

CALIFORNIA FISH AND GAME

"Conservation of Wildlife Through Education"

Volume 100

Fall 2014

Number 4

Special Fisheries Issue



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Please direct correspondence to:
Vernon C. Bleich, Ph.D.
Editor-in-Chief
California Fish and Game
Vern.Bleich@wildlife.ca.gov

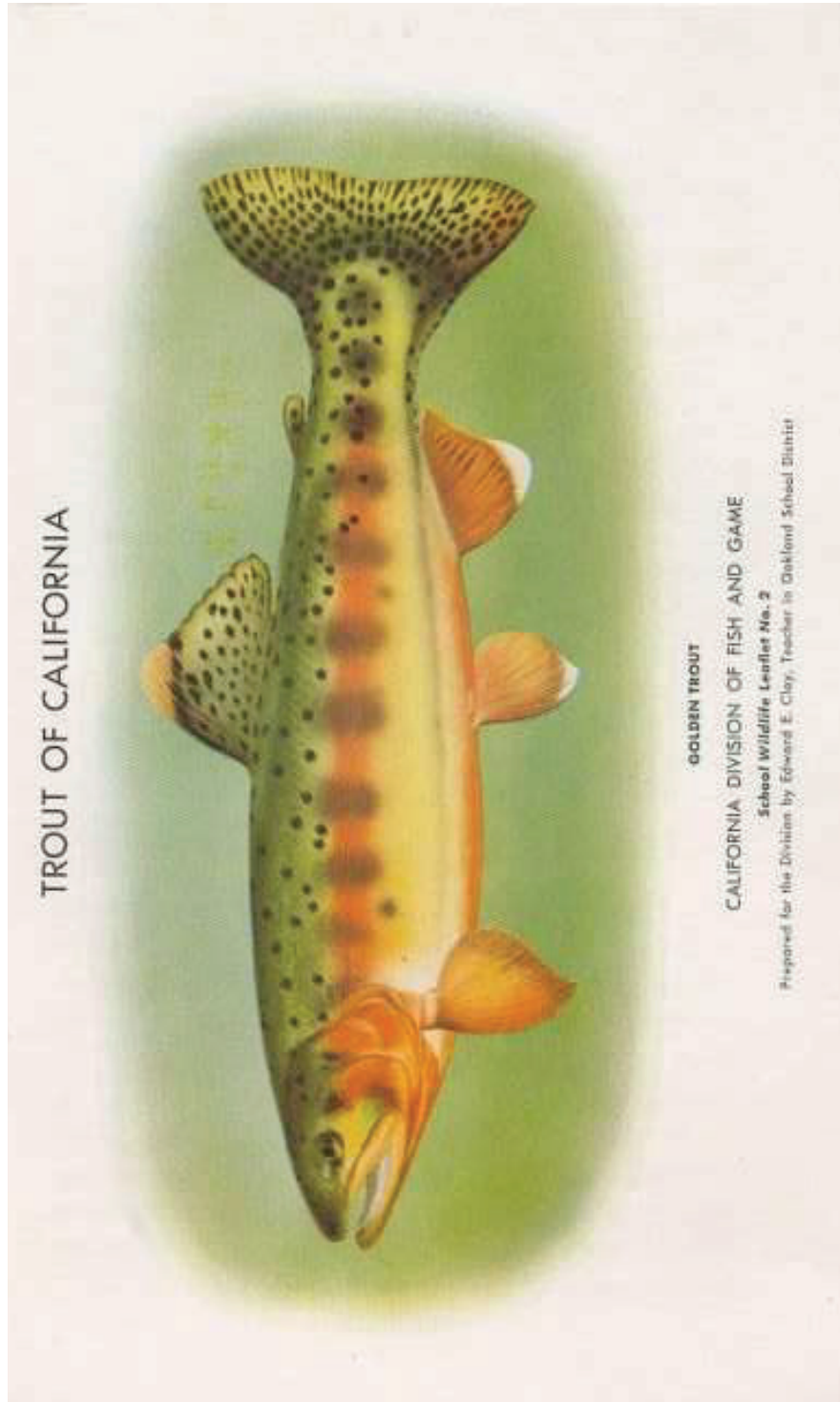


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FRONTISPIECE.—As part of an intensive educational program, the California Division of Fish and Game produced a series of wildlife leaflets for use in the schools of California. This drawing is from School Wildlife Leaflet 2, which was produced prior to 1918, although the actual year of publication has not been determined.

Notes from the Editor

Volume 100 of *California Fish and Game* is now complete. This special issue, with an emphasis on freshwater fisheries, includes a number of important contributions to our understanding of the ecology, management, or control of freshwater organisms. Among those discussed in this issue are endangered taxa, invasive species, and species native to California. This issue has been long in preparation, but the contents will be of substantial value to managers of freshwater fisheries and researchers working in the field of aquatic ecology.

It is essential that I extend my gratitude to Stafford Lehr, Kevin Shaffer, Roger Bloom, and Rob Titus, all of whom played substantial roles in arranging for reviewers, working directly with the corresponding authors, revising manuscripts, and providing me with near-final versions of each of the manuscripts. It also is appropriate to acknowledge the corresponding authors, all of whom met necessarily short deadlines associated with manuscript revisions and, especially, with respect to reading and returning page proofs. The level of professionalism reflected in those rapid responses is truly appreciated.

Director Chuck Bonham and retired fisheries biologist Phil Pister open this issue with a partial account of the evolution of fisheries management activities within the Department of Fish and Wildlife, a subject that is expanded upon in the last paper contained in this issue. Fran Pavley, Chair of the Senate Natural Resources and Water Committee also provides meaningful introductory comments.

Following the introductory material, Robert Holmes and his co-authors address spawning habitat selection by steelhead trout, Krystal Acierto and her co-authors describe a method and use it to estimate entrainment of Chinook salmon on to the Yolo Bypass, Gena Lasko et al. analyze in great detail the straying of hatchery-bred Chinook salmon into the American River, Sean Gallagher and co-authors present an analysis of the relationship between stream characteristics and salmonid abundance, Farhat Bajjaliya et al. examine morphometric differences in steelhead trout, and Dave Lentz and Mark Clifford collaborate on a history of California's inland trout management program, with an emphasis on legislation and litigation. Additionally, Quinn Granfors provides detailed information on an invasive catfish that has become locally established, along with suggestions for addressing the management of that non-native predator. Finally, Mark Clifford and his colleagues provide the results of detailed research on mechanisms involved in the early mortality of juvenile Chinook salmon exposed concurrently to Infectious Hematopoietic Necrosis Virus (IHNV) and esfenvalerate. Two book reviews also are included.

When researching material to consider as the frontispiece for this issue, I encountered numerous early papers written by well-known and influential individuals that addressed conservation and management needs. Nearly 100 years ago, conservation was at the forefront of the then Division of Fish and Game's mission, as it remains today. After selecting the image for the frontispiece, I spent a substantial amount of time reading numerous early contributions to professional journals. From those papers, I noted especially the views of four individuals, all of whom likely had a meaningful influence on the management and conservation of the freshwater and anadromous fisheries resources of the Golden State, and each of whom I quote below.

...during recent years, a public sentiment has developed which recognizes the value of the State's wildlife resources and demands that they be conserved for the benefit of those who are here to enjoy them now and for those who will come after us.

Ernest Schaeffle¹
California Fish and Game Commission

No nation can grow populous and great and long survive which, through lack of vision, continues to destroy those very resources which have made it great.

B. W. Evermann²
California Academy of Sciences

The time is here when the natural resources of our state should be conserved, for the attention of the world is upon California, both in business and a pleasure way.

F. M. Newbert³
California Fish and Game Commission

It is well known that the salmon fisheries of these [Sacramento and San Joaquin] rivers, as well as of the entire state, are greatly depleted.

G. H. Clark⁴
California Division of Fish and Game

It is not commonplace for this journal to include footnotes. Nevertheless, I have chosen to do so here because some of the readership may be interested in pursuing additional information regarding the history of wildlife and fisheries conservation in California. As this volume of *California Fish and Game* closes out its centennial year, the journal will begin its second century of publication. I trust that *California Fish and Game* will remain the valuable resource it has become, and that the papers published herein will continue to contribute in meaningful ways to the understanding, management, and conservation of California's fish and wildlife resources, all of which remain as important today as they were a century ago, but that also face challenges likely unimagined at the time the journal was founded.

Vernon C. Bleich, Ph.D.
Editor-in-Chief
California Fish and Game

¹Schaeffle, E. 1915. Fish and game: one of California's great resources. *California's Magazine* 1:159-176.

²Evermann, B. W. 1922. The conservation and proper utilization of our natural resources. *The Scientific Monthly* 15:289-312.

³Newbert, F. M. 1924. President Newbert's statement. *California Fish and Game* 10:121.

⁴Clark, G. H. 1929. Sacramento-San Joaquin salmon (*Oncorhynchus tshawytscha*) fishery of California. *Fish Bulletin* 17:1-75.

Introduction to volume 100: the special fisheries issue

In 1953 a young biologist, fresh out of graduate school, started as a Seasonal Aid with the California Department of Fish and Game. His name was Phil Pister. He is a co-author of this Introduction to the Special Fisheries Issue of the 100th year of *California Fish and Game*. Phil traces his fisheries conservation roots back to joining one of Starker Leopold's first wildlife classes at the University of California, Berkeley. We thought that this Introduction could take a tour through Phil's life and career as a way to help explain modern inland fisheries conservation in California as we know it today.

As one would expect of a brand new and grateful-to-have-a-job employee, he jumped right into the established fisheries management programs designed to supply good angling for ever-increasing numbers of California anglers following World War II. Those programs were built around extensive trout plants from a series of well-established trout hatcheries. A few years later Phil was promoted to a fishery biologist position stationed in Bishop. The job in Bishop involved aquatic management and research on the "East Slope" of the Sierra Nevada, a very diverse and huge area comprising more than a thousand waters extending from the Sierra Nevada crest across the desert through Death Valley and beyond.

Although he took his new responsibility very seriously, often having to work alone, he found that something was missing from the status quo. Some of the programs the Department was implementing presented a philosophical and ecological conflict with principles he had learned as a graduate student while at the University of California, Berkeley. Phil being Phil, he began a period of critical analysis and thinking about the status quo.

It became clear to the young biologist the Department's fishery management programs for the eastern Sierra were well-intentioned, but were often lacking in conserving the biodiversity of California's native fish fauna. For example, the Department sought to provide diverse recreational angling opportunities through widespread planting of brook, brown, and rainbow trout. However, the planting of these highly sought after gamefish likely had adverse effects to some of California's native trout and amphibian species. Parallel to the implementation of the Department's management programs was a growing recognition within the professional and academic communities of California that native fishes and amphibians possessed unique biological attributes. The native fauna warranted directed conservation actions to protect the evolutionary legacy of the State's inland aquatic resources, including non-game species.

A pivotal moment occurred when, in July of 1964, Phil received a call from Dr. Robert Rush Miller, of the University of Michigan, requesting he accompany Miller and Professor Carl Hubbs, of Scripps Institution of Oceanography, to Fish Slough north of Bishop. Dr. Miller had completed his dissertation research on pupfishes in that area, including the Owens pupfish, which was otherwise thought to be extinct. Miller wanted to determine if there was still a remnant population of Owens pupfish in existence. Hubbs and Miller came to Bishop and found that, indeed, a population existed. After this significant find the ichthyologists returned to Ann Arbor and La Jolla, respectively, and the young Department biologist changed his priorities. He shifted his emphasis to a more ecologically focused philosophy that was, in many ways, in conflict with the prevailing approaches in fishery management at that time.

As Phil shifted his views and work on the East Slope, law, policy, and societal expectations also shifted. Landmark laws passed such as the U.S. Endangered Species Act of 1973 and the California Endangered Species Act of 1984. National environmental awareness was on the rise. And, ecology as a scientific discipline saw renewed interest, all of which set the stage for a significant paradigm shift relating to resource management and species conservation.

This shift also spawned numerous conservation based groups and associated efforts that would link agency and non-government interests. One such group that evolved during this era, with help from the young biologist in Bishop, was the Desert Fishes Council. This group consisted of about 300 academic, federal, and state biologists dedicated to the conservation of

North America's desert aquatic ecosystems. These developments were a major step forward in balancing fishery management with native species conservation. Phil remains involved in the Council even as we write this Introduction now in the spring of 2015.

The Department's approach to conservation will always need to be adaptive. No serious scientist can dispute the value of good adaptive management. A recent example of this comes from the Department's stocking of high elevation lakes. Trout management throughout California's high mountain lake ecosystems has been modified in recent decades to enhance conservation of native amphibians whose decline has been associated with introduced trout stocking. Currently, trout stocking in high mountain lakes is much more selective to achieve a balance between native species conservation and maintaining traditional backcountry angling opportunities the public has enjoyed for over a hundred years.

Modification of the State's trout stocking practices reflects one of the Department's most monumental accomplishments in adapting to a modern conservation program. For well over a hundred years, hatcheries have very successfully produced large numbers of trout to meet the demands of the angling public. The Department's current trout stocking strategy, while decades in the making, reflects the result of a collaborative process that embraces equal objectives of maintaining the integrity of native aquatic ecosystems and providing abundant fish for angling. The article in this issue by Dave Lentz and Mark Clifford is an in-depth review of this evolution. This new thinking is exemplified in strong programs devoted to reestablishment of salmon runs, restoration of the California golden trout (California's State Fish), and recovery of the rare Paiute cutthroat trout. Ongoing Department programs will usually include a fish hatchery component, but no longer without factoring in the relationship at the ecological level with native fish and other aquatic fauna.

As the Department moves into the 21st century there will undoubtedly be further challenges and adaptive changes that will need to be made. Our collective understanding of past mistakes and lessons learned will inevitably shape how the Department moves forward but more importantly how we leave the landscape for future generations. As the Department continues with this endeavor, the incoming cohort of fledgling biologists raised and educated under the guiding principles of ecology will play a critical role.

However, unlike the days that challenged that young biologist so many years ago, there is now ample support both internally in the Department and outside for such efforts. Much of that outside support will come from non-government agencies, advocacy groups, and the individual sportsmen and women who played a large part in founding the conservation movement. Our new and evolving direction pleases both them and us, as anthropocentrism gives way to biocentrism, and we ask what we can do for our fish, wildlife, and plant resources rather than what they can do for us. To co-opt a phrase the non-Phil half of this co-authorship learned while working at Trout Unlimited – "if you take care of the fish, the fishing will follow."

So, to wrap up, we hope that this Introduction gives some guidance to the new generation. Take encouragement that no matter how young or how new in your career as a fish conservationist, you can make a difference. Aldo Leopold reminded us that "A thing is right when it tends to preserve the integrity, stability, and beauty of the biotic community. It is wrong when it tends otherwise." We now recognize the wisdom of Leopold's words, and we are coming closer to them every day.

If Phil had not questioned the status quo, the last population of Owens pupfish on the planet might not have been saved. If he had not questioned the status quo, the Golden Trout Wilderness might not have been created as a refugium for that native trout. Leopold summed it up, "In such matters we should not worry too much about anything except the direction in which we travel. The direction is clear..."

Introduction to the special fisheries issue from the California State Senate

One hundred years! How many journals of any kind have been around that long? Only a few can make that claim, which is why I'm particularly proud to introduce this issue of *California Fish and Game*, the scientific journal produced by the California Department of Fish and Wildlife (CDFW).

This fourth issue in the centennial volume is focused on our fresh water fisheries and ecosystems. This topic is appropriate—or perhaps ironic—in this third year of severe drought, when fresh water supply is a critical issue for all Californians. The drought's effects may be even more severe on wildlife than on humans, since fish and animals can't store, import, pump groundwater, or buy water in bottles. They live or die with what nature (and sometimes we) can provide.

Since 1914, *California Fish and Game* has kept its managers, scientific researchers, students and the public up to date on the science of environmental conservation. That year the department—then called the Fish and Game Commission—created a new branch, the Bureau of Education, Publicity and Research. The need for more scientific research and to share it with the general public must have been obvious by then, since California's natural resources had been severely damaged by both Gold Rush activities and industrialization. Compiling the latest research and publishing the results in a scientific journal was an excellent way to turn the tide toward conservation.

Despite the public's growing support for environmental protection, California has lost approximately 95 percent of its wetlands to development. All but one of our major rivers has been dammed, thus blocking the natural passage of anadromous fish to their historic spawning streams. The diversion of water from natural drainages for human use has left numerous stream and lake beds as dry as a bone in the desert. All of this has had tremendous adverse effects on California's native aquatic organisms.

Yet, against tall odds, CDFW has found ways to address these challenges and worked to maintain healthy populations of most fresh-water-dependent species. For 100 years, research published in *California Fish and Game* has helped guide fisheries programs and habitat conservation, and to support beneficial government policies and legislation.

I heartily congratulate every researcher who has contributed to this respected publication, as well as CDFW Director Charlton H. Bonham and the staff who put it all together, on *California Fish and Game's* 100th anniversary. May you continue publishing this valuable scientific information for another 100 years!

Fran Pavley, Chair
Senate Natural Resources and Water Committee

Seasonal microhabitat selectivity by juvenile steelhead in a central California coastal river

ROBERT W. HOLMES*, MARK A. ALLEN, AND SHANNON BROS-SEEMAN

California Department of Fish and Wildlife, Water Branch, 830 "S" Street, Sacramento, CA 95811, USA (RWH)

Normandeau Environmental Consultants, 890 L Street, Arcata, CA 95521, USA (MAA)

San Jose State University, Department of Biological Sciences, San Jose, CA 95192, USA (SB-S)

**Correspondent: Robert.Holmes@wildlife.ca.gov*

Microhabitat data were collected at focal positions of juvenile steelhead trout (*Oncorhynchus mykiss*) in the Big Sur River, California during spring, summer, and fall. An equal-area sampling approach was used to guide fish surveys and allocate habitat availability sampling among seasons, river reaches, and mesohabitat types. Juvenile steelhead trout habitat selection changed with fish size, season, discharge, and habitat availability. Water depth and water velocity were of primary importance in habitat selection for all size groups of rearing steelhead. Habitat Suitability Criteria (HSC) were prepared for water depth, mean water velocity, focal velocity, specific escape cover types, and distance to in-water escape cover to reflect seasonal habitat selectivity for rearing steelhead. Habitat "preference" HSC (use adjusted for availability using the U/A forage ratio) were also developed and compared with the equal-area selectivity HSC and with habitat availability. The U/A results produced extreme shifts in maximum suitability for several curves, and perhaps more significantly the U/A ratios severely deflated suitabilities where the majority of the fish were observed. With proper habitat stratification and non-limiting sampling conditions (e.g., adequate flows and non-degraded habitat), use of an equal-area sampling design for site-specific selectivity HSC development was determined to be a viable option for development of biologically relevant and representative HSC, and apt for effective environmental flow recommendations.

