
Plasma	**ET 1**	1	11.8±1.1	--	10.7±0.1	15.5±0.1	--	11.8±1.1	113
Plasma	**DF 2**	0.6±0.1	26.1±5.7	2.1±0.7	--	--	2.1±0.1	16.4	116
Plasma	**DF 1**	1	11±1.1	--	--	--	2.1±0.1	11±1.1	154.7
Liver	**ET 2**	0.6±0.1	25±7.2	2.4±1.1	9.9±0.2	14.9±0.1	--	16.1	117.7
Liver	**DF 2**	0.5±0.1	38.1±9.5	2.1±0.6	--	--	1.5±0.1	21.3	132.1
Liver	**ET 1**	1	12.3±1.5	--	10.3±0.2	14.7±0.1	--	12.3±1.5	133.1
Liver	**DF 1**	1	11.1±1.4	--	--	--	1.4±0.1	11.1±1.4	182.2
Mucus	**DF 2**	0.7±0	49.7±7.6	2.3±0.9	--	--	1.3±0.1	35.7	102.7
Mucus	**ET 2**	0.7±0.1	43.1±7.9	2.3±1.1	9.3±0.2	15±0.2	--	32.2	102.8
Mucus	**ET 1**	1	27.1±2.9	--	9.8±0.1	14.7±0.2	--	27.1±2.9	122.3
Mucus	**DF 1**	1	34.8±3.7	--	--	--	1.7±0.1	34.8±3.7	149.6
RBC	**ET 1**	1	37.6±2.5	--	9.9±0.1	15.3±0.1	--	37.6±2.5	60
RBC	**DF 2**	0.9±0	42.7±3	0.3±1	--	--	1.7±0.1	39.7	71.6
RBC	**DF 1**	1	43±2.8	--	--	--	1.9±0.1	43±2.8	78.7
Muscle	**ET 1**	1	39±3.2	--	11.2±0.1	16±0.1	--	39±3.2	68.4
Muscle	**DF 2**	0.3±0.1	657.9±1084.2	35.4±6.9	--	--	3.4±0.1	198.8	70.3
Muscle	**ET 2**	0.9±0	41.6±4.7	1.4±2.8	11.1±0.1	16.1±0.1	--	39.6	71.3
Muscle	**DF 1**	1	63.3±5.5	--	--	--	3.3±0.1	63.3±5.5	114.7
Scale	**ET 1**	1	40±2.8	--	10.2±0.1	15.3±0.1	--	40±2.8	56
Scale	**DF 1**	1	52.3±3.8	--	--	--	2.2±0.1	52.3±3.8	94.2

The ET 2, two-compartment model for RBC, and both two-compartment models for Scale failed due to singular Hessians.

FIGURES

Figure 1 δ15N tissue turnover for *O. mykiss* fin, plasma, liver, mucus, RBC, muscle, and scale (fastest to slowest with faster turnover tissues on the left and slower turnover tissues on the right) and model fits of one- (solid line) and two-compartment (dotted line) equilibrium tissue models (Eq. 1). Inset for each panel is the observed vs. expected $\delta^{15}N$