Key words: forage ratio, habitat selection, HSC, microhabitat, *Oncorhynchus mykiss*, preference, selectivity, steelhead trout

Habitat suitability criteria (HSC) are an integral biological component of an instream flow-regime needs assessment (Bovee et al. 1998; Annear et al. 2004). HSC are typically developed within the framework of the Instream Flow Incremental Methodology (IFIM) decision-making approach (Bovee et al. 1998), and then can be used through various applications to link the species and life stage(s) of interest to their physical environment. One- and two-dimensional hydraulic habitat models (Milhous et al. 1989, Waddle et al. 2000) are two such applications commonly employed to evaluate stream flow and habitat relationships for salmonids. Within the context of the IFIM, HSC are indices of characteristic behavioral traits of a species that are established as standards for comparison to modeled habitat conditions (Bovee 1986). Biologically accurate and relevant HSC are required for the models to accurately predict and reflect how the quantity and quality of habitat changes under different flow regimes (Parsons and Hubert 1988, Beecher et al. 2002).

HSC development relies on an unbiased stratified sampling strategy that reflects the spatial or temporal changes of habitat use patterns of the target species. Mesohabitat components (i.e., pools, riffles, runs, glides) typically guide the broader sampling for development of riverine HSC. Microhabitat variables, such as water depth, water velocity, cover, and substrate are the most common variables used in the development of HSC. These microhabitat variables influence the use of local stream habitats by the target species and their respective life stages, and their availability varies with flow. The range of suitability for each microhabitat variable is between 0.0 (unusable) and 1.0 (optimal; Bovee and Cochnauer 1977).

HSC curves can be developed by various levels of rigor from strictly professional judgment with no actual field data or validation for the species, life stage, or river of interest, to being developed from site-specific field observations of habitat use. Developing site-specific HSC involves collecting data from locations where target fish are observed or captured (e.g., habitat "utilization" data). To avoid bias, the habitat utilization data must account for the effects of habitat availability on fish habitat selection (Bovee 1986). Two methods commonly employed to account for effects of habitat availability include equal-area sampling, a design-based protocol (Thomas and Bovee 1993, Bovee et al. 1998, Allen 2000), and application of the forage ratio formula (Johnson 1980, Voos 1981) based upon the concept of food electivity (Ivlev 1961), a mathematical adjustment of utilization data by availability data to arrive at an estimate of a fish's habitat "preference" (Bovee 1986, Moyle and Baltz 1985, Beecher et al. 1993, Beecher et al. 1995). Other protocols for developing HSC that attempt to account for habitat availability include density sampling (Rubin et al. 1991, Aadland and Kuitunen 2006), and presence-absence sampling (Thielke 1985, McHugh and Budy 2004, Gard 2010).

Use of an equal-area sampling approach to directly account for habitat availability (Bovee et al. 1998) is more recently referred to as representing target organism "selection" (Manly et al. 2002), hereafter referred to as "selectivity." Although use of the terms "preference" and "selectivity" may seem a matter of semantics, there are broader concerns for HSC development, application, and associated biological representativeness and relevance for the target species. For example, a primary limitation of developing "preference" HSC using the forage ratio is that the mathematical adjustments for limited habitat availability may sometimes result in overcorrected HSC (Bovee et al. 1998), particularly if applied when habitat availability is not limited (Hayes and Jowett 1994). Such instances could lead to biased HSC and environmental flow recommendations that are insufficient for maintaining a robust population, or else recommendations for more water than what is naturally available.

Recurring drought conditions in California underscore the need for accurate and reliable tools to inform streamflow management decisions. Despite being an essential component of many types of flow management modeling tools, steelhead trout (*Oncorhynchus mykiss*) HSC are not available for small California coastal rivers. Further, California's South-Central Coast (SCC) steelhead trout Evolutionary Significant Unit (ESU) populations have declined from about 25,000 spawning adults per year to fewer than 500 (NMFS 2007). The free-flowing Big Sur River is thought to represent an important source population for the South-Central steelhead trout ESU that may help maintain some of the other very small populations that occur throughout the Big Sur Coast. Furthermore, the Big Sur River is considered a steelhead trout stronghold (Wild Salmon Center 2010) and, as such, a candidate coastal river for development of steelhead trout HSC.

The primary objective of this study was to investigate seasonal microhabitat selectivity by juvenile steelhead trout in a relatively pristine, unregulated coastal stream, and to fill a significant data gap in California-based steelhead trout literature. This information is critically important for designing studies to assess habitat suitability in California, where conflicts over limited water supplies are ever-increasing. Further, existing HSC data for steelhead trout in California are based on large, regulated rivers in interior California, where the application and biological relevance of those criteria to smaller coastal streams is uncertain. HSC developed from a mostly unaltered, coastal stream should help to avoid the potential biases from application of non-local HSC developed from rivers with altered flow and habitat conditions. A secondary objective was to develop, and compare and contrast HSC using two common methods intended to account for habitat availability: a design-based sampling approach (equal-area sampling) either with or without a mathematical adjustment using the forage ratio. Both methodologies are commonly employed in HSC studies, and both have strengths and weaknesses that must be considered during development and application.

The management applications of this investigation, in addition to filling a significant HSC data gap for coastal steelhead trout near the southern extent of their distribution, include developing an improved understanding of juvenile steelhead trout behavior and habitat selection in an unimpaired river system. An understanding of juvenile steelhead trout habitat selection from an unregulated coastal stream is important for designing habitat restoration efforts and identifying restoration priorities in other coastal streams that may have altered flow regimes or degraded habitat conditions, or both. Further, the HSC used in some IFIMs may originate from other studies because the stream under investigation is not a good source stream for site-specific HSC development. In such cases, assurances that the HSC are not biased by flow regulation or other habitat and sampling limitations is important in evaluating the transferability (Thomas and Bovee 1993) of those data between streams.

MATERIALS AND METHODS

Study area.—The Big Sur River is located in southern Monterey County, California (Figure 1) and has a watershed of approximately 155 km² with no major dams, diversions, or reservoirs. The Big Sur River, which has limited access, originates in the steep canyons of California's Ventana Wilderness within the Los Padres National Forest, and flows northwesterly through federal and private lands, two state parks (Pfeiffer Big Sur and Andrew Molera), and a small lagoon before joining the Pacific Ocean about 4.5 km southeast of Point Sur. Significant tributaries include Pfeiffer-Redwood Creek, Juan Higuera Creek, Post Creek, and Pheneger Creek.



FIGURE 1.—Map of study reaches referenced in this paper along the Big Sur River, Monterey County, California.

The hydrology of the Big Sur River is typical of many coastal California rivers, experiencing high winter flows, low summer flows, and variable annual discharges. Most of the annual flow occurs in the winter with stream discharge reflecting local and watershed-wide rainfall patterns. Flows in winter may rise and recede rapidly in association with rainfall events, while flows in the summer tend to be more stable and predictable as they recede into the fall months.

Percent exceedance flows are typically used as a guideline for describing the watershed hydrology, as well as for making informed decisions about water resources

planning and management. The percent exceedance flows between 20 and 80 percent reflect the most commonly observed flows in the stream, with the 50 percent exceedance flow reflecting the stream's natural benchmark. The 20, 50, and 80 percent exceedance flows for the Big Sur River are 2.83, 0.82, and 0.39 m³/s, respectively. The Big Sur River is predominately a single-salmonid species river, where steelhead trout use the study area year-round for migration, spawning, incubation, rearing, or emigration, or all of these. Further, the Big Sur River is free-flowing, in relatively pristine condition with limited anthropogenic impact.

Sampling strategy.—Sampling effort was stratified by season, reach, study site, and mesohabitat type. Seasonal stratification was important to reflect juvenile steelhead trout life-history characteristics during the rearing period on a coastal stream and how they may change as the fish grow during this period. The study area includes three reaches (i.e., Lower Molera, Molera, and Campground), each representing generally homogenous stream segments based upon gradient, geomorphology, hydrology, riparian zone type, flow accretion, and channel metrics (Figure 1). The reaches extend approximately 12 km from the lower-most part of the river at the lagoon-river transition upstream to Pfeiffer Big Sur State Park near USGS gage 11143000.

Summer sampling took place in June 2010 in the Lower Molera Reach, and in August 2010 in the Molera and Campground reaches. The survey (fish use) data were combined to reflect the equal area sampling design and represent juvenile steelhead trout microhabitat distributions during the summer time period. Fall sampling took place in all three reaches during October 2010 and represents the fall time period for rearing juvenile steelhead trout. Sampling resumed in May 2012 on all reaches to identify fry microhabitat distributions during spring.

Mesohabitat classification consisted of partitioning the reaches into low-gradient riffle, pool, glide, run (and shallow run) mesohabitat types (Flosi et al. 2010). Study sites were selected using a stratified random sampling design. First, each study reach was partitioned into three approximately equal sub-reaches based upon the number of mesohabitat units. A study site was then randomly selected in the lower third, middle third, and upper third of each sub-reach. This process was repeated until each sub-reach contained one of each mesohabitat type. Additional mesohabitat units, beyond the initial random draw, were also randomly selected from each reach or mesohabitat type stratum if needed to achieve equal-area (i.e., square meter) sampling and adequate sample numbers of fish (Bovee et al. 1998).

The equal-area sampling approach was intended to account for the influence of habitat availability on fish selectivity by sampling the same surface area of mesohabitats composed of different depths and velocities, then allowing the relative density of observations in each microhabitat to dictate the shape of the final HSC curve (Thomas and Bovee 1993, Allen 2000). The Big Sur River was not intensively mapped into discrete cells of specified depth or velocity categories; instead we opted to utilize a more simplified and rapid approach that associated conventional mesohabitat types with combinations of depth and velocity. For example, pools can generally be characterized as having an abundance of deep and slow microhabitats, whereas riffles are dominated by shallow and fast microhabitats. In like manner, runs are relatively deep and fast, whereas glides are comparatively shallow and slow. These four mesohabitat types thus approximate the four combinations of depth and velocity, and were the basis for the equal-area sampling design within the mesohabitat stratum.

Although pools also contain shallow depths along their margins, and slow velocities may occur near the banks of riffles, if a fish demonstrates a true preference for deep and

slow habitat, it will likely occur at highest densities in the deeper and slower portion of the pool (i.e., not along the shallow margins). Likewise, a fish preferring fast velocities will occur most often in the swifter portions of a riffle or run, not in the calmer margin areas. If each of these mesohabitats is sampled at equal intensity, combining the target species or lifestage depth and velocity measurements among the mesohabitats will yield an HSC curve that represents its habitat selectivity by virtue of the density of observations in deep, shallow, fast, or slow microhabitats.

Fish observation techniques.—We sampled for steelhead trout fry (<6 cm) and juvenile (6–9 cm and 10–15 cm) life stages during three seasons (summer, fall, and spring). Habitat use data were collected for all undisturbed steelhead observed via direct underwater observation. Potential diving scenarios for collecting HSC data depended upon (1) fry/juvenile densities; (2) water clarity; and (3) channel width. Where narrow channel widths and adequate water visibilities allowed, a single diver collected HSC data with support from a data recorder. Where channel widths prevented a single diver from fully covering the entire sampling area, two divers or more worked upstream together, communicating to avoid replicate observations. Each diver transferred HSC data to one or two data recorders.

Water visibility was estimated using an 8-cm juvenile trout rapala. The recorder would suspend the rapala mid-depth in the water column using a sinker and monofilament line. The snorkeler would move away from the rapala until they were as far away as possible while still being able to see color markings on the rapala. Visibility was determined to be the maximum distance the underwater observer could see the rapala and color markings.

In each sampling (mesohabitat) site, the observers entered the water about 6 m downstream of the site, and moved slowly upstream through the site, observing steelhead and determining their focal positions. Location markers (weights with numbered flags) were placed where undisturbed steelhead (1 or more) were observed. Where large groups (>20 individuals) of fry or other juveniles were distributed over a larger (0.30 m²) area that encompassed different water depths and velocities, they received several measurements that were treated as individual observations to characterize the different microhabitats and different sizes of fish within the groups.

Divers attempted to move around, rather than move through, fish positions to avoid herding fish within or out of the site. Fish that were disturbed by the diver prior to identification of the fish's focal position were not marked, but were noted as present and not included in subsequent analyses. Fish marker number, number of fish, estimated size (fork length[s] to nearest cm for each fish by reference to an underwater ruler), fish activity (e.g., holding, feeding), and focal height (i.e., actual distance above the substrate or relative height in the water column) were recorded for each observation. A numbered marker was placed underneath individual fish or sub-group focal position and the data were transmitted to the nearby data recorder. The observer then proceeded upstream and marked all undisturbed fish in the sampling unit.

After the dive was completed, habitat characteristics were measured at all observation markers. Habitat characteristics recorded for each marked fish location were: water depth, mean column water velocity (mean velocity), focal velocity, overhead cover (in-water and out-of-water cover type) presence, distance to escape cover, and distance to bank (Table 1). Escape cover was defined as any object capable of concealing a juvenile steelhead from aquatic or terrestrial predators, including unembedded cobbles and boulders, woody debris, instream branches, or overhead branches within 46 cm of the water surface. When multiple cover types were present at a fish focal position, the object type possessing

the greatest concealment opportunity for a fish was recorded. Distance to that cover object was then measured to the nearest 1.5 cm; cover objects >3.1 m from a focal position were considered no cover. Water depth was measured with a graduated top-setting rod to nearest 30.5 mm. Velocity was measured with a Marsh McBirney electromagnetic water velocity meter to the nearest 3.0 mm/sec following standard U.S. Geological Survey procedures (Rantz 1982). River stage was monitored to assess potential changes in stage during the surveys using USGS 11143000 and USGS 11143010.

TABLE 1.—Vegetative codes and substrate codes referencing environmental conditions associated with the Big Sur River, Monterey County, California.

Vegetative Codes		Substrate Codes		Size (cm)
0	None	20	None	
1	Filamentous algae	21	Clay	
2	Non-emergent rooted aquatic vegetation	22	Sand or silt/sand	< 0.25
3	Emergent rooted aquatic vegetation	23	Coarse sand/DG	0.25-0.5
4	Grass	24	Small gravel	0.5-2.5
5	Sedges/rushes	25	Medium gravel	2.5-5
6	Vines/ poison oak	26	Large gravel	5-7.6
7	Branches &/or small vegetation < 10 cm, IW	27	Gravel/cobble	7.6-10
8	Branches &/or small vegetation < 10 cm, OW	28	Small cobble	10-15.3
9	Branches > 10 cm, IW	29	Medium cobble	15.3-23
10	Branches > 10 cm, OW	30	Large cobble	23-30
11	Tree trunks < 10 cm dbh, IW	31	Small boulder	30-61
12	Tree trunks < 10 cm dbh, OW	32	Medium boulder	61-122
13	Tree trunks > 10 cm dbh, IW	33	Large boulder	>122
14	Tree trunks > 10 cm dbh, OW	34	Bedrock	
15	Roots and root-wads	35	Undercut bank	
16	Shrubs < 10 cm			
17	Duff, leaf litter, organic debris			
18	Small woody debris (< 10 cm), dead			
19	Large woody debris (> 10 cm), dead			

Habitat availability techniques.—Habitat availability data were collected in each sampled mesohabitat unit during each seasonal sampling event immediately upon conclusion of fish observation and data collection. Field procedures followed a random point sampling design that consisted of (a) random selection of cross-sectional transects, and then (b) random selection of measurement points along each transect. To keep the level of effort for habitat availability data consistent with the effort for fish habitat selection data (i.e., according to the equal-effort design), the number of availability measurement points in each sampled habitat unit was roughly proportional to the size of that habitat unit (e.g., larger individual mesohabitat units have more availability points than smaller units, but the overall number of availability points were equal among the mesohabitat types). This design provided a minimum of three habitat availability measurements from each of two to six transects per sampling unit. The total number of measurements per unit was based on unit size in order to maintain an equal effort in both the habitat availability and the fish habitat use datasets.

A second set of habitat availability measurements were also obtained from survey data collected from 118 transects spanning the three-reach study area in 2011. The transect

locations were selected through a stratified random process to be used as part of a one-dimensional (1D) physical habitat hydraulic model analysis (Bovee 1997). The 1D data were based upon proportional, not equal, area habitat representation for hydraulic habitat modeling and are useful for comparisons with the habitat availability data collected in conjunction with the fish surveys.

HSC development.—Separate HSC were developed for each size class (e.g., <6 cm, 6–9 cm, 10–15 cm) and each seasonal period, and data were pooled among reaches and mesohabitat types in order to produce more generalized HSC representing the entire anadromous reach of the Big Sur River. Data were compiled into frequency histograms using bin size intervals of 0.03 m for water depth, and 3.0 cm/s for mean water and focal water velocity, respectively. The spring sample event was elected to identify rearing microhabitat selectivity for <6 cm steelhead fry, which represent the steelhead size class most representative of spring young-of-year rearing conditions. The summer and fall sampling events were elected to identify rearing microhabitat selectivity for larger juvenile steelhead in the 6–9 cm and 10–15 cm size groups.

Kernel-smoothing techniques (Jowett 2002, Jowett and Davey 2007) were used to develop HSC curves from the frequency of habitat selectivity, habitat availability, and preference (U/A) HSC curves, using the curve-fitting component of System for Environmental Flow Analysis (SEFA), an instream flow modeling toolkit (Payne and Jowett 2012). All smoothed curves were standardized by dividing them by their maximum values to provide suitability indices ranging from 0 to 1. For depth, some practitioners choose to subjectively maintain suitability at 0.5, 1.0, or at some intermediate value for depths beyond the last observation; we chose to maintain suitability at the value from the last observation into deeper water.