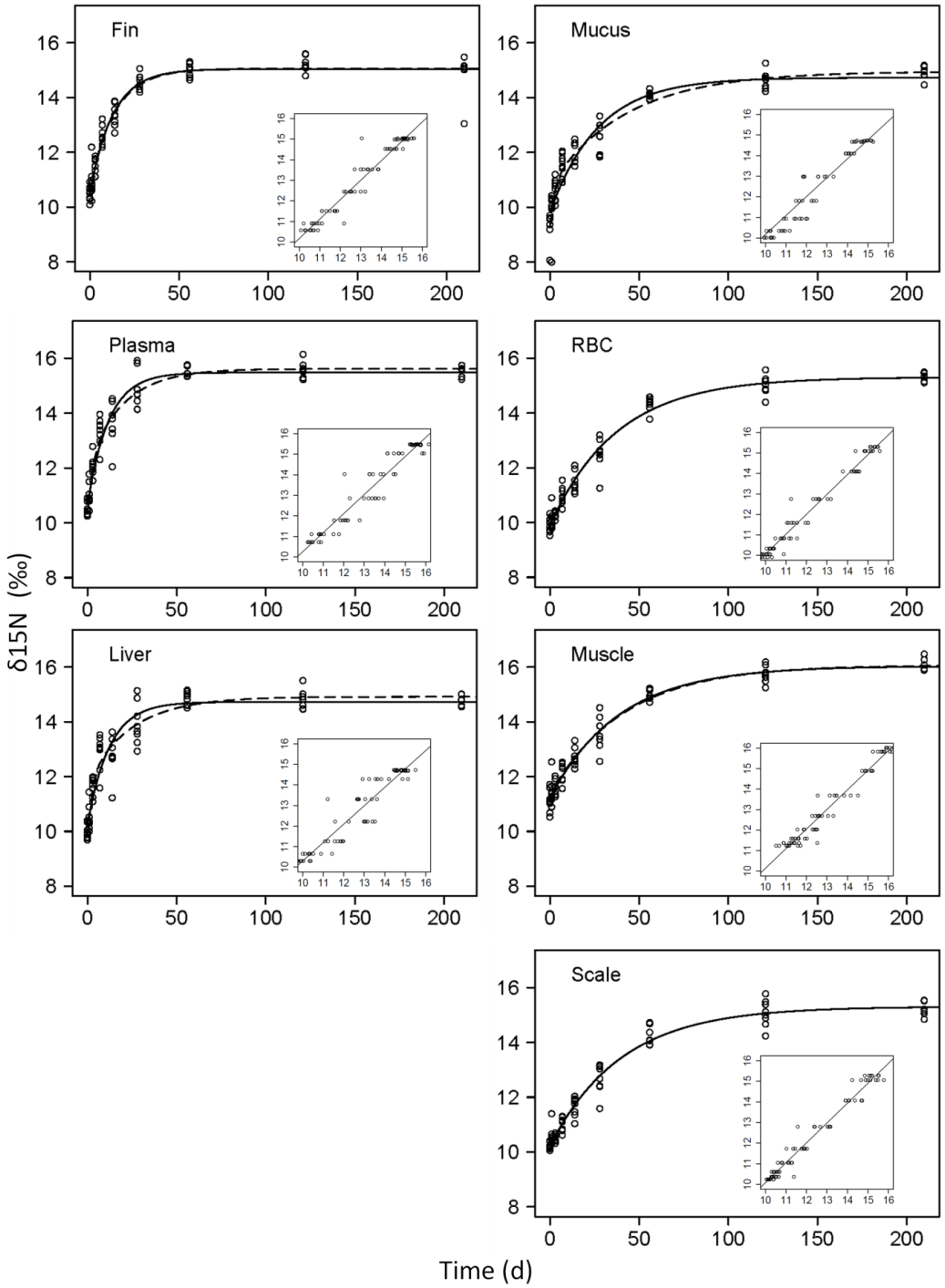


Figure 2 Mass (g) through time of *O. mykiss* sampled 0 to 200 days during the diet-switch experiment and best fit of a "specialized" von Bertalanffy growth equation: $(Mass_t = 557 * (1 - 0.78\ exp(-0.00595 * Day))^{2.27})$

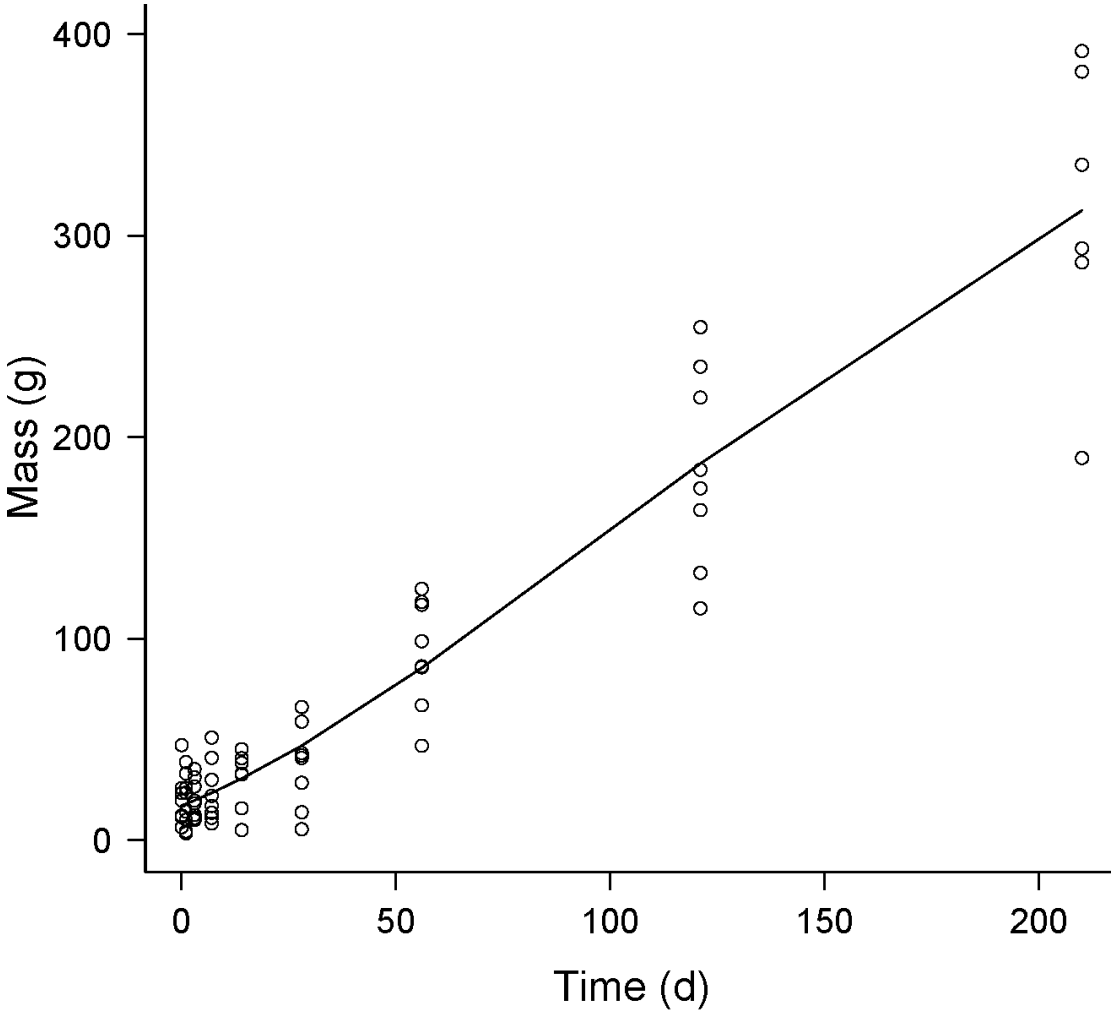


Figure 3 *O. mykiss* one-compartment discrimination factors (Δ) Eq. 2 (top), and average residence times (τ) (bottom) based on equilibrium tissues (Eq. 1, open circles) and diet and discrimination factors (Eq. 2, closed circles); error bars are SE

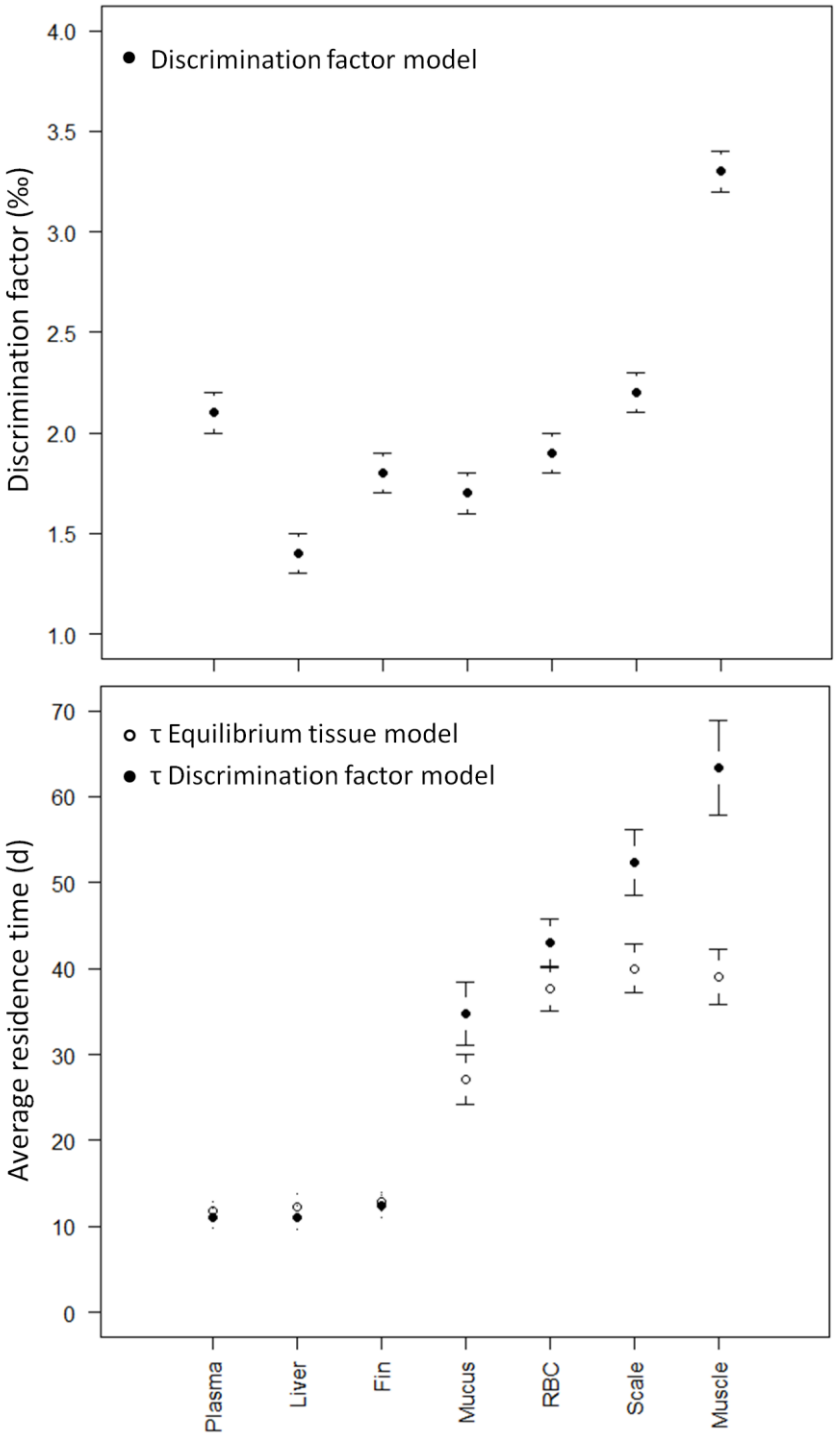


Figure 4 Estimated time (test) vs. actual time (tactual) for one-compartment equilibrium single-tissue clocks (Eq. 3) ordered fastest turnover to slowest. To better represent each clock, axes for panels are scaled differently. The 1:1 line is shown for reference. Circles are calculated t_{est} from our diet-switch fish. Lines are bootstrapped 95% prediction intervals and median t_{est}. Solid lines represent t_{est} for which no log(0) was returned, dashed lines represent t_{est} for which at least one log(0) was returned