To further evaluate the representativeness of the equal-area selectivity HSC curves and the potential effects of habitat availability on these curves, alternative HSC curves were derived using the U/A forage ratio methodology. While the equal-area HSC are intended to reflect habitat selectivity (i.e., habitat choice) by the fish, the forage ratio criteria (Moyle and Baltz 1985) are also intended to reflect fish “preference,” or habitat use adjusted for habitat availability (i.e., U/A). The U/A forage ratio is the proportion of habitat of a particular microhabitat category (e.g., water depths between 0.3 meters and 0.34 meters) selected by a fish, divided by the proportion of habitat units of that category available (Manly et al. 2002). Smoothed preference HSC were calculated within SEFA using the forage ratio formula as outlined and described by Jowett and Davey (2007).

Statistical analyses.—Statistical analyses assessed whether habitat availability differed from the habitat characteristics where fish were observed (habitat selected). Separate two-way for steelhead <6 cm and three-way Analysis of Variance (ANOVA) tests for larger juveniles (6–9 cm, and 10–15 cm) were conducted for each of the fish length classes. The factors in the statistical analysis were depth and velocity selection (fish habitat use, habitat available), mesohabitat (runs, riffles, pools and glides) and sample period (spring, summer, and fall for 6–9 cm fish, summer and fall only for 10–15 cm fish). Fish <6 cm were only abundant in the spring so sample period was not assessed. Significant effects ($P < 0.05$) associated with selection (habitat used vs. habitat available) would indicate habitat selectivity. Holmes et al. (2014) outlined the complete statistical analyses of habitat use variables other than depth and velocity (i.e., fish focal velocity, fish focal position, overhead cover, escape cover distance, distance to bank).

RESULTS

Approximately equal areas of mesohabitat types were sampled in each reach and season (Table 2). Steelhead trout were observed in all the mesohabitat types sampled in all seasons. Flows during sampling ranged from 0.99-1.44, 0.88-1.76, and 0.65-0.74 m³/s for the spring, summer, and fall sample periods, respectively. Water visibility ranged from 2.7-6.0 m (mean 4.7 m). Water temperature ranged from 10-18°C with means of 15°C, 16°C, and 14°C for spring, summer, and fall, respectively. River stage did not change during each site survey.

TABLE 2.—Summary of total area sampled and total number of juvenile steelhead trout observed among mesohabitat types in the Lower Molera, Molera, and Campground reaches of the Big Sur River, Monterey County, California in 2010 and 2012. Sampling flows and corresponding monthly exceedance probabilities are outlined for each season.

Lower Molera Reach			
Habitat Type	Spring 2012 Area (m ²)/Fish	Summer 2010 Area (m ²)/Fish	Fall 2010 Area (m ²)/Fish
RUN	2,000/85	1,632/74	1,592/113
LGR	1,530/300	1,515/98	1,349/53
POOL	1,805/170	1,694/81	1,734/130
GLD	1,427/110	1,543/14	1,434/13
Total:	6,762/665	6,384/267	6,109/309
Molera Reach			
RUN	1,456/295	1,452/116	1,460/48
LGR	1,013/144	1,837/91	1,612/43
POOL	1,180/103	1,483/101	1,398/74
GLD	1,840/101	1,510/24	1,560/10
Total:	5,489/643	6,283/332	6,030/175
Campground Reach			
RUN	1,352/758	1,710/306	1,472/69
LGR	1,059/244	1,785/175	1,651/37
POOL	1,680/1,569	1,840/202	2,127/175
GLD	2,371/281	2,126/90	2,162/5
Total w/o RUN(S) ¹ :	6,462/2,852	7,461/773	7,412/286
RUN(S)	755/184	1,797/71	1,729/19
Total w/ RUN(S):	7,217/3,036	9,258/844	9,141/305
Total Area (m ²)/Total Fish:	19,468/4,344	21,925/1,443	21,280/789
Sampling Flows (m ³ /s):	0.99-1.44	0.88-1.76	0.65-0.74
Monthly Exceedance (%):	50-65	5-24	9-15

¹ RUN(S) are a mesohabitat type observed in the Campground Reach described as shallow runs with swiftly flowing water, little surface agitation, and no major flow obstructions.

Habitat Availability

Habitat availability data were also equally allocated among reaches and seasons. A total of 1,452 habitat availability samples were collected with 414, 522, and 516 samples collected in the spring, summer, and fall sample seasons, respectively (Table 3). Sample sizes were also generally consistent among reaches. Habitat availability statistics represent the availability measurements made at the same mesohabitat sites where the fish surveys were conducted. The 1D transect data, collected from the 118 transects as outlined earlier, were collected at comparable flows (i.e., 0.68–0.85 m³/s) to the flows (0.65–0.74 m³/s) that existed when the fall fish survey and associated habitat availability data were collected (Table 3).

TABLE 3.—Statistics for water depth and water velocity habitat availability measurements from the Big Sur River, Monterey County, California during spring 2012, summer 2010, and fall 2010 fish observation sampling events and from measurements at 118 stratified random transects used for a 1D hydraulic habitat model from fall 2011.

Season	Statistic	N	Minimum	Maximum	Average	Median	SD
Spring	Water Depth (m)	414	0.02	1.16	0.33	0.30	0.20
	Water Velocity (cm/s)	411	0	190.2	35.7	32.3	27.4
Summer	Water Depth (m)	522	0.02	1.22	0.34	0.30	0.20
	Water Velocity (cm/s)	522	0	172.8	41.8	38.4	28
Fall	Water Depth (m)	516	0.03	1.22	0.28	0.24	0.18
	Water Velocity (cm/s)	516	0	131.7	28	24.7	21
1D (Fall)	Water Depth (m)	4,273	0.02	1.07	0.26	0.24	0.15
	Water Velocity (cm/s)	4,273	0	135.6	27.7	23.8	22

Generally, minimum and maximum water depth habitat availability data were comparable during the spring, summer, and fall sample events (Table 3). Maximum water velocity, on the other hand, showed a general decrease from spring through summer and fall. Similarly, water depth and water velocity were less in fall when compared to the spring and summer sample events. Because the 1D availability data represent a much larger data set ($N = 4,273$) compared to the availability data from the fall fish surveys ($N = 516$), these data allow greater insight into habitat availability conditions at the flows when the fall fish surveys were conducted. Comparing the 1D habitat availability data to the fall fish survey habitat availability data indicates the same general occurrence of habitat availability conditions and further indicates a decrease in availability of the higher velocities in fall when compared to summer, and the rarity of depths greater than 1.07 m.

Seasonal Fish Observations

Sample sizes of fish frequencies for spring, summer, and fall sampling events were 4,344, 1,443, and 789, respectively. Most steelhead trout were observed feeding, as opposed to holding.

Steelhead trout <6 cm – spring habitat use.—Steelhead trout <6 cm were found in all habitat types, with approximately 70% occurring in pool and run mesohabitat types

in spring. Over 75 percent of the <6 cm fish observed in spring were smaller individuals, 2–3 cm in length, which were observed in locations with water depths ranging from 0.02 to 1.16 m, with a mean of 0.24 m (Table 4).

TABLE 4.—Habitat use statistics for juvenile steelhead trout observed in the Big Sur River, Monterey County, California in spring 2012, summer 2010, and fall 2010.

Season, size	Statistic	<i>N</i>	Minimum	Maximum	Average	Median	<i>SD</i>
Spring <6 cm	Water Depth (m)	3,921	0.02	1.16	0.24	0.18	0.17
	Water Velocity (cm/s)	3,920	0	107	15	9.8	14.6
	Fish Focal Point Height	3,921	0	10	6.92	8.00	2.32
	Fish Focal Point Water Velocity (cm/s)	3,905	0	81.4	11.3	7.9	11
	Distance to Escape Cover (m)	3,767	0	3.05	0.44	0.30	0.47
	Distance to Bank (m)	3,921	0	10.0	2.18	1.37	2.0
Summer 6-9 cm	Water Depth (m)	748	.09	1.45	0.41	0.37	0.17
	Water Velocity (cm/s)	748	0	131.4	43.6	42.4	19.8
	Fish Focal Point Height	748	6	10	8.91	9.00	0.82
	Fish Focal Point Water Velocity (cm/s)	740	0	99.1	27.1	25.3	16.8
	Distance to Escape Cover (m)	650	0	3.05	0.92	0.76	0.70
	Distance to Bank (m)	738	0.30	8.84	3.3	3.05	1.50
Fall 6-9 cm	Water Depth (m)	166	0.14	1.31	0.52	0.47	0.26
	Water Velocity (cm/s)	166	0.91	83.5	35.1	34.4	17.4
	Fish Focal Point Height	166	6	10	9.04	9.00	0.84
	Fish Focal Point Water Velocity (cm/s)	166	0	73.8	21.6	21.0	14.3
	Distance to Escape Cover (m)	146	0	3.05	1.17	1.07	0.88
	Distance to Bank (m)	166	0.30	7.32	2.70	2.44	1.44
Summer 10-15 cm	Water Depth (m)	609	0.18	1.45	0.49	0.46	0.19
	Water Velocity (cm/s)	609	1.83	159.7	44.8	43.6	22.3
	Fish Focal Point Height	609	6	10	8.50	9.00	0.82
	Fish Focal Point Water Velocity (cm/s)	605	0	114.3	31.1	29.6	18
	Distance to Escape Cover (m)	523	0	3.05	0.94	0.76	0.69
	Distance to Bank (m)	608	0.30	8.53	3.14	3.05	1.34
Fall 10-15 cm	Water Depth (m)	570	0.17	1.49	0.55	0.52	0.24
	Water Velocity (cm/s)	570	0	136.4	38.7	34.7	24.7
	Fish Focal Point Height	570	6	10	8.74	9.00	0.84
	Fish Focal Point Water Velocity (cm/s)	570	0	102.1	24.4	19.8	17.4
	Distance to Escape Cover (m)	500	0	3.05	1.02	0.91	0.87
	Distance to Bank (m)	570	0.15	7.32	2.60	2.44	1.23

Steelhead trout <6 cm were observed in locations with mean water velocities ranging from 0.0 to 107 cm/s, with a mean of 15 cm/s (Table 4). The focal position of steelhead <6 cm ranged throughout the water column from 0 (surface) to 10 (bottom), but the median fish focal position was 8. Water velocities at the fish focal position ranged from 0.0-81 cm/s with a mean of 11 cm/s.

Greater than 95% of <6 cm steelhead trout were observed at locations near (0.15–0.30 m) some type of escape cover either in form of vegetative or hard substrate types, with hard substrate types (large gravel to large cobble sizes) as the most common (>65%) types. Although <6 cm steelhead trout were observed at locations near escape cover, over 95% occurred at locations with no direct overhead cover. In addition, most <6 cm steelhead were observed within 1.5 m of the bank.

Steelhead trout 6–9 cm – summer habitat use.—Steelhead trout 6–9 cm were found in all habitat types, with most (>65%) occurring in run and riffle mesohabitat types in summer. Steelhead trout 6–9 cm were observed in locations with water depths ranging from 0.09 to 1.45 m, and a mean water depth of 0.41 m (Table 4). Steelhead trout 6–9 cm were observed in locations with water velocities ranging from 0.0 to 131 cm/s, and a mean water velocity of 44 cm/s. The focal position of steelhead trout 6–9 cm ranged from 6 to 10, with a median near-bottom position of 9. Water velocities at the fish focal position ranged from 0.0–99 cm/s.

Steelhead trout 6–9 cm were observed in proximity to a variety of escape cover types, with the most common types being cobble and boulders (65%), followed by branches in water (10%). Although most steelhead trout 6–9 cm were observed to be within approximately 0.6 m of escape cover, only about 13% were observed selecting habitat locations not near (>3 m) any type of escape cover. Further, 99% of all steelhead trout 6–9 cm observations in summer occurred at locations with no overhead cover. In addition, steelhead trout 6–9 cm were observed at a mean distance of 3 m from the bank.

Steelhead trout 6–9 cm – fall habitat use.—Steelhead trout 6–9 cm were found in all habitat types in fall, with most (73%) occurring in pool and run mesohabitat types. Juvenile steelhead trout 6–9 cm were observed in locations with water depths ranging from 0.14 to 1.30 m, and a mean of 0.52 m (Table 4). Steelhead trout were observed in locations with water velocities ranging from 0.91 to 84 cm/s, and a mean of 35 cm/s. The focal position of steelhead trout 6–9 cm ranged from 6 to 10, and a median of 9 (near-bottom). Water velocities at the fish focal position ranged from 0.0 to 104 cm/s.

Steelhead trout 6–9 cm were observed in proximity to a variety of escape cover types in the fall. The most common types of escape cover near the fish observation locations were branches and/or small vegetation (both in-water and out-of-water; 37%) and boulders (13%). Although distance to escape cover ranged from 0 to 3 m, most juvenile steelhead trout were observed to be within approximately 0.9 m of escape cover. Further, over 95% of all steelhead trout 6–9 cm observations in fall occurred at locations with no overhead cover. In addition, steelhead trout 6–9 cm were observed from about 0.30 to 7.3 m from the bank.

Steelhead trout 10–15 cm – summer habitat use.—Steelhead trout 10–15 cm were fairly evenly distributed among run (35%), low gradient riffle (26%), and pool (30%) habitat in summer. Only 9% percent of juvenile steelhead trout 10–15 cm were observed in glide habitat in summer. Juvenile steelhead trout 10–15 cm were observed in locations with water depths ranging from 0.18 to 1.45 m, and a mean of 0.49 m (Table 4). Steelhead trout 10–15 cm were observed in locations with water velocities ranging from 1.8 to 160 cm/s, and a mean of 45 cm/s. The focal position at which the fish were observed ranged from 6 to 10, and had a median of 9 (near-bottom). Water velocities at the fish focal position ranged from 0.0 to 114 cm/s.

Steelhead trout 10–15 cm were observed in proximity to a variety of escape cover types during summer, with the most common being cobble and boulders (54%), followed by branches in water (12%). Most juvenile steelhead trout were observed to be within

approximately 0.6 m of escape cover, with a mean distance to escape cover of 0.9 m (Table 4). Further, 99% of all steelhead trout 10–15 cm observations in summer occurred at locations with no overhead cover. In addition, steelhead trout 10–15 cm were observed at distances ranging from 0.3 to 8.5 m from the bank in the summer.

Steelhead trout 10–15 cm—fall habitat use.—Steelhead trout 10–15 cm were found in all habitat types in fall, with most (77%) occurring in pool and run mesohabitat types. Steelhead trout 10–15 cm were observed in locations with water depths ranging from 0.17 to 1.49 m, and a mean of 0.55 m (Table 4). Steelhead trout 10–15 cm were observed in locations with water velocities ranging from 0.0 to 136 cm/s, and a mean of 39 cm/s. The focal position at which the fish were observed ranged from 6 to 10, and had a median of 9 (near bottom). Water velocities at the fish focal position ranged from 0.0 to 102 cm/s.

Steelhead trout 10–15 cm were observed in proximity to a variety of escape cover types in fall. The most common types of escape cover near the fish observation locations were branches and/or small vegetation (both in-water and out-of-water; 44%) and boulders (16%). Although distance to escape cover ranged from 0 to 3 m, most fish were observed to be within approximately 0.0–0.9 m of escape cover (Table 4). Further, over 95% of all steelhead trout 10–15 cm observations in fall occurred at locations with no overhead cover. In addition, steelhead trout 10–15 cm were observed at a range of 0.15–7.3 m from the bank.

Habitat Selection vs Habitat Availability

Water depth (<6 cm steelhead trout).—Depth selection was highly significant (Table 5), since the mean water depth at which steelhead trout <6 cm were found (0.24 m) was significantly shallower than the mean water depth of available habitat (0.33 m). The mesohabitat effect was highly significant (Table 5), with depth use greater in pools and glides than in runs or riffles. Also, there was no significant interaction between depth selection and mesohabitat (Table 5), indicating that differences between habitat used by steelhead <6 cm and available habitat were consistent among mesohabitats.

Water velocity (<6 cm steelhead trout).—There was a highly significant interaction between velocity selection and mesohabitat (Table 5), as steelhead <6 cm generally selected slower moving water (mean 15.0 cm/s) than was available (mean 35.7 cm/s), especially in runs and riffles.

Water depth (6–9 cm steelhead trout).—There was a significant interaction between depth selection, sample period, and mesohabitat type for steelhead 6–9 cm (Table 5). In the run mesohabitat, steelhead 6–9 cm increased their selectivity for deeper water over time. In the riffle and pool mesohabitats, steelhead trout 6–9 cm were found in the deeper water (mean 0.52 m) relative to what was available particularly in the fall (mean 0.28 m) and, to a lesser extent, in summer (mean use and available depths 0.41 m and 0.34 m, respectively; Tables 3 and 4).

Water velocity (6–9 cm steelhead trout).—The interaction between velocity selection, sample period, and mesohabitat type was not significant for steelhead trout 6–9 cm, nor was the interaction between sample period and mesohabitat type (Table 5), indicating that the differences in the availability of water velocity among mesohabitats remained consistent among sample periods. The interaction between velocity selection and mesohabitat type was also not significant (Table 5), indicating that selectivity for water velocity was consistent among mesohabitats. However, the interaction between velocity selection and sample period

TABLE 5.—Results of two-way and three-way ANOVA for testing effects of water depth and water velocity selection, mesohabitat, and sample period for juvenile steelhead trout in the Big Sur River, Monterey County, California. Significant effects are in bold italics. If interactions are significant, however, ignore the single effects within the interaction, which have been lined out (e.g., $\theta.032$) in the table.