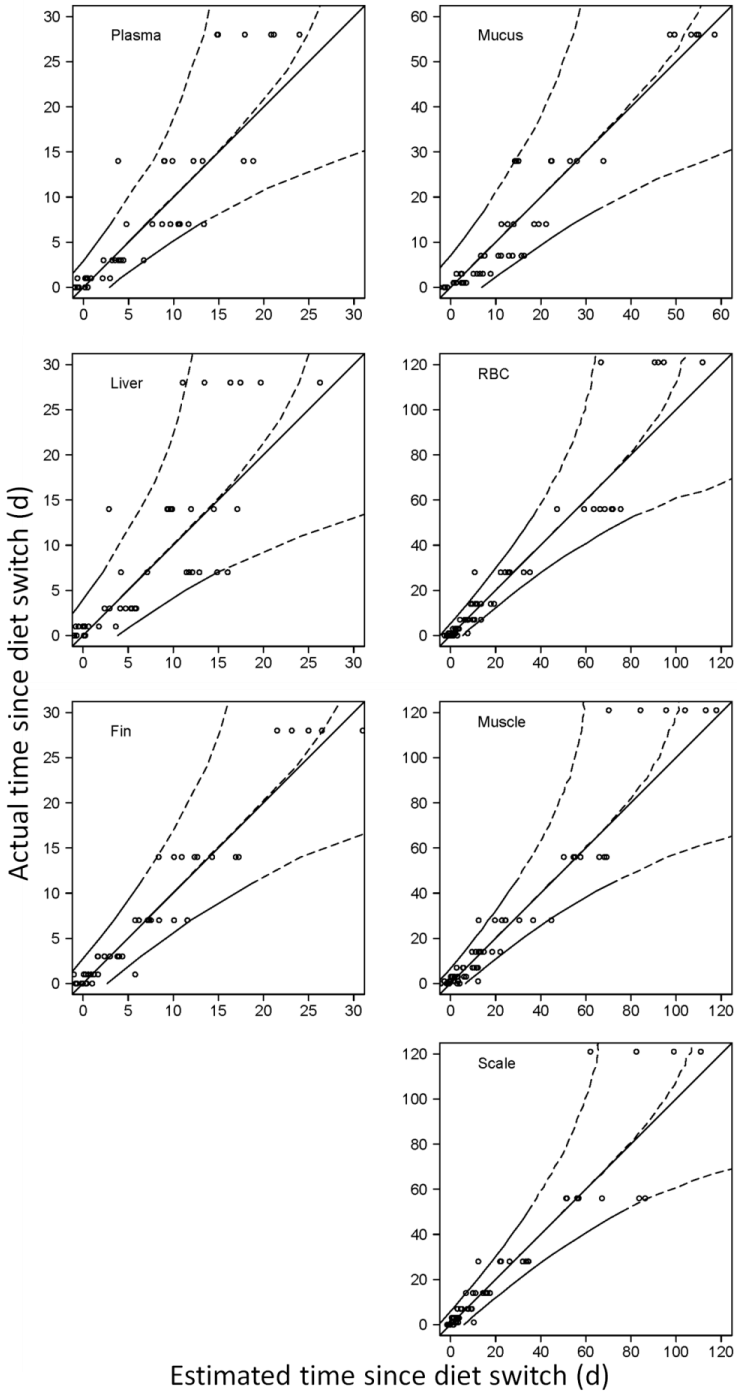
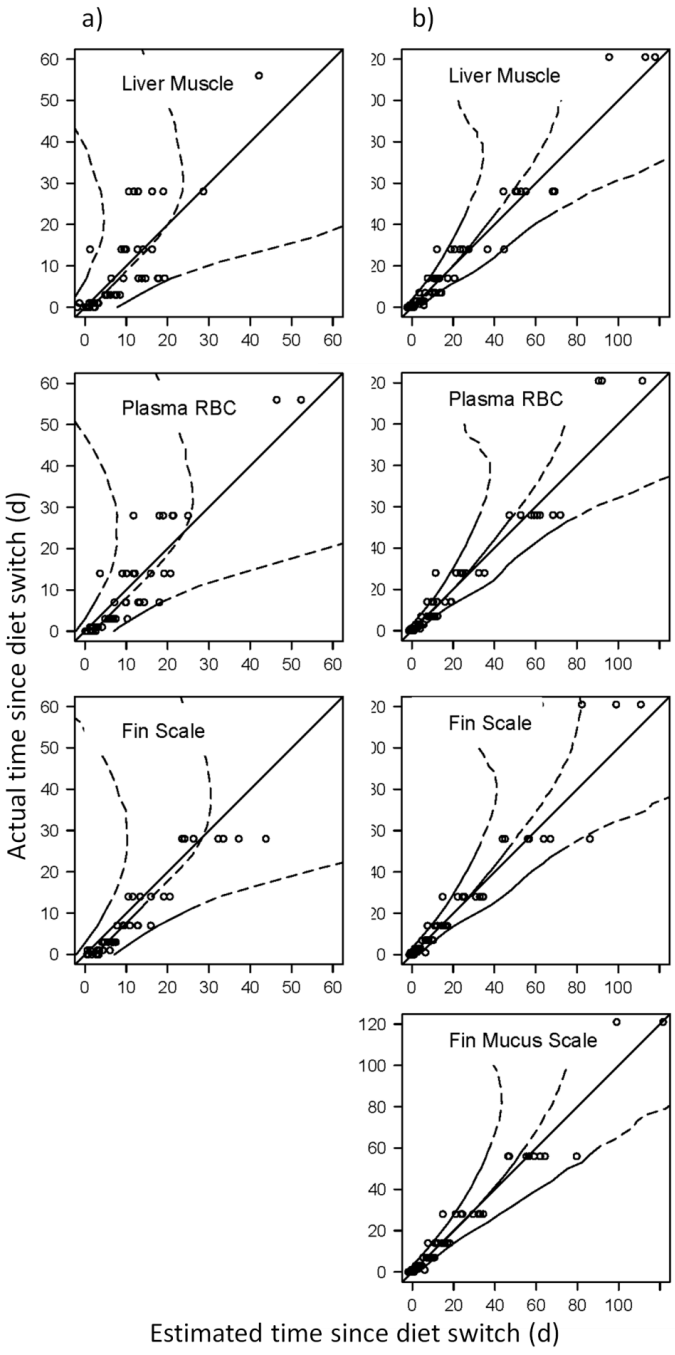


Figure 5 Estimated time (test) vs. actual time (tactual) for one-compartment equilibrium a) algebraic two-tissue and b) averaged clocks for four different tissue combinations. To better represent each clock, axes for panels are scaled differently. The 1:1 line is shown for reference. Circles are calculated t_{est} from our diet-switch fish. Lines are bootstrapped 95% prediction intervals and median t_{est}. Solid lines represent t_{est} for which no log(0) was returned, dashed lines represent t_{est} for which at least one log(0) was returned



APPENDIX 1

Figure A1 Seasonal variation in discharge from Camanche Dam in cubic meters per second (m3 s-1) averaged over the period from 1965 to 2010. Data were collected from USGS National Water Information System.