Size (cm)	Variable	Factor	df	<i>F</i>	<i>P</i>
<6	Water Depth	Selection	1, 2266	145.978	<0.001
		Mesohabitat	3, 2266	101.889	<0.001
		Selection*Mesohabitat	3, 2266	0.950	0.416
<6	Water Velocity	Selection	1, 2265	475.533	<0.001
		Mesohabitat	3, 2265	30.600	<0.001
		Selection*Mesohabitat	3, 2265	27.521	<0.001
6-9	Water Depth	Selection	1, 2340	65.560	<0.001
		Sample Period	2, 2340	4.898	0.008
		Mesohabitat	3, 2340	242.718	<0.001
		Selection*Sample Period	2, 2340	8.246	0.051
		Selection*Mesohabitat	3, 2340	2.392	<0.001
		Sample Period*Mesohabitat	6, 2340	2.332	0.026
		Selection*Sample Period*Mesohabitat	6, 2340	65.560	0.030
6-9	Water Velocity	Selection	1, 2340	3.501	0.061
		Sample Period	2, 2340	17.404	<0.001
		Mesohabitat	3, 2340	75.812	<0.001
		Selection*Sample Period	2, 2340	4.999	0.007
		Selection*Mesohabitat	3, 2340	2.477	0.060
		Sample Period*Mesohabitat	6, 2340	1.173	0.318
		Selection*Sample Period*Mesohabitat	6, 2340	0.700	0.650
10-15	Water Depth	Selection	1, 1920	305.050	<0.001
		Sample Period	2, 1920	9.563	0.002
		Mesohabitat	3, 1920	254.211	<0.001
		Selection*Sample Period	2, 1920	10.220	0.001
		Selection*Mesohabitat	3, 1920	8.980	<0.001
		Sample Period*Mesohabitat	6, 1920	4.008	0.007
		Selection*Sample Period*Mesohabitat	6, 1920	0.808	0.489
10-15	Water Velocity	Selection	1, 1920	40.795	<0.001
		Sample Period	2, 1920	49.118	<0.001
		Mesohabitat	3, 1920	98.523	<0.001
		Selection*Sample Period	2, 1920	13.252	<0.001
		Selection*Mesohabitat	3, 1920	0.305	0.822
		Sample Period*Mesohabitat	6, 1920	1.248	0.291
		Selection*Sample Period*Mesohabitat	6, 1920	3.836	0.009

was highly significant indicating that selectivity for water velocity differed among sample periods. Steelhead trout 6–9 cm showed no selectivity in summer (mean 43.6 cm/s), and selectivity for faster water (mean 35.1 cm/s) than what was available in fall (mean velocity available 41.8 cm/s and 28 cm/s in summer, and fall, respectively; Tables 3 and 4). The

mesohabitat effect was also highly significant (Table 5), indicating that available water velocities differed among mesohabitats, generally with the greatest velocities occurring in riffle and run mesohabitats.

Water depth (10–15 cm steelhead trout).—The interaction between depth selection and mesohabitat was highly significant with steelhead trout 10–15 cm selecting deeper water (0.49 m and 0.55 m use in summer and fall, respectively; Table 4) than was available (0.34 m and 0.28 m available in summer and fall, respectively; Table 3), especially in pool mesohabitat (Table 5). Similarly, the interaction between sample period and mesohabitat was highly significant. Water depth in riffle, glide and run mesohabitats was slightly deeper in summer but water depth in pool mesohabitat was consistent between sample periods. The interaction between depth selection and sample period was also highly significant indicating steelhead trout 10–15 cm generally selected deeper water than was available, but the difference was most pronounced in fall (mean of 0.55 m and 0.28 m use and available, respectively; Tables 3 and 4).

Water velocity (10–15 cm steelhead trout).—The interaction between water velocity selection, sample period, and mesohabitat was highly significant for steelhead trout 10–15 cm (Table 5). In summer, there was a slight selection for faster water in run, pool and glide mesohabitats, and in fall there was a stronger selection for faster water than was available in all mesohabitat types.

Habitat Suitability Criteria

The equal-area selectivity HSC were developed from the fish frequency data for water depth and water velocity (Figure 2A–J). HSC were developed for steelhead trout <6 cm from the spring sampling event only. In contrast, seasonal umbrella HSC were developed for steelhead trout (6–9 cm and 10–15 cm) for water depth and water velocity to encompass selectivity in both summer and fall rearing periods (Figure 3A and B; Figure 3D and E). Depth HSC remained as separate curves for each size group because of the difference in avoidance of shallow depths between the two size groups (Figure 3C). However, the 10–15 cm velocity curve encompassed the 6–9 cm curve and is representative of both size classes (Figure 3F).

The following selectivity HSC account for (1) differences in fish size; (2) sampling period effects by using spring data for fry, and summer vs. fall umbrella curves for larger juveniles; and (3) for mesohabitat and habitat availability effects through the use of the equal-area sampling approach. All HSC curve points for each size group of juvenile steelhead trout for water depth, water velocity, focal velocity, and distance to escape cover are available in Holmes et al. 2014.

Water depth.—Juvenile steelhead trout avoided shallow water and progressively used deeper water with increasing size. HSC for steelhead trout <6 cm indicate no use of water <0.02 m deep (Figure 2A). Water depth is most suitable (i.e., an index of 1.00) for <6 cm steelhead trout at 0.14–0.16 m. The umbrella HSC for 6–9 cm steelhead trout indicate no use of water <0.10 m (Figure 3A and Figure 3C). Further, water depth is most suitable for 6–9 cm steelhead trout at 0.36–0.46 m during the summer and fall rearing period. The umbrella HSC for 10–15 cm steelhead trout indicate no use of water <0.18 m (Figure 3B and Figure 3C). Finally, water depth is most suitable for 10–15 cm steelhead trout at 0.44–0.51 m during the summer and fall rearing period.

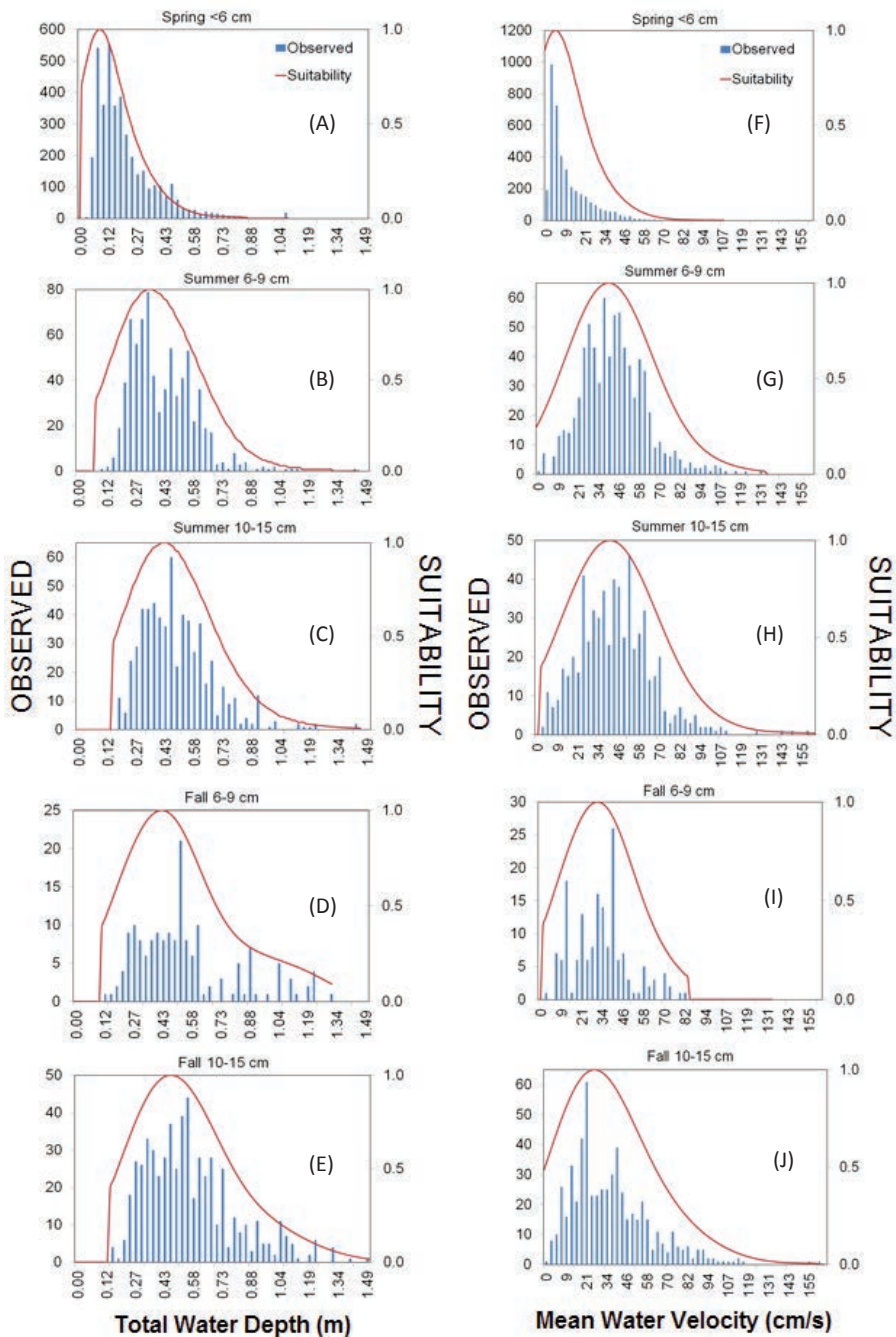


FIGURE 2.—Total water depths and mean water velocities at focal positions selected by juvenile steelhead trout (bars) according to season and size of juvenile steelhead trout. The solid line is the normalized-kernel smoothed suitability of total water depth and mean water velocity.

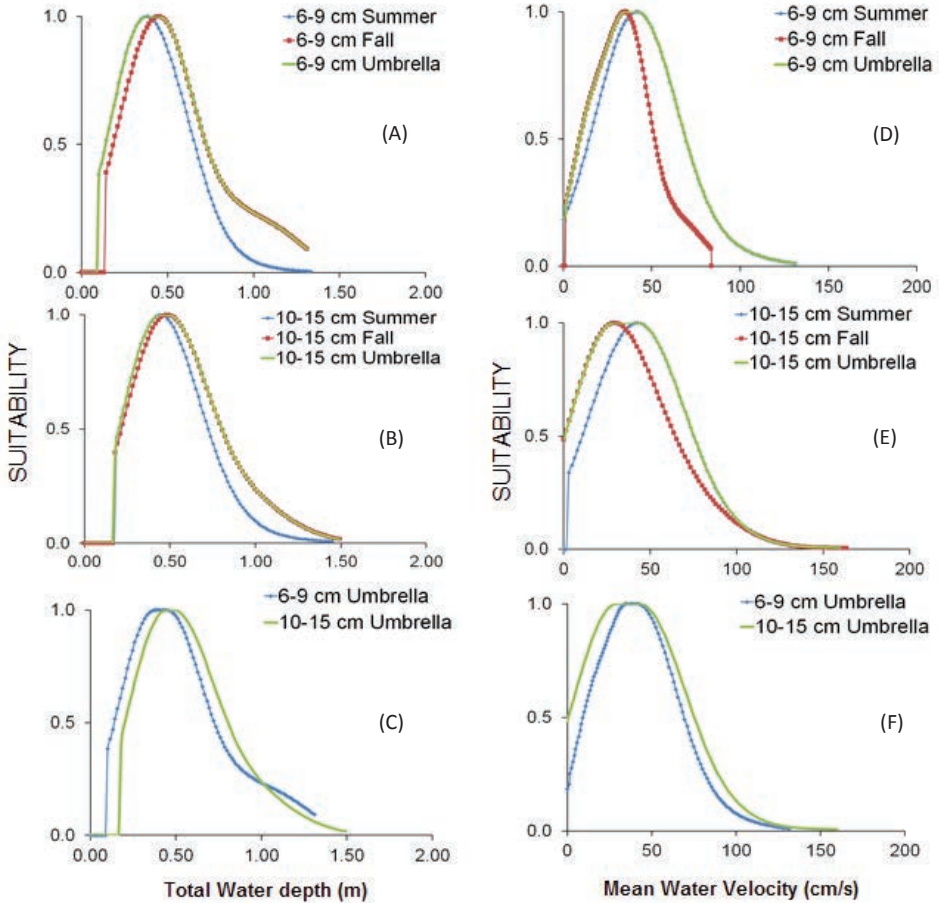


FIGURE 3.—Comparison of seasonal total water depth and mean water velocity habitat suitability criteria for 6–9 cm and 10–15 cm steelhead trout in the Big Sur River, Monterey County, California. Umbrella habitat suitability criteria curves reflect seasonal use patterns for each size group of juvenile steelhead trout.

Water velocity.—Suitability for water velocity is 1.00 from 5.5 to 7.6 cm/s for steelhead trout <6 cm (Figure 2F). The umbrella HSC for 6–15 cm steelhead trout indicate suitability for water velocity is 1.00 from 27.7 to 44.8 cm/s during the summer and fall rearing period (Figure 3D and Figure 3F).

Fish focal velocity.—Fish focal water velocity HSC for steelhead trout <6 cm is 1.00 from 4.9 to 6.4 cm/s. Fish focal water velocity HSC for 6–9 cm steelhead trout is 1.00 from 22 to 24.7 cm/s. Fish focal water velocity HSC for 10–15 cm steelhead trout is 1.00 from 26 to 29.6 cm/s.

Distance to escape cover.—Distance-to-escape-cover HSC for steelhead trout <6 cm have a 1.00 suitability from 0.24 to 0.27 m. Steelhead trout 6–9 cm distance to escape cover HSC is 1.00 suitability in summer and fall from 0.46 to 0.55 m and 0.58 to 0.73 m, respectively. Steelhead trout 10–15 cm distance to escape cover HSC is 1.00 from 0.55 to 0.64 m and 0.12 to 0.21 m in summer and fall, respectively.

Escape cover types.—In general, hard substrate types (large gravel to large cobble sizes) were the most common types of escape cover observed near the fish observation locations and had the highest HSC. Steelhead trout <6 cm escape cover HSC are 1.00 for small cobble. Steelhead trout 6–9 cm escape cover HSC are highest for large cobble in the summer, and highest for small branches or in-water vegetation <10 cm in the fall. Steelhead trout 10–15 cm escape cover HSC are 1.00 for small boulders in the summer, and highest for small branches or in water vegetation <10 cm in the fall.

Selectivity vs Preference (U/A) HSC Curves

To further evaluate the representativeness of the equal-area selectivity HSC curves, and the potential effects of habitat availability on these curves, alternative HSC curves were derived using the U/A forage ratio methodology (Figure 4, A–J). The smoothed habitat availability curves for depth and velocity were deeper and faster than the fish selectivity curves for steelhead trout <6 cm (Figure 4A and Figure 4F), and resulted in shifts of the preference curves to the left into shallower and slower water. In contrast, the smoothed habitat availability curves were shallower and slower than the fish selectivity curves for steelhead 6–9 cm and 10–15 cm, and frequently resulted in radical shifts of the preference curves to the right (Figures 4B and Figure 4C, and Figure 4G and Figure 4H, respectively). These shifts for steelhead trout 6–9 cm and 10–15 cm were particularly extreme for the fall data, and resulted in high suitability for depths greater than 1.2–1.5 m and velocities greater than 107 cm/s while severely deflating the suitabilities where the majority of fish were observed (Figure 4D and Figure 4E, and Figure 4I and Figure 4J, respectively). Trimming or truncating (or both) the U/A data was (were) unsuccessful at producing preference curves that were not radically shifted to the right for 6–9 cm and 10–15 cm steelhead trout.

DISCUSSION

Steelhead trout life history tactics and thresholds.—Big Sur River steelhead trout were observed selecting faster velocity habitats as the rearing fish grew during the spring and summer seasons, consistent with Everest and Chapman (1972) as well as by more recent observations on the Klamath River (Hardy and Addley 2001, Hardin et al. 2005). Interestingly, the fastest velocities selected by all steelhead (fry and larger juveniles) on the Big Sur River were observed to occur in the summer, not the fall rearing period. These findings are consistent with Allen (2000), who found that juvenile spring-run Chinook salmon (*O. tshawytscha*) selected faster velocities in summer over fall in the Yakima River, Washington. There was also good overlap of the Big Sur River HSC velocity curves for both larger size groups of steelhead trout, and the resultant velocity umbrella curve was comparable in peak and overall shape with historical steelhead HSC (Bovee 1978).

The 10–15 cm steelhead trout showed a slightly increased selectivity for faster velocities greater than 61–91 cm/s over the 6–9 cm steelhead trout in the summer, while also showing higher selectivity for slower velocities than the 6–9 cm fish in the fall. These results are generally consistent with Spina (2003), who reported that larger juvenile steelhead trout, ages 1 and 2, selected slower water velocity habitats than young-of-year in Santa Rosa Creek, approximately 129 km south of the Big Sur River. As flows receded in the Big Sur River during fall, larger juvenile steelhead trout showed higher selectivity for deeper, slower

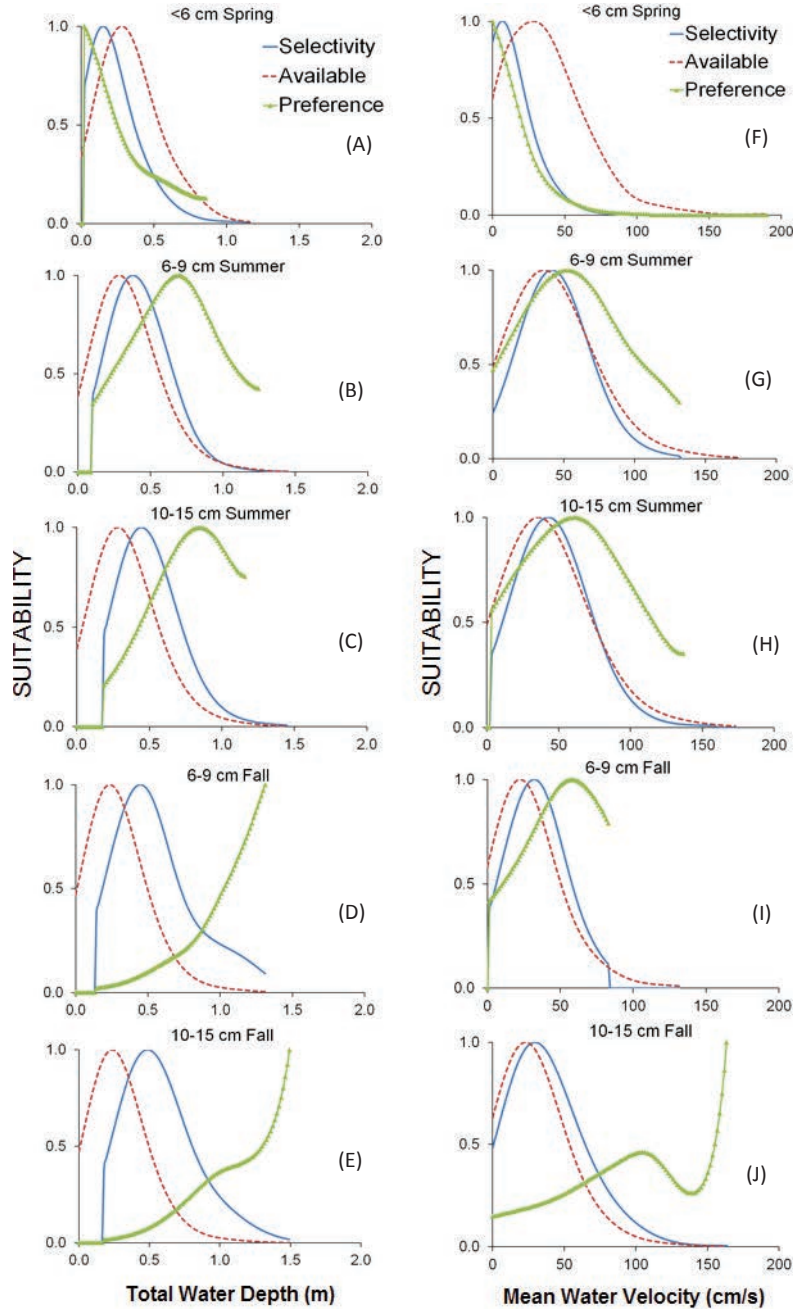


FIGURE 4.—Comparison between juvenile steelhead trout (according to size) selectivity habitat suitability criteria using equal-area sampling with habitat availability and preference habitat suitability criteria using forage ratio mathematical adjustments; Big Sur River, Monterey County, California.

water velocity habitats that occur in pools. The smaller, 6–9 cm young-of-year steelhead trout, on the other hand, selected faster velocity habitats despite the rare occurrence of such habitat in the fall compared to summer. Increased use of pools and deeper habitats by the larger juveniles in the fall may be related to other non-hydraulic habitat factors such as bioenergetics, predation, or temperature.

Steelhead trout temperature tolerance varies among life stages (Bell 1986, Bjornn and Reiser 1991), and differences in seasonal water temperatures may affect habitat selection (Reeves et al. 2009). The drop in mean temperatures from 16° C in summer to 14° C in fall could be associated with increased use of deeper and slower pool habitats. A similar change in water temperatures may have influenced a shift in microhabitats selected by juvenile spring-run Chinook salmon in the Yakima River, Washington (Allen 2000). However, these temperatures are well above the 5–10° C temperatures known to elicit significant shifts in behavior in steelhead and rainbow trout in colder, interior climates (Chapman and Bjornn 1969, Bustard and Narver 1975, Riehle and Griffith 1993).

Hardy and Addley (2001) also observed juvenile steelhead trout selecting deeper water habitats in fall versus spring on the Klamath River. Thus, it is apparent that steelhead trout select deeper water (and faster velocity) habitats as they grow. However, the depth thresholds (minimum depth avoidance) observed by the rearing (non-fry) steelhead trout in the Big Sur River have not been found by other researchers (Waite and Barnhart 1992; Hardy and Addley 2001) or to be as distinct between juvenile size groups in studies on other coastal California rivers. On the Big Sur River, 95% of all juvenile steelhead ≥ 6 cm FL ($N = 2,093$) avoided water depths shallower than 0.23 m during the core rearing period of summer and fall.

In addition to hydraulic microhabitat conditions (i.e., water depth and velocity), rearing site selection of Big Sur River steelhead trout was influenced by factors such as proximity and type of in-water escape cover. Despite some juvenile steelhead trout not being observed near (i.e., < 3 m) any type of escape cover, all size groups of juveniles were predominately observed in close proximity to some type of in-water escape cover, with types ranging from gravel/cobble for < 6 cm steelhead trout to larger cobble and small boulders for larger juvenile steelhead trout. Although proximity and type of escape cover shifted with fish size, it also shifted with season and associated flow conditions (Holmes et al. 2014).

We observed juvenile steelhead trout shifting selection of rearing sites in close proximity to hard substrate escape cover types (i.e., cobble and boulder) in summer to selection of rearing sites in close proximity to predominately vegetative escape cover components (i.e., branches < 10 cm diameter in-water) in the fall. This seasonal shift was apparently not directly due to respective availability of sites in proximity to those escape cover types between summer and fall. Instead, we attributed this shift to decreased availability of faster water velocities in the fall or the faster areas becoming too shallow, or both. For example, juvenile steelhead trout were observed selecting feeding locations in the summer with faster water velocities near hard substrates, which may act as both in-water escape cover and water velocity shelter. In the fall, however, flow levels decline naturally on coastal California streams and rivers and the corresponding water velocities also slow making such faster velocity habitats rare or too shallow for larger juveniles.

Hardy and Addley (2001) also observed seasonal shifts in proximity of steelhead trout to hard substrates (i.e., small boulders) and vegetative-type (e.g., shrubs, grass, sedges, herbs) escape cover on the Klamath River. However, the trend they observed was opposite of what we observed on the Big Sur River. The opposing trends are likely related to the fact

that Klamath River vegetative cover was only available under high spring flows as well as differences in the physical channel and riparian habitat between the much larger Klamath River Basin (i.e., 40,790 km²) and the smaller redwood-dominated Big Sur River Watershed (i.e., 160 km²). Waite and Barnhart (1992) cautioned applying HSC from one river system to another without consideration of site-specific hydrology and habitat characteristics.

Equal-area sampling vs. forage ratio adjustments.—Flow conditions during the fish surveys, with the exception of the fall sampling event, occurred at annual exceedance probability flows below the Big Sur River's 50% annual exceedance probability benchmark. However, comparison of timing of fish surveys with monthly exceedance probability flows indicates summer and fall sampling occurred at above average flows ranging from 5 to 24% exceedance probability. We conclude habitat availability was good to optimal based upon site-specific water availability since the sampling flows during the core rearing period of summer and fall were comparable to those of above average or wet months (Table 2). A central tenet of developing HSC is that all micro- and macrohabitats should be equally available for the organism to select from (Bovee 1986). Since stream flow is associated with juvenile steelhead survival (Grantham et al. 2012) and to salmonid habitat use (Ptolemy 2013), sampling for HSC development at lower than average natural flows may not provide equal availability of all habitats and may limit the effectiveness of an equal-area sampling approach. In such cases, corrective methods to adjust for habitat availability, such as application of the forage ratio, may be necessary.

Big Sur River steelhead trout HSC, which far exceeded minimum sample size requirements as outlined by Bovee (1986), were developed using habitat utilization data that were not mathematically adjusted for habitat availability. Instead, we employed a rigorous effort to maintain equal-area sampling among mesohabitat types, river reaches, and sampling seasons. Equal-area sampling within mesohabitat types helps minimize biases by allowing relative quality of the different habitat types to dictate the form of the HSC (Allen 2000). Further, use of the equal-area sampling design under natural unimpaired flow conditions accounts for potential biases of flow-related habitat availability (i.e., avoids confusing selection or use of optimal habitat with selection or use of merely tolerable habitat) on development of site-specific HSC. Our study design using equal-area sampling allowed the species and its respective life stages to inform us of its biological habitat requirements, without the need for mathematical adjustments (i.e., forage ratio adjustments) of habitat use with habitat availability data.

Using the equal-area selectivity HSC approach avoids potential pitfalls associated with development of preference HSC other researchers have identified (Bovee and Zuboy 1988, Hayes and Jowett 1994, Payne and Allen 2009). For example, small sample sizes, particularly at the tails or extremes of the frequency distributions of habitat parameters, can result in potential overcorrection for habitat availability when using the forage ratio adjustments, as seen with the Big Sur River depth and velocity HSC. Our observations (Figure 4) were, therefore, consistent with those of Hayes and Jowett (1994), which indicate performing the forage ratio adjustment for habitat availability when populations are not limited by habitat or when sampling bias is not suspected (Payne and Allen 2009) may result in over-corrected HSC (Bovee et al. 1998). Other researchers have also justified use of HSC based upon the utilization data without a preference adjustment for habitat availability (Johnson 1980).

We contend that development of preference HSC may well be a viable option for development of HSC in those instances when sampling conditions are known or suspected to be limited by habitat availability, or where inequalities in sampling effort among habitat types leads to biases in the use data. In such cases, selectivity HSC based solely on equal-area sampling may not yield HSC that are unbiased by habitat limitations. Equal area sampling may also be highly inefficient where a species or life stage is largely confined to limited habitat conditions, such as salmonid spawning which is limited to specific locations where appropriate substrate is available, or for obligate pool- or riffle-dwelling species that rarely occupy other habitats.

We observed juvenile steelhead trout habitat selectivity changing with fish size, season, discharge, and habitat availability. Biologically accurate and unbiased HSC are critical for valid and biologically representative hydraulic habitat modeling of flow and habitat relationships. There are many potential pitfalls in developing site-specific HSC that could contribute to defective HSC and hence unreliable instream flow modeling efforts, which include (1) inadequate overall sample sizes; (2) unequal or insufficient representation of habitat use; (3) habitat availability being unaccounted for, which may mask flow-linked constraints on habitat use; (4) limited temporal sampling such as during one timeframe or season of an important life history component of a species (although one timeframe or season may be fine for certain applications such as spring sampling for salmon fry that emigrate soon after emergence); and (5) uncritical application of ratio-based curves that bear little resemblance to the underlying use data. Our sampling strategy and the overall ecologically favorable stream conditions of the Big Sur River minimized the potential bias of sampling techniques and habitat availability. Use of corrective mathematical methods (i.e., using availability data) were evaluated, but were not effective or warranted based upon the enhanced flow conditions observed during sampling and the overall ecologically favorable habitat conditions of the Big Sur River. With proper habitat stratification and non-limiting sampling conditions (e.g., adequate flows and non-degraded habitat), use of an equal-area sampling design for site-specific HSC development is, therefore, a viable option for development of biologically relevant and representative HSC and, ultimately, for effective environmental flow recommendations.

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Potential mechanisms in the early mortality of juvenile Chinook salmon exposed concurrently to Infectious Hematopoietic Necrosis Virus (IHNV) and esfenvalerate

MARK A. CLIFFORD*, LIZABETH BOWEN, JEFFRY L. STOTT, INGEBORG WERNER,
AND RONALD P. HEDRICK

*California Department of Fish and Wildlife, Fisheries Branch, #3 North Old Stage Road,
Mt. Shasta, CA 96067, USA (MAC)*

*Department of Medicine and Epidemiology, School of Veterinary Medicine, University of
California, Davis, California 95616, USA (RPH)*

*Department of Pathology, Microbiology, and Immunology, School of Veterinary Medicine,
University of California, Davis, California 95616, USA (JLS, LB)*

*Aquatic Toxicology Program, Department of Anatomy, Physiology, and Cell Biology, School
of Veterinary Medicine, University of California, Davis, California 95616, USA (IW)*

*Correspondent: mark.clifford@wildlife.ca.gov

Replicate groups of juvenile Chinook salmon (*Oncorhynchus tshawytscha*) were exposed to infectious hematopoietic necrosis virus (IHNV), sub-lethal levels of esfenvalerate, or to both agents concurrently. A lethal synergistic effect of concurrent exposure to IHNV and esfenvalerate resulted in 24.1% mortality by 68 h post-virus exposure with no mortality observed in any other treatment groups at this time. Analyses of spleen samples from fish sampled at 68 h following exposure to both IHNV and esfenvalerate was suggestive of a disruption of transcription, and demonstrated a significant decrease in the production of two early, non-specific anti-viral genes (Mx-1 and Vig-8). Analyses of blood serum suggested that osmolality was not a contributing factor to the observed early mortality event. Examinations of stained sections of the gill and anterior kidney from fish in all treatment groups at 68 h did not reveal pathologic microscopic changes. This study suggests that the lethal synergistic effect of exposure to IHNV and esfenvalerate to juvenile Chinook salmon may be related to inhibited transcription of early, non-specific, anti-viral cytokines.

Key words: IHNV, esfenvalerate, synergy, Mx-1, Vig-8, Chinook salmon, pesticides

Exposure to chemical pollutants can disrupt normal physiologic and immunologic processes of fish resulting in greater mortality than exposure to microbial pathogens alone (Hetrick et al. 1979, Dunier 1996, Arkoosh et al. 1998, Clifford et al. 2005). The recent decline in wild salmon populations worldwide has been attributed to many anthropogenic activities including the introduction of agricultural pesticides to aquatic environments. The rivers and Delta region of California's Central Valley (USA) is one area where exposure to pesticides is believed to contribute to recent declines in fish populations (Moyle 1994, Hinton 1998).

California's Central Valley region is one of the most agriculturally productive areas in the world, but one unfortunate consequence is the inadvertent introduction of pesticides into aquatic environments (Hinton 1998, Werner et al. 2002, Weston et al. 2004). The Sacramento and San Joaquin rivers drain the Central Valley and join to form the Sacramento-San Joaquin Delta, which empties into the San Francisco Bay, forming the largest estuary on the west coast of North America. Historically, these waters supported enormous populations of Chinook salmon (*Oncorhynchus tshawytscha*) at an estimated 1–2 million spawning fish annually (Fisher 1994). However, all natural Chinook salmon populations have declined in California, some even to extinction (Fisher 1994).

Pesticides can disrupt immune system functions in fish (Dunier 1996, Banerjee 1999, Eder et al. 2004) and increase mortality when these fish are exposed to microbial pathogens (Clifford et al. 2005). Thus, exposure to Central Valley agricultural pesticides may be contributing to the decline of wild Chinook salmon populations in the Sacramento-San Joaquin Delta. The term "pollution" is used to describe the presence of agents that potentially exert a negative effect on ecosystems and can include hydrocarbons, heavy metals, and pesticides. The use of the word "pollutant" fails to recognize the vastly different effects compounds may, or may not have on fish immune function and overall fish health. Pesticides have many different modes of action affecting both target and non-target species (Miller and Adams 1982). Studies of definite immune effects from specific pesticides on target and non-target species are required to establish direct cause and effect relationships (Austin 1999). Depending on the microbial pathogen involved, immunosuppressive effects suffered from pesticide exposure may or may not influence the incidence and severity of infectious disease. In the current study, we examined in more detail certain features of the physiologic and immunologic effects observed among juvenile Chinook salmon concurrently exposed to sublethal levels of esfenvalerate and to infectious hematopoietic necrosis virus (IHNV). The combination of these two agents induces a reproducible and lethal synergism resulting in mortality by mechanisms of unknown cause (Clifford et al. 2005).

Esfenvalerate is a synthetic pyrethroid insecticide derived from compounds produced by certain species of chrysanthemum. All pyrethroids are neurotoxins that affect central nervous system function by disrupting normal voltage-dependent sodium and other ion channels (Miller and Adams 1982, Bradbury and Coats 1989, Burr and Ray 2004). Pyrethroids are used extensively on row crops, orchards, forest spray applications, and in urban areas for structural pest control and pet sprays (Haya 1989, Oros and Werner 2005). These insecticides can enter waterways via agricultural and urban runoff, spray drift, direct application for mosquito control and the release of agricultural tail waters (Oros and Werner 2005). Modeling results indicate that greater than 1% of total pyrethroids applied to agricultural lands alone may be available for transport through the California Delta and into the San Francisco Bay (Oros and Werner 2005). Pyrethroids were detected in 75% of sediment samples taken from small creeks and irrigation canals in the Central Valley of California (Weston et al. 2004) and winter precipitation events were reported to transport

esfenvalerate from agricultural lands to surface waters in February and March of 2003 (Bacey et al. 2005), a time when juvenile Chinook salmon are residing in Central Valley rivers and tributaries.

IHNV is a single-stranded negative-sense RNA virus of the family *Rhabdoviridae* and is a serious pathogen of many species of salmon and trout (Wolf 1988) and is listed as a "catastrophic disease" in the California Code of Regulations (California Code of Regulations, Title 14, Natural Resources, Division 1. Fish and Game Commission. Department of Fish and Wildlife, Section 245). IHNV is endemic to the Pacific Northwest of North America and has been reported in the Sacramento River since the 1940's (Wolf 1988). The virus is associated with significant mortality of hatchery-reared as well as wild salmon populations and the principal target tissues are the hematopoietic organs, the kidney and spleen (Wolf 1988, Drolet et al. 1994). Young fish are most susceptible and succumb to the disease due to kidney failure, anemia or fluid and electrolyte imbalances (Amend and Smith 1974, 1975; Williams and Amend 1976; Wolf 1988; Bootland and Leong 1999). IHNV alone induces mortality among young susceptible salmonids 5–10 days post exposure (dpe) and early survival is likely dependent on innate rather than acquired immune mechanisms (LaPatra 1998, Hattenberger-Baudouy et al. 1995, Cain et al. 1996, Purcell et al. 2004). The early innate immune response to IHNV infection includes increased transcription of two important type-1 interferon regulated genes, Mx-1 and Vig-8 (Purcell et al. 2006a, 2006b).

Horisberger et al. (1983) first reported Mx genes to provide mouse cells resistance to myxovirus infections. Homologues of Mx genes were later discovered and reported in rainbow trout (*Oncorhynchus mykiss*) (Staeheli et al. 1989, Trobridge and Leong 1995). Mx proteins block the transcription of viral mRNA (Caipang et al. 2003) and interfere with the transport of viral proteins to the site of viral assembly (Haller and Kochs 2002). Boudinot et al. (1999) first discovered Vig genes in rainbow trout cell lines infected with viral hemorrhagic septicemia virus (VHSV) and thus their designation as VHSV-induced genes (Vig). Vig genes are upregulated after viral infection and code for proteins that have characteristics of CXC chemokines (O'Farrell et al. 2002) that attract activated T-lymphocytes to the site of viral infection (Laing and Secombs 2004). Both Mx-1 and Vig-8 are transcribed in an early response to virus infection and type 1 interferon production, a vital step in the antiviral cascade (Congleton 1996). Significant increases in both Mx-1 and Vig-8 gene transcription result when rainbow trout and Chinook salmon are exposed to IHNV (Purcell et al. 2004).