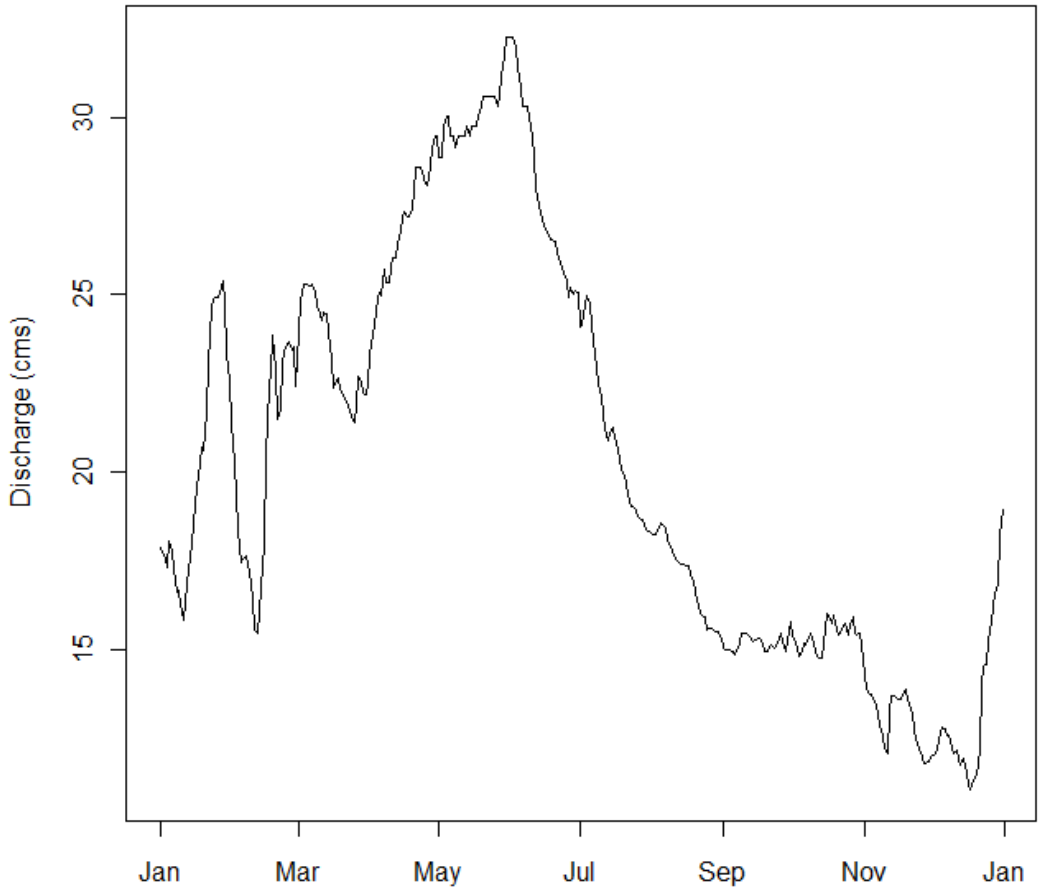


Figure A2 Frequency histograms of fork lengths (mm) of resident fish in 2007, residents in 2008, and emigrants in 2008. The single emigrant in 2007 (FL = 225 mm) was not plotted. All emigrants in 2008 were captured with rotary screw traps below WIDD.

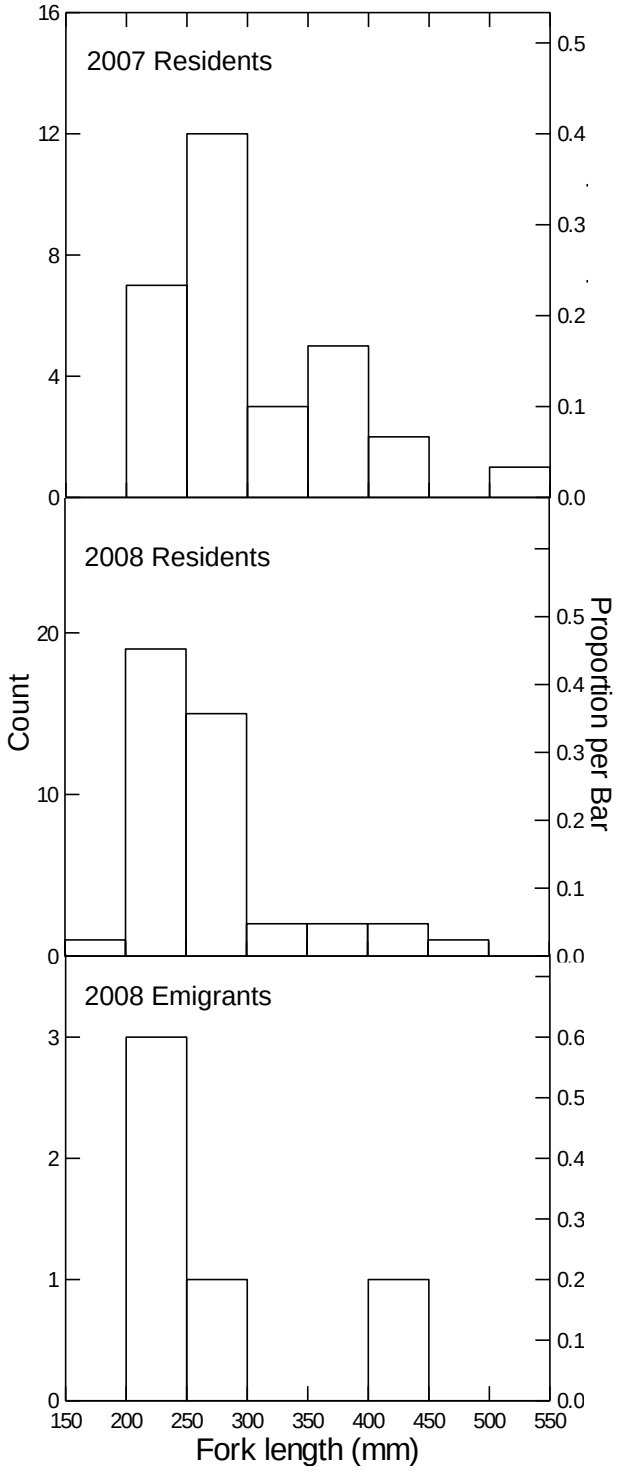


Figure A3 Frequency histograms of standardized movement (km/mo) of individual resident *O. mykiss* in 2007 and 2008.