Juvenile Chinook salmon undergo an early and unexplained mortality when concurrently exposed to sublethal levels of esfenvalerate and to IHNV (Clifford et al. 2005). In the study presented here, we examined potential causes of this mortality event including blood osmolality, microscopic pathological changes in the gill and anterior kidney and changes in the transcription of selected cytokines (Mx-1 and Vig-8) as indicators of physiologic, pathologic, or immunologic impairments resulting from the lethal synergism of esfenvalerate and IHNV.

MATERIALS AND METHODS

Fish.—Fall-run Chinook salmon were obtained as fertilized eyed eggs and hatched at the Fish Health Laboratory (University of California, Davis) and maintained for experimental purposes. Fish were approved for research purposes under an Institutional

Animal Care and Use Protocol. Hatched fish were reared in fiberglass tanks receiving 12° C, aerated, single-pass well water. This is a temperature at which both IHNV and esfenvalerate demonstrate effects on fish (LaPatra 1998, Kumaraguru and Beamish 1981) and is within the normal range of wild juvenile salmon habitat. Fish received a commercial diet at approximately 3–5% body weight per day. At 12 weeks post-hatch, 14 fish were randomly selected for each of 4 replicates per treatment group (control, esfenvalerate only, IHNV only and esfenvalerate/IHNV). Fourteen fish were used per replicate to achieve an approximate ratio of 0.008 µg esfenvalerate per g of fish, which proved critical in preliminary studies regarding the propensity of esfenvalerate to be bound by organic material (see discussion section). Replicate groups were held in 16-L static, glass aquaria provided with aeration and partially submerged in 12° C chilled water baths as described elsewhere (Clifford et al. 2005). These aquaria provided environments in which fish could be exposed to nominal amounts of esfenvalerate and IHNV. Every 24 hours 75% of water and any uneaten food or fecal material was siphoned out of aquaria and replaced with fresh 12° C well water. The mean fork length and weight of fish was 4.85 cm and 0.96 g, respectively. During esfenvalerate exposures, fish in all treatment groups were not fed for ≥ 1 hour before water changes to minimize esfenvalerate binding to organic material in the tanks. Following esfenvalerate and IHNV exposure in static aquaria, all treatment groups were transferred to 15-L flow-through tanks receiving aerated, single pass 12° C well water. Fish were held in the 15-L flow-through tanks for the remainder of the experiment (21 days) and total cumulative mortality was recorded. Water temperature and appearance of fish were monitored one or more times per day.

Esfenvalerate exposures.—Fish transferred to 16-L static-system aquaria were held for 24 h, after which the first water change took place. Upon completing the first water change, the first esfenvalerate test and control treatments were administered to aquaria. Water changes and esfenvalerate dosing were conducted 4 times at 24-h intervals to complete a 96-h pesticide exposure time. Methanol served as the solvent for esfenvalerate and thus also for the control (non-pesticide) groups (10 ml per aquarium per dosage). Solid, crystalline and 98% pure esfenvalerate (ChemService, West Chester, PA, USA) was diluted in methanol to make a stock solution of 160.0 mg/L. Ten ml of this stock solution was added to the 16-L static-system aquaria for a final nominal concentration of 0.1 mg/L. Because 100% of esfenvalerate was assumed to breakdown or adsorb to glass after 24 h, 10.0 ml of stock solution was added after each subsequent water change to keep concentrations as close to 0.1 mg/L as possible for a period of 96 h. Composite water samples from all esfenvalerate groups were taken on the fourth day of pesticide dosing immediately after administration, and again 24 h later (just prior to water change) to record actual esfenvalerate concentrations in aquaria. These water samples were analyzed by the Fish Wildlife Water Pollution Control Laboratory of the California Department of Fish and Wildlife using gas chromatography with dual electron capture detectors, and with positive samples confirmed using gas chromatography mass spectrometry. Esfenvalerate concentrations were 0.06 µg/L at initial dosing and 0.02 µg/L 24 h later. After 96 h of pesticide exposure, water in all 16 L static-system aquaria was changed for 1 additional day, allowing fish to remain in clean and pesticide-free water after which all groups of fish were transferred to 15-L flow-through aquaria receiving 12° C well water supplied with aeration for the remainder of the experiments.

IHNV propagation.—IHNV was propagated in the CHSE-214 line as previously described (Clifford et al. 2005). The IHNV isolate used in this study originated from adult

winter-run Chinook salmon in the upper Sacramento River in July 2001 and was passed 4 times on the CHSE-214 cell line and is designated as Type Q from the L genogroup by Kelley et al. (2006). Virus in culture medium was cleared of cell debris by centrifugation at 1,300g for 10 min at 10° C. The resulting supernatant was kept on ice until used for the fish exposures. Because viral culture media (minimal essential media, MEM) contains organic material that can bind esfenvalerate, the virus was further purified by ultracentrifugation at 30,000g for 1 h at 10° C. The virus pellet was re-suspended in 4° C double-distilled water. Concentrations of virus used in the exposure studies were determined by plaque titration as previously described (Clifford et al. 2005).

IHNV Exposures.—Virus was added to the static-system aquaria during the daily water change beginning after the first 24 h of esfenvalerate exposure. Virus groups were exposed to 3.2×10^5 plaque forming units/ml/day while control groups received 37 ml of double distilled water. Groups were exposed to treatments for 1 h (at 12° C), after which aquaria were replenished to 16 L with fresh water and the toxicant or methanol control dosages resumed as described. All trials were conducted for 21 days to observe total cumulative mortality of non-sampled tanks.

Tissue sample timing and numbers.—Four replicates were used for each treatment group: two for obtaining tissue samples at a designated time point and two for observing total cumulative mortality. For baseline measurements, 10 fish were randomly selected from the original stock tank used to hold related juvenile Chinook salmon prior to selecting fish for replicate treatment and control groups. These fish were euthanized with an overdose of anesthetic (100 ppm benzocaine, no more than 3 fish at a time), and immediately weighed, measured and dissected aseptically to obtain blood, gill, anterior kidney and whole spleen samples. Fish were visually inspected during the procedure for external and/or internal signs of disease and observations were recorded. This same procedure was followed to obtain tissue samples from 10 fish in each replicate aquarium designated for sampling at 68 h post-virus exposure. This time point was chosen as it is just prior to the anticipated early mortality event (72 h post-virus exposure) induced by concurrent exposure to esfenvalerate and IHNV as reported by Clifford et al. (2005).

Total blood serum osmolality.—As described above, 10 fish from each replicate designated for tissue sampling for each treatment group, were euthanized at 68 h post-virus (or control) exposure. Immediately following euthanasia and recording weight and fork length, fish were bled by caudal severance and blood collected in 20 µl capillary tubes without heparin (Drummond Microcaps, Drummond Scientific Company, Broomall, PA, USA). Blood was centrifuged in a micro-hematocrit centrifuge (Clay Adams, Benton Dickson and Company, Parsippany, NJ, USA) for 15 min and serum collected in autoclaved 0.5 ml microcentrifuge tubes and frozen at -20° C until analyzed (approximately 1 week). All serum samples were thawed and analyzed within a 3-h period. Serum samples were centrifuged briefly to collect contents, kept on ice and then diluted in RNase-free autoclaved sterile water (Ultra Pure, USB Corporation, Cleveland, OH, USA) at a ratio of 2.5 µl serum to 7.5 µl water just prior to testing osmolality. The dilution was necessary as the vapor pressure osmometer (Vapro model 5520, Westcor Inc., Logan, Utah, USA) requires 10 µl of sample and many blood samples did not yield that volume of serum. In trial studies, serial dilutions of catfish serum and water demonstrated a consistent linear relationship in blood osmolality (M. Clifford, California Department of Fish and Wildlife [CDFW], unpublished data).

Histopathology.—A sagittal incision from posterior of the operculum to the base of dorsal fin was made on all euthanized fish. This section included both the gills and anterior kidney, while preserving the spleen for subsequent cytokine transcription analyses. The anterior portion of the fish was placed whole into Davidson's fixative for 24–48 h, after which the fixative was removed and replaced with 70% ethanol. Fixed samples were divided into two along a midsagittal plane and then processed by standard paraffin embedding. Tissue sections were affixed to microscope slides and stained with hemotoxylin and eosin.

Spleen sampling and preservation.—Individual spleen samples aseptically dissected from fish were placed directly into autoclaved 1.5-ml microcentrifuge tubes containing 350 μ l of Buffer RLT (Qiagen, Valencia, CA, USA) with beta-mercaptoethanol. These spleen samples were kept on ice and subsequently frozen at -80°C within hours. Between each sampled fish, instruments were wiped, dipped in 70% ethanol, and flamed. Instruments used to dissect fish were changed frequently and always between replicate groups of fish, as were the anesthetic baths.

RNA/cDNA preparation.—Total RNA was extracted from spleen samples using RNeasy Minikit (Qiagen) following the manufacturer's instructions. Total RNA was frozen at -80°C for later synthesis into cDNA using the QuantiTect Reverse Transcription kit with DNase treatment (Qiagen), following the manufacturer's instructions.

Primer design.—Published primers for Mx-1 and Vig-8 in rainbow trout (Purcell et al. 2004) were used for amplifying sequences in Chinook salmon, due to the close taxonomic relationship of those species. To validate these primers in Chinook salmon, conventional PCR was performed using Platinum Taq DNA Polymerase (Invitrogen, Carlsbad, CA, USA) and a MJ Research PTC-200 thermocycler (Bio-Rad, Hercules, CA, USA) under the following conditions: 95°C for 3 min, then 40 cycles of 95°C for 30 s, 55°C for 1 min, 70°C for 1 min with a final extension step of 70°C for 10 min. Amplified products were separated by electrophoresis in 2% agarose gels and visualized by ethidium bromide staining. Resulting amplicons were single bands of correct size and were purified using QiaQuick PCR purification kit (Qiagen) and submitted for sequencing. Sequences were evaluated using computer applications from MacDNASIS (Mirai-Bio., Alameda, CA, USA), Amplify 1.2 (University of Wisconsin Genetics, WI, USA) and NCBI GenBank BLAST to verify correct amplicon and primer binding. Validated sequences were used for designing Mx-1 and Vig-8 primers for Chinook salmon. The primer sequences are: Mx-1 forward primer: 5'-GGG TAG CTG TCA AGG GTC AA-3', Mx-1 reverse primer: 5'-GTC CAC CTC TTG TGC CAT CT-3', Vig-8 forward primer: 5'-AGA AGC TCC ATT TGC CAA GA-3', and Vig-8 reverse primer: 5'-TTC ATT ATT TTC TTA ATG GTT TTC TGA-3'. These primers and resulting amplicons were validated with conventional PCR, sequencing, and software applications as described above with the exception that the annealing temperature was increased to 60°C . Degenerate primers for the ribosomal subunit S9 housekeeping gene were used for designing Chinook salmon S9 primers as described above with the exception that the PCR reactions were 94°C for 3 min, then 40 cycles of 94°C for 1 min 30 sec, 55°C for 30 sec and 70°C for 1 min.. Sequences for Chinook-specific S9 primers are: S9 forward primer: 5'-GAC AGC CAG AAG CAC ATT GA-3', and S9 reverse primer: 5'-TGG CGT TCT TTC TCT TGA CA-3'.

Real-time PCR.—Quantitative analysis of Mx-1, Vig-8 and S9 transcripts were completed with the ABI 7300 Real Time PCR system (Applied Biosystems, Foster City, CA, USA) using Quantitect SYBR Green PCR Master Mix (Qiagen) plus 0.5 units of

uracil DNA glycosylase (Invitrogen). All primer pairs were run in separate wells and in duplicate for all tissue samples with water as a control. Because the comparative C_T method ($\Delta\Delta C_T$ method) was used for quantifying gene transcription, primer efficiency curves were conducted using 10-fold serial dilutions of cDNA to assure equal priming ability of all primer pairs (Leutenegger et al. 1999). The Chinook salmon ribosomal subunit S9 housekeeping gene was used to normalize transcription of the immune genes by correcting for variation in reverse transcriptase efficiency, template quantity, or both. Transcription amounts were calibrated using the weakest transcription signal of one individual fish for Mx-1 or Vig-8 and all data was expressed as fold-increase transcription relative to this fish. Amplicons synthesized with real time PCR were verified by analysis of dissociation curves and by sequencing multiple wells for each primer set in duplicate using methods described above for conventional PCR. Infrequently, samples were excluded from comparative analysis wherever duplicate real time PCR reactions yielded a difference in cycle threshold crossing greater than 1.5 for either housekeeping or immune genes. This was true for no more than 1 fish in any given group.

Statistical analysis.—Statistical analysis was performed using Number Crunching Statistical Software (NCSS, Kaysville, UT, USA). Differences in mortality, mean day to death, serum osmolality and Mx-1 and Vig-8 transcription among all treatment groups were evaluated with GLM ANOVA with P -values ≤ 0.05 considered statistically significant.

RESULTS

Mortality.—Dead fish were detected among groups of Chinook salmon exposed to both IHNV and to esfenvalerate by 68 h post virus exposure. Cumulative mortality among replicate groups ranged from 14.3% to 28.6% (Table 1). Mortality among fish exposed only to IHNV began 9 dpe and dead fish were detected subsequently up to day 21 when the experiment was terminated with an average mortality of 17.8 % (Table 1). There was

Treatment Group	Sampled at 68 h?	Replicate	Cumulative Mortality (%)	Mean Time to Death (days)
Control	no	A	0	NA
	no	B	0	NA
	yes	C	0	NA
	yes	D	0	NA
IHNV	no	A	21.4	14.6
	no	B	28.6	15.5
	yes	C	14.3	17.5
	yes	D	7.1	16.0
Es	no	A	0	NA
	no	B	0	NA
	yes	C	0	NA
	yes	D	0	NA
IHNV+Es	no	A	21.4	3.0
	no	B	14.3	3.0
	yes	C	28.6	3.0
	yes	D	21.4	3.0

TABLE 1.—Mean time to death (days) of juvenile Chinook salmon exposed to infectious hematopoietic necrosis virus (IHNV) only, esfenvalerate (Es) only, Es and IHNV concurrently, or to controls. Each replicate initially contained 14 fish, while 10 fish were removed from designated replicate groups for sampling at 68 hours post-virus exposure. Mortality on day 3 was recorded prior to sampling procedure and no other fish died that day. Groups that had no mortality have no mean time to death (NA).

nomortality among any other groups of fish, including unexposed control groups at any time during the study. Total cumulative mortality was not significantly different between groups of fish exposed to IHNV only, or to esfenvalerate and IHNV, though both these groups had higher mortality ($F_{3,16} = 17.49$; $P < 0.001$) than control groups or groups exposed to esfenvalerate alone. Mean day-to-death was lower ($F_{1,4} = 915.06$; $P < 0.001$) in groups of fish exposed to both esfenvalerate and IHNV (3.0 d) as compared to groups exposed to IHNV alone (15.9 d).

Osmolality.—Fish sampled from the stock tank (time zero) had the highest serum osmolality with a mean of 88.0 mM/kg, the greatest value when compared to all treatment groups, including controls. The control groups had the next highest serum osmolality ($\bar{x} = 79.5$ mM/kg), which was greater than groups exposed to esfenvalerate or IHNV alone. The remaining treatment groups (esfenvalerate only, IHNV only, and esfenvalerate/IHNV) had mean serum osmolalities of 70.1, 71.0 and 78.0 mM/kg, respectively (Table 2).

Treatment Group	Mean Serum Osmolality (mMoles/kg)
Stock tank	88.0
Control	79.2
IHNV	70.5
Es	69.5
Es + IHNV	76.9

TABLE 2.—Mean serum osmolality of juvenile Chinook salmon from stock tank and from replicate groups exposed to, infectious hematopoietic necrosis virus (IHNV) only, esfenvalerate (Es) only or to Es and IHNV concurrently. All fish were sampled at 68 h post-virus exposure except stock tank fish, which were sampled before any fish were allocated for replicate groups; control fish were not exposed to either virus or esfenvalerate.

Histopathology.—No obvious microscopic pathological changes were observed in the gill and anterior kidney in any experimental or control groups sampled at 68 h post virus exposure. Separation of the epithelial from supporting pillar cells was noted, but this occurred inconsistently and among all groups suggesting this was an artifact of the sampling procedures.

Mx-1 and Vig-8 transcription.—All replicate groups exposed to IHNV had elevated levels of Mx-1 transcripts, but only groups exposed to IHNV alone were significantly higher ($F_{4,43} = 6.69$; $P < 0.001$) than all other treatment groups (Table 3). Though transcription of Mx-1 was elevated in groups exposed to esfenvalerate and IHNV concurrently, it was not significantly greater than any other treatment group (Table 3). Similar to Mx-1, all groups exposed to IHNV had elevated levels of Vig-8, but only groups exposed to IHNV alone were significantly higher ($F_{4,42} = 4.15$; $P < 0.007$) than all other treatment groups including groups exposed to IHNV and esfenvalerate (Table 3). Vig-8 transcripts were elevated in esfenvalerate-only groups relative to stock tank fish, but not significantly (Table 3).

TABLE 3.—Mean-fold transcription of Mx-1 and Vig-8 ($\pm SE$) in juvenile Chinook salmon from stock and control tanks, and in replicate test groups: esfenvalerate (Es) only, infectious hematopoietic necrosis virus (IHNV) only, or IHNV and Es concurrently. Significantly different ($P < 0.05$) groups are designated with an asterisk.