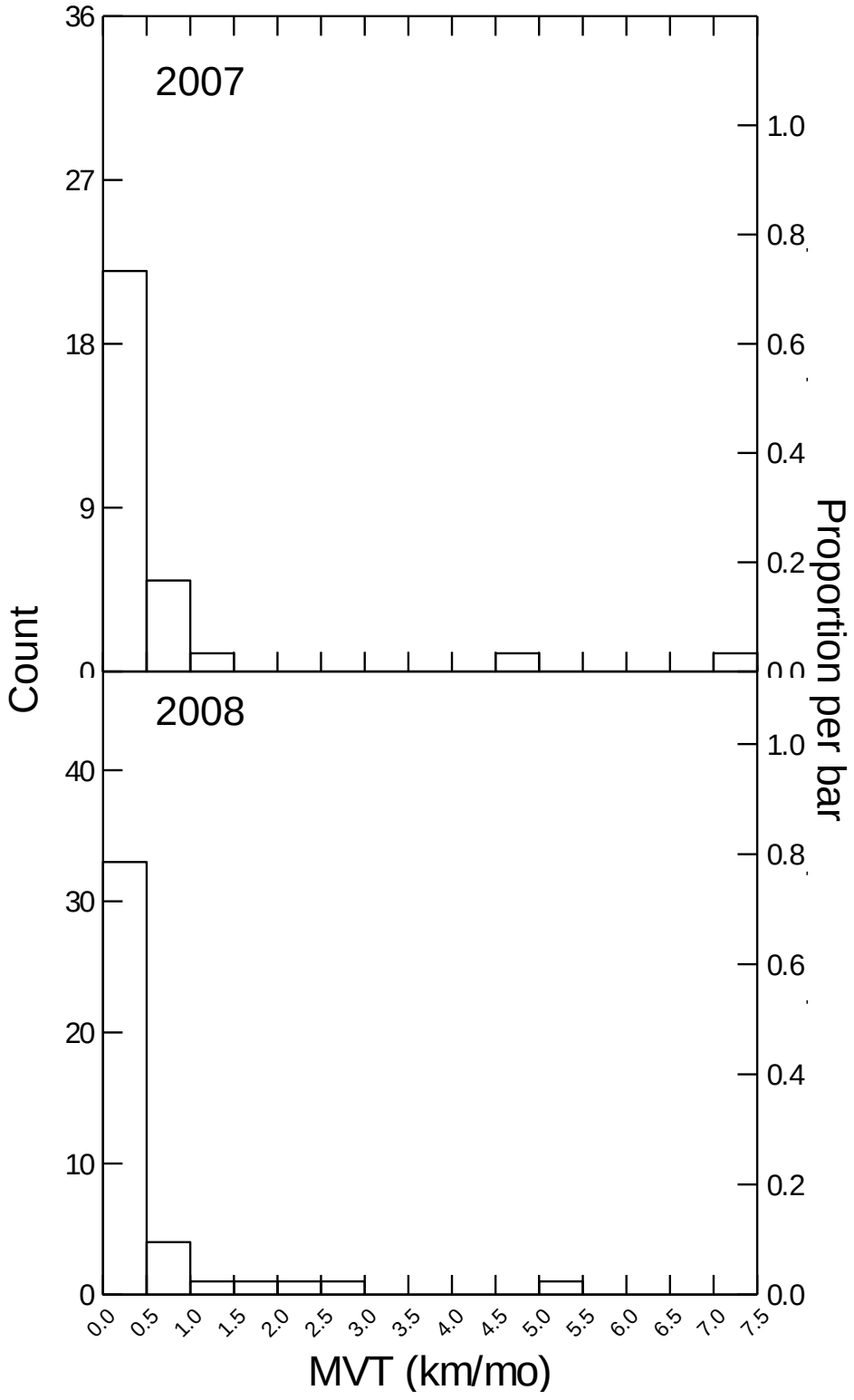
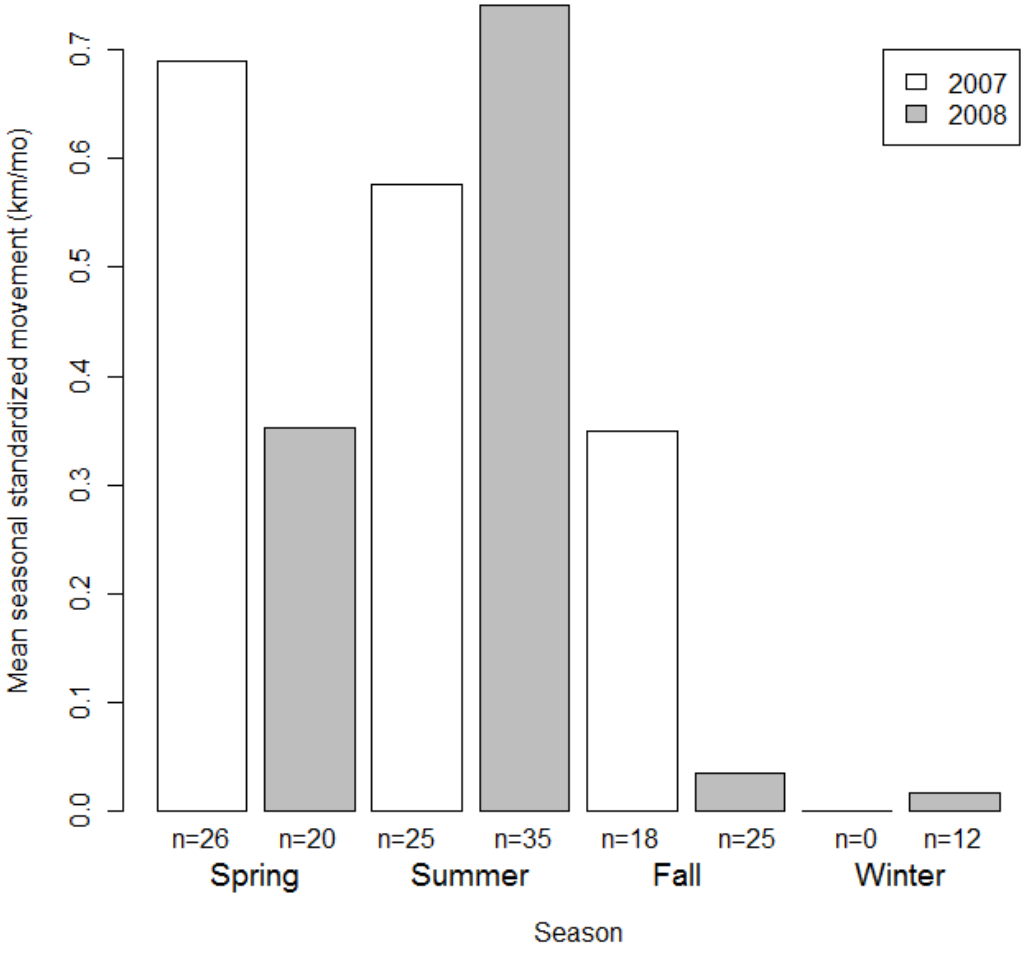


Figure A4 Means of seasonal movement (mvts km / mo) for each season for 2007 and 2008 (white and grey respectively). Sample sizes of resident fish within each season are below each bar.



APPENDIX 2 (ESM)

Tissue turnover and stable isotope clocks to quantify resource shifts in anadromous rainbow trout

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Growth effects on isotopic tissue turnover

Tissue turnover can reflect rates of growth and catabolic tissue replacement. While our study wasn't explicitly designed to examine the relative importance of growth on turnover, there was enough emergent variation in individual growth to explore these relationships. We explored the relationship between growth rate and isotopic tissue turnover within our data set with three main approaches: 1. we modeled growth of our laboratory fish, 2. plotted the three-way relationship between $\delta^{15}N$, individual growth rate and time after our diet-switch, and 3. modeled isotopic tissue turnover as the sum of individual fractional net growth *(k)* and the fraction of turnover due to catabolic tissue replacement *(m)* ($1/\tau = \lambda = k+m$; Hesslein et al. 1993).