Gene	Treatment group				
	Stock Tank	Control	Es only	IHNV only	Es + IHNV
Mx-1	20.2 \pm 8.2	4.9 \pm 0.8	5.0 \pm 1.9	2,898.8 \pm 1,018.4*	861.9 \pm 183.9
Vig-8	10.0 \pm 3.6	8.4 \pm 2.7	79.4 \pm 33.4	3,751.6 \pm 1,675.6*	519.1 \pm 233.7

DISCUSSION

As demonstrated in prior studies, replicate groups of juvenile Chinook salmon exposed to both IHNV and to sub-lethal levels of esfenvalerate experienced an unusual and significant early mortality event not seen in any other treatment group (Clifford et al. 2005). Initial investigations of the causes of this early mortality in the current study suggest a potential role for an inhibition in the transcription of important anti-viral gene transcription in fish exposed to IHNV and esfenvalerate, compared to fish exposed to the virus or the pesticide alone.

Preliminary results in our lab demonstrated the amount of biomass per aquaria and the tendency of pyrethroids to adsorb to organic materials greatly affect the outcome of concurrent esfenvalerate and IHNV exposure (Clifford, CDFW, unpublished data). A nominal concentration of 0.00322 μg esfenvalerate per g of fish resulted in 0% mortality, but when increased to 0.00812 μg esfenvalerate per g of fish (by reducing amount of fish per replicate), the early mortality event was observed. Given the hydrophobic nature of pyrethroids, these values will most likely vary under different experimental conditions regarding the composition and surface area of tanks, the amount of suspended materials in water, methods of aeration, and any other surfaces in the experimental vessels. For example, toxic levels of a pyrethroid insecticide resulted in 100% mortality of rainbow trout by 24 h post exposure, but when water containing 15 mg/L suspended solids was used with the same pyrethroid concentrations there was 0% mortality observed (Hill 1985). Carefully accounting for these factors and using replicate groups, we have repeatedly witnessed the synergistic mortality event using different ages, weights and strains of fish, and different strains and concentrations of IHNV.

Investigating the potential causes of the early mortality following exposure of Chinook salmon to both esfenvalerate and IHNV did not indicate that disruption in osmoregulation was a contributing factor. Both pyrethroids and IHNV are reported to disrupt gill-ion regulation (Kumaraguru and Beamish 1981, Kumaraguru et al. 1982, Bradbury et al. 1987, Symonik et al. 1989, Congleton 1996), but in our trials no significant differences in blood osmolality were detected at 68 h between any of the experimental or control groups. This suggests at the sampling time (68 h) osmotic imbalances are unlikely to underlie the early synergistic mortality episodes that have been observed.

Apart from their primary neurotoxic effects, pyrethroids are reported to disrupt non-neural mechanisms, including gill and renal function, leading to lethal toxicity to fish species (Bradbury et al. 1987). Histopathological studies of gill surfaces in rainbow trout exposed to pyrethroids, including esfenvalerate, reported epithelial separation and necrosis, mucous cell hyperplasia, clubbing and fusion of secondary lamellae, and damage consistent with gill irritation (Kumaraguru et al. 1982, Bradbury et al. 1987). These results were reported whether pyrethroids were administered directly by aqueous exposure or indirectly through dietary intake. Histopathological examinations of fish in our studies did not reveal any obvious cellular changes to gill or anterior kidney. The absence of microscopic changes in our investigation compared to prior studies may be due to differences in experimental design. Kumaraguru et al. (1982) exposed fish to higher pyrethroid concentrations (0.09–35.0 µg/L) and exposed fish for 20–40 d before taking tissue samples. Bradbury et al. (1987) investigated acute lethal toxicity and exposed fish to very high concentrations of pyrethroids ($\bar{x} = 412 \pm 50$ µg/L) and performed extensive surgical procedures (spinal transection, catheterization, copper electrodes). The use of much lower concentrations of pyrethroid and the shorter exposure periods in our study are likely explanations for the lack of histopathological changes in the gill and anterior kidney in our study.

Exposure to IHNV is known to result in the transcription of many early, non-specific anti-viral cytokines including Mx-1 and Vig-8 (Purcell et al. 2004, 2006). While we observed this in all IHNV-exposed groups, concurrent esfenvalerate exposure resulted in significantly lower Mx-1 and Vig-8 transcription, suggesting an immune suppression or inhibition effect (Table 3). Eder et al. (2004) reported that exposure of juvenile Chinook salmon to 0.08 µg/L esfenvalerate led to a slight but significant decrease in Mx protein transcription to below basal levels, a trend consistent with our results (Table 3). Clifford et al. (2005) first reported the observed lethal synergy of juvenile Chinook salmon exposed to IHNV and esfenvalerate, an event occurring in the absence of detectable increases in virus replication above that of fish exposed only to IHNV. In our study, fish exposed to both IHNV and esfenvalerate demonstrated significant decrease in the transcription of type-1 interferon-regulated early non-specific anti-viral genes (Mx-1 and Vig-8), which may indicate a less adequate immune response to IHNV.

In the trials presented here, concurrent esfenvalerate and IHNV exposure resulted in a significant and rapid synergistic mortality event that may be linked to the disruption of the innate, early anti-viral response of juvenile Chinook salmon. Additional environmental stressors including water quality parameters (e.g., temperature) and co-contaminant or pathogen exposures would likely result in even greater losses of juvenile salmonid fish. Further studies of the observed lethal synergism are needed to define potential mechanisms involved. These include more sensitive molecular detection methods for virus (Purcell et al. 2006) and more comprehensive gene transcription analyses (von Schalburg et al. 2005, Purcell et al. 2006). Lastly, our study supports the importance and difficulty of taking into account the many variables required to establish lawfully acceptable concentrations of pesticides in aquatic environments.

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Estimating juvenile winter-run and spring-run Chinook salmon entrainment onto the Yolo Bypass over a notched Fremont Weir

KRYSTAL R. ACIERTO*, JOSHUA ISRAEL, JOE FERREIRA, AND JASON ROBERTS

California Department of Fish and Wildlife, Water Branch, 830 S Street, Sacramento, CA 95811, USA (KRA)

United States Bureau of Reclamation, Bay-Delta Office, 801 I Street, Suite 140, Sacramento, CA 95814, USA (JI)

California Department of Fish and Wildlife, Fisheries Branch, 830 S Street, Sacramento, CA 95811, USA (JF)

California Department of Fish and Wildlife, Northern Region, 601 Locust Street, Redding, CA 96001, USA (JR)

**Correspondent: krystal.acierto@wildlife.ca.gov*

In this study, a proposed notching of the Fremont Weir was analyzed compared to existing conditions using empirical data to estimate the proportion of juvenile Sacramento River winter-run and Central Valley spring-run Chinook salmon (*Oncorhynchus tshawytscha*) entrained onto the Yolo Bypass. Using historic flow and rotary screw trap data from water years 1997-2011, we found that entrainment of listed juvenile salmon onto the Yolo Bypass was higher on average across all water year types under evaluated notch conditions than occurred under existing conditions. We found that notching the weir resulted in increased listed juvenile salmon entrainment onto the Yolo Bypass in the months of November through March, but not in April. Our results indicate that lowering the required river stage for Sacramento River flows to enter the Yolo Bypass by notching the Fremont Weir is likely to increase entrainment of listed juvenile salmon onto the bypass for the majority of the listed juvenile salmon emigration seasons.

Key words: Fremont Weir, Chinook salmon, *Oncorhynchus tshawytscha*, notch, Yolo Bypass, entrainment, winter-run, spring-run

Construction of dams and levees for flood control and water distribution in the Central Valley (California, USA) has resulted in substantial decreases in the available floodplain habitat for native fish species (Sommer et al. 2001, NMFS 2009a). Restoration of floodplain habitat has been identified as a key action to contribute to the recovery of Sacramento River winter-run Chinook salmon and Central Valley spring-run Chinook salmon (*Oncorhynchus tshawytscha*), which are listed under the state and federal endangered species acts (NMFS 2009a, 2009b). The Yolo Bypass (Figure 1), which is an integral part of the

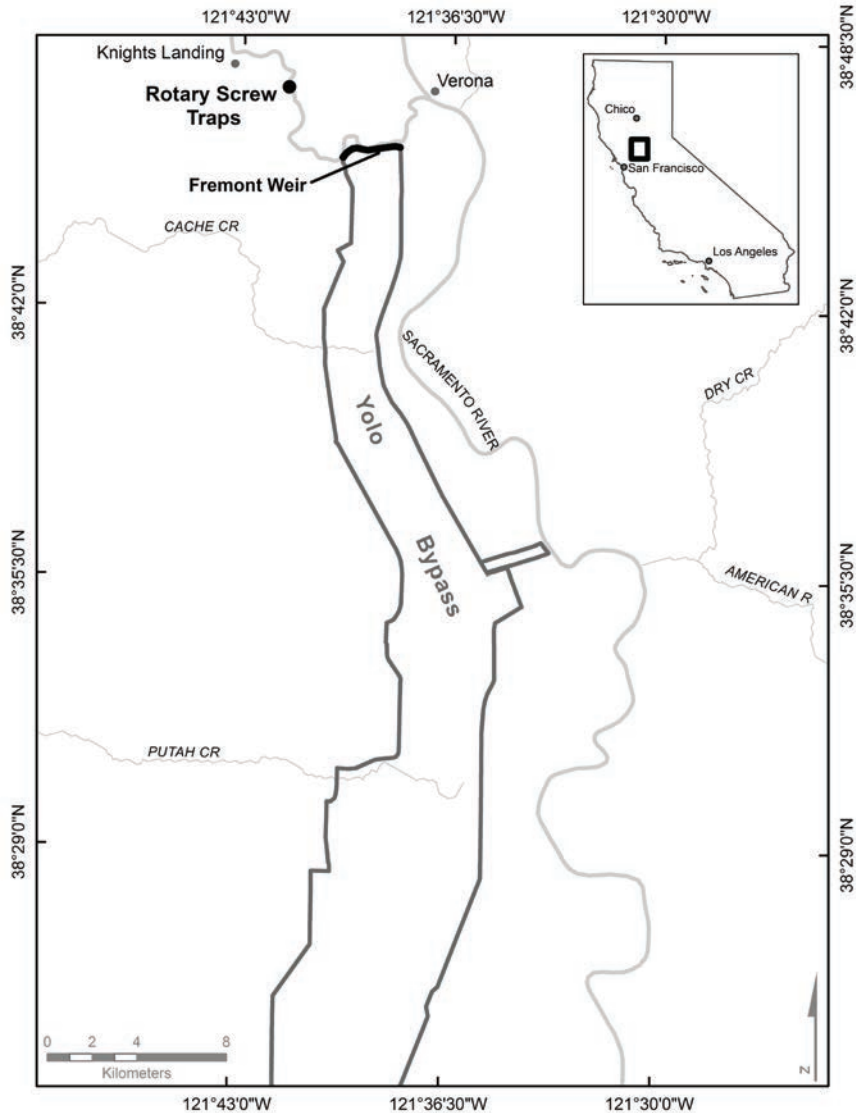


FIGURE 1.—Location of the California Department of Fish and Wildlife’s Juvenile Salmonid Emigration Monitoring Program rotary screw traps (38° 47’ N, 121° 41’ W) and Fremont Weir (38° 45’ N, 121° 38’ W).

flood control system in the Sacramento River basin of the Central Valley, has been shown to provide habitat conducive of enhanced growth and survival of juvenile salmon (Sommer et al. 2001). Currently, floodwaters passively enter the Yolo Bypass over the Fremont Weir when the Sacramento River stage, or height of the river, reaches an elevation of 10 m North American Vertical Datum 1988 (NAVD88). Modifying a section of the Fremont Weir (evaluated notch) to allow for inundation of the Yolo Bypass at lower Sacramento River flow stages has been proposed as a regionally important restoration action necessary to increase the frequency and duration of flooding events and, thus, increase the availability of beneficial floodplain rearing habitat for winter-run and spring-run Chinook salmon (listed juvenile salmon) (NMFS 2009a). The purpose of this study is to evaluate a proposed notch configuration in the Fremont Weir to determine how lowering the Sacramento River stage requirements to overtop Fremont Weir might contribute to listed juvenile salmon entrainment onto the Yolo Bypass. Decreasing the river stage required for overtopping the weir is hypothesized to allow greater numbers of listed juvenile salmon to access the beneficial floodplain in all water year types, and in all months when listed juvenile salmon are emigrating past Fremont Weir.

METHODS

The Fremont Weir is located on the Sacramento River at River Kilometer (RK) 132.77 (38° 45' N, 121° 38' W) (Figure 1). Flows currently enter the 61 km long Yolo Bypass when the Sacramento River stage exceeds the existing weir elevation of 10 m NAVD88, which occurs in approximately 60–70% of years depending on the historical time period used (Feyrer et al. 2004, DWR 2012). When the Sacramento River overtops the Fremont Weir into the Yolo Bypass, emigrating listed juvenile salmon can potentially leave the main stem river and enter the bypass to use the floodplain as highly-productive rearing habitat. Lowering the Fremont Weir elevation to allow flows to enter the bypass at lower river stages may provide increased access to rearing habitat for emigrating juveniles. In 2009, a technical team evaluated a Fremont Weir notch configuration with a 68.58 meter wide channel bottom, 2 to 1 side slopes, and an invert elevation of 5.33 m NAVD 88 (BDCP Integration Team 2009). In this study, we evaluated this notch's ability to divert water over the weir at lower river stages compared to existing conditions in order to estimate listed juvenile salmon entrainment onto the Yolo Bypass. We used rotary screw trap data, historic flow data, and the assumption that listed juvenile salmon are equally distributed throughout the water column and enter the Yolo Bypass proportionally to the flow split at the weir to derive this flow-entrainment relationship.

Salmon data.—The California Department of Fish and Wildlife (CDFW) has operated rotary screw traps on a daily basis from October through June since 1996 to monitor juvenile salmonid emigration timing, composition, and abundance on the Sacramento River near Knight's Landing at RK 132.77 (38° 47' N, 121° 41' W). The rotary screw traps are located approximately 8 kilometers upstream of the Fremont Weir (Figure 1). We analyzed rotary screw trap data for water years 1997–2011 to determine the daily catch by run (winter-run or spring-run Chinook salmon) based on size-at-date criteria (Fisher 1992, Rosario et al. 2013), as well as trap effort, in order to determine the daily catch per unit effort (CPUE). Observed raw catch data from rotary screw traps is affected by variability in trapping efficiency, diel migration patterns, and water quality. Since these data contain periods

affected by these factors, catch per unit effort provides a better measurement of potential fish entrainment than raw catch data because it reduces the influence of trap servicing, diel operations, and high debris loads.

Daily catch per unit effort (by run) was calculated using the following equation:

$$CPUE_i = C_i / (E_i / 24) \quad (\text{Eq. 1})$$

where $CPUE$ is daily catch per unit effort (by run), C is daily catch, E is daily effort, and i is day index. Daily proportion of salmon catch was calculated using the following equation:

$$P_i = CPUE_i / \sum_i CPUE_i \quad (\text{Eq. 2})$$

where P is the daily proportion of salmon catch (by run).

Spring-run sized salmon catch was adjusted to take into account fall-run hatchery releases from Coleman National Fish Hatchery (CNFH); these spring-run sized fish are not easily distinguishable from the similarly sized CNFH fall-run hatchery fish. Therefore, after CNFH release fall-run hatchery fish, which typically occurs in April, all juvenile Chinook salmon within this size range were considered hatchery fall-run fish.

Flow data.—Historic flow data for water years 1997–2011 was derived from Sacramento River stage data at Fremont Weir, spill data at Fremont Weir, and Sacramento River flow at Verona, Sutter County (Figure 1). Daily Sacramento River stage and Fremont Weir spill data were collected by the personnel from the California Department of Water Resources and the Sacramento River flow at Verona data were collected by personnel from the United States Geological Survey. Daily Sacramento River flow at Fremont Weir was calculated per the rating table developed by the National Weather Service California-Nevada River Forecast Center, using the Sacramento River flow stage level at Fremont Weir. Flow data that were not recorded on the California Data Exchange Center for Fremont Weir spills into the Yolo Bypass was calculated using the following surrogate equation (Jones and Stokes 2001):

$$\text{Fremont Weir Spill} = 0.06(\text{Flow at Verona} - 56,000)^{1.5} \quad (\text{Eq. 3})$$

Flow relationships.—The daily proportion of Sacramento River flow entering the Yolo Bypass under existing conditions was calculated using the following equation:

$$F_e = F_w / (F_w + F_v) \quad (\text{Eq. 4})$$

where F_e is the proportion of Sacramento River flow entering the Yolo Bypass when the weir was overtopping under existing conditions, F_w is the flow over Fremont Weir, and F_v is the flow at Verona.

Notch flow, defined as the flow of the Sacramento River at the Fremont Weir that could have entered the Yolo Bypass had the evaluated notch been in place from 1997 to 2011, was derived per the rating curve developed by the Bay Delta Conservation Plan Integration Team (BDCP Integration Team (2009)). Per the BDCP Integration Team Report, the daily proportion of Sacramento River flow entering the Yolo Bypass under the evaluated notch conditions can take two different forms depending on the river stage at the Fremont Weir.

If the river stage was between 5.3 and 10.0 m NAVD 88 (i.e. the weir was not overtopping under existing conditions, but flows would have entered through the evaluated notch), the following equation was used:

$$F_p = F_n / (F_n + F_f) \quad (\text{Eq. 5})$$

where F_p is the proportion of Sacramento River flow that would have entered the Yolo Bypass from the evaluated notching of the Fremont Weir, F_n is the flow through the evaluated notch, and F_f is the Sacramento River flow at Fremont Weir. If the river stage was above 10 m NAVD 88 (i.e. the weir was overtopping under existing conditions and flows would have entered through the evaluated notch), the following equation was used:

$$F_p = (F_n + F_w) / (F_n + F_w + F_v) \quad (\text{Eq. 6})$$

where F_p is the proportion of Sacramento River flow that would have entered the Yolo Bypass from the evaluated notching of the Fremont Weir, in addition to the spill that would have occurred under existing conditions.