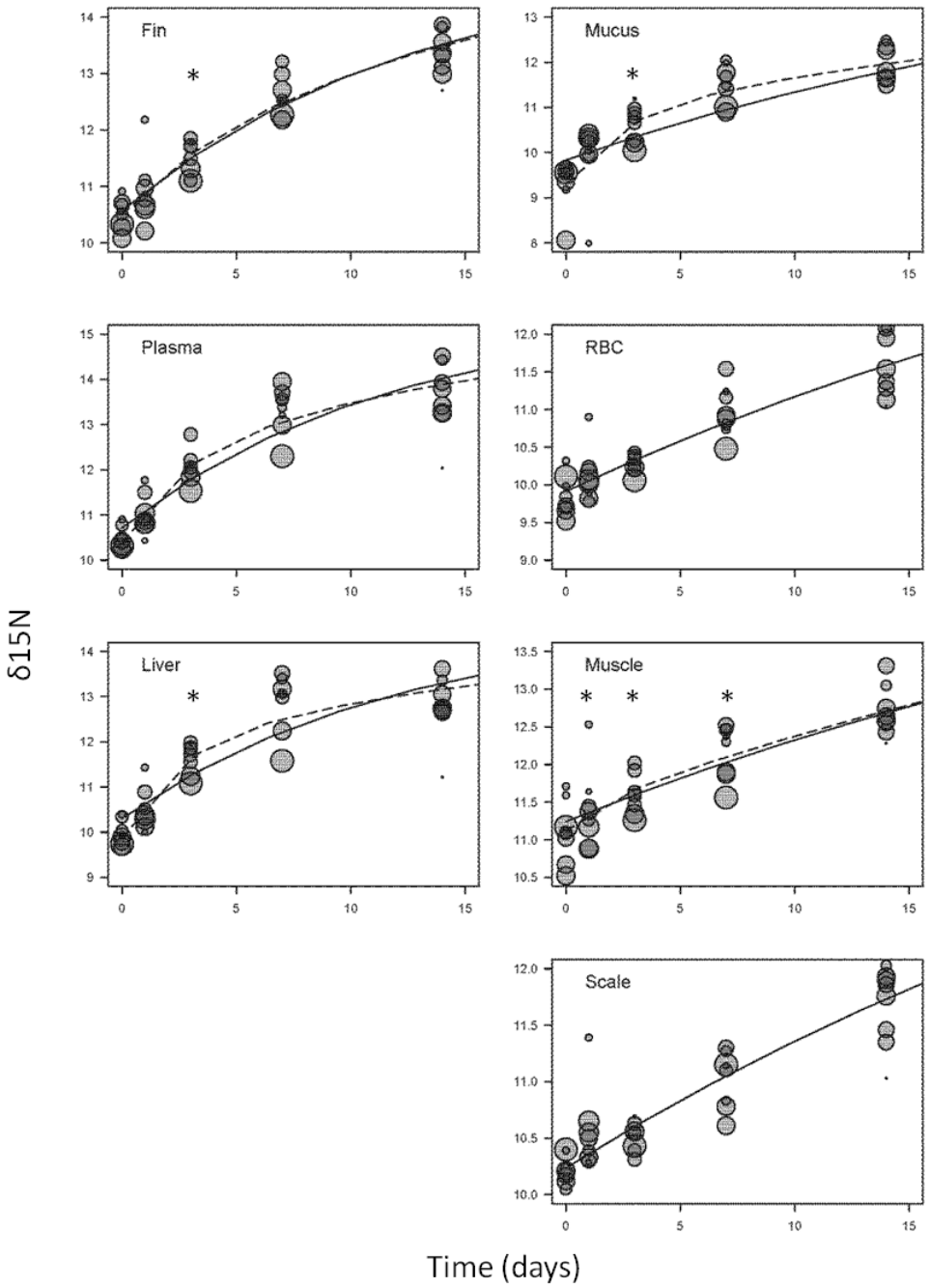
To describe the pattern of growth for our experimental fish, we compared relative support for three growth models common to fisheries studies: exponential, von Bertalanffy and Richards using AICc scores (Burnham and Anderson 2010). The mass of the experimental fish was best described by the Richards model (Table S1).

Table S1. Growth models and best estimated parameters using mass of individual fish from our laboratory experiment. AICc scores are shown for comparison of relative model support, with lover AICc scores indicating higher model support.

		AIC_c
Richards	$Mass\ at\ time\ t = 557 * (1 - 0.78 * \exp(-0.00595 * t))^{2.27}$	681.8
von Bertalanffy	$Mass\ at\ time\ t = 5048 * (1 - \exp(-0.0015 * t))^{3}$	682.2
exponential	$Mass\ at\ time\ t = 38.8 * \exp(0.01 * t)$	705.0

We also calculated Z-scores of relative growth for each fish within each sampling period as *Z = (growth – mean growth)/SD growth*. Fig. S1. presents the relationship between isotopic tissue turnover, individual relative growth (Z-score) and time, with bubble size related to relative growth rate. Interestingly, tissue-day combinations that a significant relationship was found using independent linear regressions (marked with an *) showed counterintuitive patterns. Accelerated growth is expected to increase isotopic turnover rate (Phillips and Eldridge 2006), yet in Fig. S1, the most striking pattern shows evidence of individuals with relatively higher growth rates to show lower than expected $\delta^{15}N$ values. This could be due to allometry (McIntyre and Flecker 2006). However, this could also simply be due to individual variation (Deniro and Epstein 1981), and a reflection that we did not explicitly design this experiment to look at growth effects on isotopic tissue turnover (Carleton and Martínez del Rio 2010).

Fig. S1 δ15N tissue turnover for *O. mykiss* fin, plasma, liver, mucus, RBC, muscle, and scale (fastest to slowest with faster turnover tissues on the left and slower turnover tissues on the right) and model fits of one- (solid line) and two-compartment (dotted line) equilibrium tissue models (Eq. 1) for days 0 through 15. Bubbles represent Z-scores of individual relative growth of sampled fish with larger bubble size indicating higher relative growth. Asterisks indicate days for which there was a significant relationship between growth and turnover (Table S2)



Lastly, we investigated the relative contribution of growth *(k)* and catabolic tissue replacement *(m)* to isotopic tissues turnover ($1/\tau = \lambda = k+m$; Hesslein et al. 1993). We used growth *(k)* calculated for each individual fish $k = (ln(W/W_o)/t)$ as an input to our isotopic turnover modeling using Eq. 1 and estimated catabolic replacement *(m)* (Hesslein et al. 1993). Estimated contributions of fractional growth and catabolic tissue replacement are presented for each tissue in Table S1. This modeling did not affect estimates or standard error of either equilibrium tissue value. Dividing turnover rate into the relative contribution of growth and catabolic tissue replacement did not affect model support for fin, RBC, and scale as indicated by ΔAICc scores < 2 (Table S1, Burnham & Anderson, 2010). The relative lack of support for dividing turnover into growth and catabolic tissue replacement as indicated by ΔAICc > 10 for liver and mucus is most likely due to their data supporting the use of two-compartments when modeling tissue turnover. With ΔAICc of approximately seven, the data itself, and other ecological and physiological insight must be considered for plasma, also best supporting two-compartment turnover, and muscle. Calculated growth rates varied among individuals from 0.014 to 0.034 * d^{-1} with a mean of 0.024 * d^{-1}. By dividing isotopic tissue turnover into growth and catabolic tissue turnover we found that turnover in more structural tissues is dominated by growth, and that catabolic tissue replacement increases turnover rate in other tissues like liver, plasma and fin. It is interesting that turnover in fin, a tissue of assumed structural importance is dominated by catabolic tissue replacement. Elevated

catabolic tissue replacement and resulting increased isotopic tissue turnover may result from an increased need to repair this important structural tissue from continual degradation due to abrasion, friction or removal by other con-specifics or other species.

Table S1. Proportional contribution of growth and metabolism to isotopic tissue turnover modeled using Eq.1 with $\lambda = 1/\tau$. We modeled λ as $(k + m)$, where k were input as calculated individual growth $k = (ln(W/W_o)/t)$(Hesslein et al. 1993), and m was estimated. We also modeled λ without dividing it into $(k + m)$ to calculate the % contribution of growth and metabolism to isotopic tissue turnover. We present AICc values for each tissue turnover model and ΔAICc as the difference between this model and the most supported tissue turnover model from Table 1 within each tissue.

		%growth	% metabolism	AICc	ΔAICc
Fin	$N15 = 15 - 4.5 * \exp -(k + 0.0526) * t)$	32.0	68.0	84.5	1.6
Plasma	$N15 = 15.5 - 4.8 * \exp -(k + 0.0580) * t)$	31.7	68.3	110.9	7.4
Liver	$N15 = 14.7 - 4.4 * \exp -(k + 0.0533) * t)$	34.3	65.7	131.6	13.9
Mucus	$N15 = 14.7 - 4.9 * \exp -(k + 0.0118) * t)$	68.0	32.0	120.8	18.1
RBC	$N15 = 15.5 - 5.4 * \exp -(k + 0.0018) * t)$	93.4	6.6	58.1	-1.9
Muscle	$N15 = 16.0 - 4.8 * \exp -(k + 0.0016) * t)$	93.9	6.1	76.3	7.9
Scale	$N15 = 15.3 - 5.1 * \exp -(k + 0.0002) * t)$	99.3	0.7	53.1	-2.9

Multiple compartment clocks

We compared single-tissue clocks derived both one- and two-compartment versions of Eqs. 1 and 2. To do this we used the same clock approach described in the main text but allowed for two-compartments as:

Single-tissue clock using equilibrium tissue values:

$$\boldsymbol{t_{est} = -(p\tau_1 + (1-p)\tau_2) * \ln\left(\frac{\delta X_{Post} - \delta X_t}{\delta X_{Post} - \delta X_{Pre}}\right).}$$

Single-tissue clock using a single discrimination factor:

$$\boldsymbol{t_{est} = -(p\tau_1 + (1-p)\tau_2) * \ln\left(\frac{(\delta X_{diet_2} + \Delta) - \delta X_t}{(\delta X_{diet_2} - \delta X_{diet_1})}\right).}$$

For a one-compartment model p=1. We used a bootstrapping routine to investigate how parameter uncertainty affects error in calculating t_{est}. To do this we took simultaneous random draws of parameters, (i.e. δX_{Pre}, δX_{Post}, p, τ_1 and τ_2 for Eq. 1, or Δ, p, τ_1 and τ_2 for Eq. 2) from the estimated multivariate normal defined by the parametric variance-covariance matrix of each model fit. Associated values of δX_t were drawn from a normal distribution produced from our model fitting. We iterated this 10,000 times for each true time (t_{true}) to produce sets of parameter estimates to be used for each different clock's calculation. Thus, we produced distributions (n=10,000) of t_{est} for each clock derived from the same parameter sets at each t_{true}.

We produced the median and the 95% prediction intervals from each clock's resampled distribution of t_{est}. Prediction intervals delineate the area within which 95% of all future calculated t_{est} will occur considering variability from measurement, environmental conditions, or individual physiology (Ott 1993). Thus, more precise clocks will have narrower prediction intervals and more accurate clocks will have median t_{est} values closer to the 1:1 line of observed to expected values.

Figure S2 shows that a one-compartment version of Eq. 1 using equilibrium tissue values returned the most accurate and precise t_{est} over the longest period of time for all tissues. This one-compartment clock using equilibrium tissue values even performed better for tissues where a two-compartment version of Eq. 1 was better supported by AICc to model tissue turnover (plasma, liver and mucus).

Fig. S2. Estimated time (test) vs. actual time (tactual) for single-tissue clocks derived from four different turnover models: a) a one-compartment version of Eq. 1 (with tissues ordered vertically from the fastest turnover tissue to the slowest; tissue order is maintained for the other three model frameworks for comparison); b) a two-compartment version of Eq. 1; c) a one-compartment version of Eq. 2; d) a two-compartment version of Eq. 2. Note that axes are not scaled the same for each tissue to better represent each clock. The 1:1 line is shown for reference. Circles are actual t_{est} calculated from our diet switch fish. Lines are 95% prediction intervals and median t_{est} from t_{est} resampled (n=10,000) from the estimated multivariate normal distributions of parameters. Solid lines represent t_{est} for which no log(0) was returned, dashed lines represent t_{est} for which at least one log(0) was returned. Tissue turnover curves were not solvable for scale for two-compartment models or for RBC for Model 1.2 and so clocks are not possible.