Entrainment calculations.—Using the salmon and flow data above, we estimated the daily percentage of listed juvenile salmon potentially entrained onto the Yolo Bypass under existing and evaluated notch conditions. The daily percentage of listed juvenile salmon entrained onto the Yolo Bypass under existing conditions was calculated using the following equation:

$$P_e = (F_e \times P_i) \times 100 \quad (\text{Eq. 7})$$

where P_e is the percentage of listed juvenile salmon entrained under existing conditions. The daily percentage of listed juvenile salmon entrained onto the Yolo Bypass from the evaluated notching of Fremont Weir was calculated using the following equation:

$$P_n = (F_p \times P_i) \times 100 \quad (\text{Eq. 8})$$

where P_n is the percentage of listed juvenile salmon entrained from the evaluated notching of the Fremont Weir and F_p is entrainment calculated from either Eq.5 or Eq.6 above, depending on the river stage at Fremont Weir.

Data analysis.—The estimated daily percentage of listed juvenile salmon potentially entrained onto the Yolo Bypass was summed under existing conditions and the evaluated notch by water-year-type based on the Sacramento Valley Water Year Hydrologic Classification (SWRCB 2006) and by month.

Using the Shapiro-Wilk statistical test (Shapiro and Wilk 1965), it was found that the data failed to meet the normal distribution assumption. Therefore, Mann-Whitney-Wilcoxon non-parametric tests were conducted to test for differences between listed juvenile salmon entrainment under existing conditions and the evaluated notch. Differences were considered significant at $P \leq 0.05$.

RESULTS

Using historic flow data and rotary screw trap data from water years 1997–2011, the evaluated notch significantly ($W=8758.5$, $P < 0.001$) increased the proportion of listed juvenile salmon entrained onto the Yolo Bypass compared to existing conditions for all water-year-types (Table 1; Figure 2). In wet years, above normal years, and when averaging across all years, the number of listed juvenile salmon entrained increased by 155–280% compared to existing conditions, which amounts to an approximately 9–10% increase in the proportion of listed juvenile salmon populations entrained (Table 1; Figure 2). In dry years, entrainment onto the bypass went from effectively no entrainment under existing conditions (0.02%) to approximately 8% of each of the populations with the evaluated notch, which is an increase of over two orders of magnitude compared to existing conditions (Table 1; Figure 2).

TABLE 1.—Percentages of juvenile winter-run (WRC) and spring-run (SRC) Chinook salmon entrained onto the Yolo Bypass under existing conditions (P_e) and the evaluated notching of Fremont Weir (P_n) by water year type for water years 1997–2011.

Water Year Type	WRC P_e	WRC P_n	P -value	SRC P_e	SRC P_n	P -value
Wet & Above Normal	6.19	15.74	0.014	6.16	16.10	<0.00
Dry & Critical	0.02	8.32	0.005	0.02	7.65	0.005
All Years	3.38	12.53	<0.001	3.35	12.66	<0.001

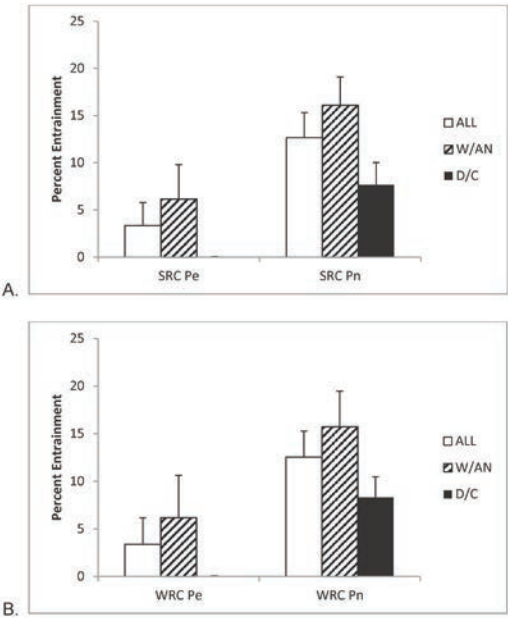


FIGURE 2.—Mean percentage of (A) juvenile spring-run (SRC) and (B) winter-run (WRC) Chinook salmon entrained onto the Yolo Bypass (38° 45' N, 121° 38' W) under existing conditions (P_e) and the evaluated notching of the Fremont Weir (P_n) by water year type (ALL=average of all water years, W/AN=wet and above normal, and D/C=dry and critical) for water years 1997–2011.

The number of listed juvenile salmon entrained was also significantly higher under evaluated notch conditions compared to existing conditions for the months of November through March (Table 2; Figure 3). There was no significant difference in listed juvenile salmon entrainment between the evaluated notch and existing conditions in the month of April (Table 2; Figure 3).

TABLE 2.—Percentages of juvenile winter-run (WRC) and spring-run (SRC) Chinook salmon entrained onto the Yolo Bypass under existing conditions (P_e) and the evaluated notching of Fremont Weir (P_n) by month for water years 1997–2011.

Month	WRC P_e	WRC P_n	P -value	SRC P_e	SRC P_n	P -value
November	0.00	0.52	0.04	0.00	0.10	0.04
December	0.52	3.96	0.004	0.95	4.28	0.011
January	1.12	3.71	0.001	0.48	2.60	<0.001
February	1.09	3.08	0.002	0.75	2.92	<0.001
March	0.62	1.21	0.002	0.87	2.16	0.001
April	0.03	0.04	0.203	0.31	0.60	0.071

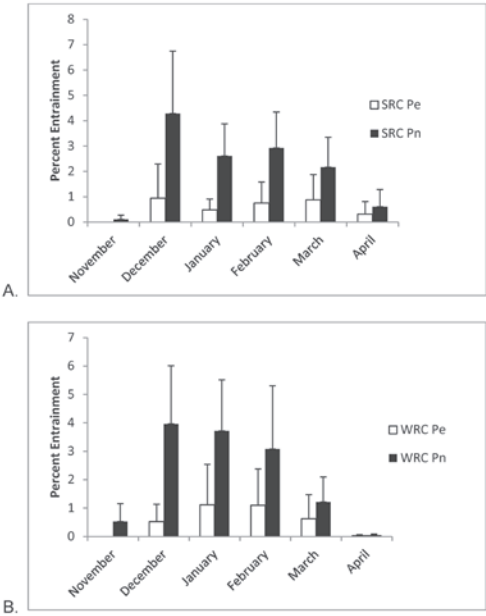


FIGURE 3.—Mean percentage of (A) juvenile spring-run (SRC) and (B) winter-run (WRC) Chinook salmon entrained onto the Yolo Bypass (38° 45' N, 121° 38' W) under existing conditions (P_e) and the evaluated notching of the Fremont Weir (P_n) by month for water years 1997–2011.

DISCUSSION

Our results indicate that the evaluated notch would significantly increase the percentage of listed juvenile salmon entrained onto the bypass in all water-year-types, most notably in dry and critical water years. Furthermore, the notch would also allow for increased entrainment of listed juvenile salmon in the months of November through March,

indicating that through all water-year-types, more juveniles will likely be entrained over numerous hydrologic conditions observed in the Sacramento Valley. This result emphasizes the importance of development and implementation of adaptive management strategies for operation of a notched weir that could be planned as part of a multi-year conservation and restoration action.

The number of listed juvenile salmon entrained onto the bypass was not significantly higher in the month of April under evaluated notch conditions when compared to existing conditions (Table 2; Figure 3). A majority of CNFH fall-run hatchery Chinook are released in April without any visual mark signifying their hatchery origin. Thus, after this date, all observed juvenile Chinook salmon captured at the rotary screw trap within the spring-run length-at-date criteria are considered unmarked hatchery origin fall-run and no spring-run are recorded. This censoring of spring-run Chinook observation during April may explain why spring-run salmon entrainment was not significantly higher under evaluated notch conditions than existing conditions. Winter-run salmon entrainment was likely not significantly higher under evaluated notch conditions because winter-run typically emigrate past Knights Landing between November and March, extending into April and May only in some years (Snider and Titus 2000a, 2000b).

The assumption that fish are equally distributed throughout the water column and enter the Yolo Bypass proportionally to the flow split at the weir was used to derive the flow-entrainment relationship. There are studies to suggest that juvenile salmonids show certain habitat preferences depending on various environmental conditions and developmental stage and may not be equally distributed in the water column (Williams 2006). Additional investigations of the ways that environmental conditions influence behavior of various juvenile lifestages is an area of research important to accurately predicting entrainment at different locations and river stages at Fremont Weir.

These results suggest that notching the Fremont Weir to increase the flow volume onto the Yolo Bypass could increase the percentage of listed juvenile salmon entrained during the majority of their emigration seasons. However, additional studies and finer scale analyses are necessary to assess how the migratory behavior of fishes and location of notches may more directly influence the percentage of fish entrained, and to determine the effectiveness of the evaluated notch post-restoration.

Increasing the percentage of listed juvenile salmon entrained onto floodplain habitat will provide a larger proportion of the population an alternate emigration corridor with potential population-level benefits. Entraining fish onto the bypass is a critically important fishery management action and may be necessary to derive increases in growth rates and survival (which ultimately may result in an increased contribution to adult production) compared to the main stem of the Sacramento River (Sommer et al. 2001). Further studies to compare survival between the Yolo Bypass and Sacramento River migration corridors are necessary to evaluate the survival benefits of floodplain entrainment.

Studies of frequency, duration, and timing of flooding events over the weir for restoration actions are needed in order to assess how each factor contributes to increased growth rates and survival, and how increased growth rates may benefit adult returns by reducing the number of smaller sized juveniles, which tend to be more susceptible to mortality (Beckman et al. 1999). In addition, it is also necessary to determine how entrainment and inundation events are timed and integrated to assess stranding risks for listed juvenile salmon. Field monitoring is necessary to evaluate when the magnitude and duration of inundation events are insufficient to provide appropriate connectivity to existing waterways, which can

create stranding risks. In water years that do not provide sufficient flows onto the bypass, operation of the evaluated notch will need to be adaptively managed based on real-time monitoring.

The framework for this study can be used for management and restoration purposes as a preliminary step to assess the potential benefits from notching the Fremont Weir with the intent of increasing the proportion of juvenile salmon entrained onto the Yolo Bypass, and it utilizes fish and flow data unique to that system. Further, this approach can be used in conjunction with monitoring efforts and lifecycle models to help determine the effectiveness of fishery restoration actions in the Yolo Bypass and similar floodplain habitats.

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Assessment of quagga mussel (*Dreissena bugensis*) veliger survival under thermal, temporal and emersion conditions simulating overland transport

JAMES P. SNIDER*, JAMES D. MOORE, MARTHA C. VOLKOFF, AND SHAUNA N. BYRON

California Department of Fish and Wildlife, Shellfish Health Laboratory, Bodega Marine Laboratory, P. O. Box 247, Bodega Bay, CA 94923 USA (JPS, JDM, SNB)

California Department of Fish and Wildlife, Habitat Conservation Planning Branch, 1416 9th Street, Sacramento, CA, 95814 USA (MCV)

**Correspondent: James.Snider@wildlife.ca.gov*

Invasive quagga mussels (*Dreissena bugensis*) were first detected on the lower Colorado River at Lake Mead in January 2007 and have spread to a number of southern California reservoirs. Though it is highly likely that larval transportation along connected waters was the primary cause of these infestations, little is known about the potential for larval conveyance in association with trailered watercraft. We conducted laboratory experiments at the Lake Mead fish hatchery to determine the potential for larvae (veliger) survival under immersion and emersion conditions that simulate those potentially encountered on recreational watercraft when trailered from infested to uninfested waterbodies. Our results demonstrate that at or above an air temperature of 35°C there is no practical risk of conveyance under any condition of emersion or low volume immersion. For emersed veligers in high relative humidity microenvironments there is risk of conveyance over a 'next day' time frame at temperatures up to 25°C. For immersed veligers in volumes as small as 31 µl there is risk of conveyance for at least 20 hours at 30°C and for at least seven days at 25°C or lower. Larval densities in infested waters are low enough that a volume on the order of one droplet is unlikely to contain veligers, but low volume itself does not appear to be a significant impediment to survival. These studies support the development and implementation of robust decontamination methods for watercraft moving from infested or potentially infested waters to those assumed to be uninfested.

Key words: veliger, conveyance, *Dreissena bugensis*, emersion, immersion, invasive species, Lake Mead, quagga mussels, watercraft

The quagga mussel (*Dreissena bugensis*, Andrusov 1897) first appeared west of the Continental Divide in Lake Mead, Nevada, USA in January 2007 (Turner et al. 2011). Since that time numerous agencies and stakeholders have committed to stopping the spread of quagga mussels and limiting the damage these invasive, biofouling mollusks can cause (Turner et al. 2011). The quagga mussel life history is characterized by byssaceous attachment, planktotrophic larvae, and high fecundity, facilitating dispersal (Johnson and Carlton 1996). Byssally attached adults can pose an overland conveyance risk (Ricciardi 1995) and larval entrainment through contiguous waters is a primary dispersive mechanism in the geographic spread of dreissenid mussels (McMahon et. al. 1993). Larval entrainment is believed to be the cause of infestation in a number of southern California reservoirs, all of which received untreated Colorado River water known to harbor quagga mussel veligers (CDFW 2014). Few studies have focused on the potential overland conveyance of viable immersed veligers, and to our knowledge no other studies or publications address the survival and risks of transferring viable emersed veligers associated with overland conveyances. Immersion, as we apply it to dreissenid mussel larvae, means that veligers have not been removed from natal waters while emersion means veligers have been removed from water and are exposed to ambient air conditions in association with microhabitat. Craft and Myrick (2011) demonstrated that under experimental conditions immersed quagga veligers from the Willow Beach National Fish Hatchery, Willow Beach (Lake Mojave), Arizona, USA exhibited 100% mortality when exposed to a water temperature of 35°C for 26 hours. Their study also demonstrated that at experimental temperatures of 10°C, 15°C and 30°C, there was a negative correlation between veliger survival and temperature, with veligers surviving for 43.3 hours at 30°C, 163.3 hours at 15°C, and to the experimental endpoint of 210.9 hours when held at 10°C. In another immersion study conducted at Lake Mead, Choi et al. (2013) found that quagga veligers could survive up to five days during warm summer months and for 27 days during cooler autumn months. In this study summer air temperatures ranged from 25°C to 40°C and autumn air temperatures ranged from 6°C to 18°C, while summer water temperatures ranged from 27°C to 36°C and autumn water temperatures ranged from 8°C to 16°C.

The purpose of our study was to further investigate the potential for quagga mussel veligers to survive conditions likely to be encountered on watercraft during overland transport. We focused on what we believed to be realistic scenarios for trailered watercraft transported between infested and uninfested waterbodies in California, examining survival potential at various air temperatures at four hours (same-day conveyance), 20 hours (overnight conveyance) and at moderate temperatures for up to seven days. We examined immersion survival in small droplets of water and emersion survival on moist substrate in order to simulate vessel microhabitat, such as small pockets of water or water-saturated materials like sponges or cloth.

Surviving an experimental condition is distinct from maintaining viability. Viability implies that a veliger is competent for settlement or can proceed with development to that point under certain conditions, while survival simply means that the organism is not dead at the point of examination. Given the complexities of determining viability, our aim was to use survival as a means of assessing conveyance risk. We use transportation and conveyance interchangeably to describe the potential to move live veligers between waterbodies within trailered watercraft. No assumptions were made about the events that would have to occur for the placement of veligers upon watercraft.

MATERIALS AND METHODS

Location of study.—Experiments were conducted at the Nevada Division of Wildlife Lake Mead fish hatchery indoor laboratory facility (245 Lakeshore Road, Boulder City, NV 89005) from 11 June 2013 through 18 June 2013. Air temperature and relative humidity in the air-conditioned laboratory were measured each morning (between 0800 and 1100) and afternoon (between 1600 and 1630) and ranged from 23°C to 25°C and 26% to 39%, respectively, throughout the study period.

Veliger collection.—A plankton net (Aquatic Research Instruments, Hope, ID) was used to collect vertical plankton tows at the end of the Lake Mead Marina dock between 0830 and 0930 daily and the experiments set up each day used only veligers collected that morning. Net dimensions were 30 cm in diameter \times 90 cm in length, with mesh of 64 μ m. The cod end was two-piece with a 64 μ m mesh. Tows were pulled from a depth of 25 m to the surface and decanted from the cod end into a single 250 ml wide-mouth Nalgene container. Four to six containers were collected each day with 2–3 tows per container. After collection the containers were placed in an 8 L cooler with several frozen gel packs to prevent overheating and transported to the Lake Mead fish hatchery where they were stored in a refrigerator at 4°C until veliger harvesting later that day.

Veliger harvesting.—Plankton tows were gently poured through stainless steel sieves (Fisher Scientific, U.S.A.; Standard Test Sieves, ASTM E-11 Specifications) stacked in a column in order of descending mesh sizes of 1 mm, 300 μ m, 150 μ m, 106 μ m, and 63 μ m. The 150 μ m sieve was back-flushed with unfiltered Lake Mead water into a finger bowl, while both the 106 μ m and the 63 μ m sieves were back-flushed with unfiltered Lake Mead water into a separate finger bowl. In this way, two size class populations of veligers were obtained: a large size class (i.e., those retained on the 150 μ m mesh) and a small size class (i.e., those retained on the 63 and 106 μ m meshes). The bowls were manually swirled in a gentle clockwise motion until the veligers were concentrated centrally, which facilitated harvest and reduced contamination with other material present in the tow, such as microalgae and crustacea. A stereomicroscope fitted with a cross-polarizing filter (Johnson, 1995) was used to identify veligers in finger bowls so they could be harvested with an Eppendorf pipettor fitted with 100 μ l disposable tips. All experiments were conducted on each size class separately.

Determination of veliger survival.—Veliger survival in lake water was determined by observation using light microscopy at 100 \times magnification. Veligers were scored as live on the basis of any movement of the velum, cilia, or musculature, or any other movement that would indicate post-treatment survival.

Experiment 1: Acute immersed thermal tolerance.—A volume of 31 μ l of lake water containing a target number of 30–50 veligers was pipetted from finger bowls into 200 μ l PCR tubes (Eppendorf, Cat. No. 951010022). An MJ Research PTC-200 gradient thermal cycler was used to subject veligers to target temperatures for one-hour exposures. The cycler was programmed for a 10-minute, 25°C acclimation period prior to exposure and a 30-minute, 25°C recovery period after exposure. In a preliminary study, the thermal cyclers' gradient function was used to screen for veliger thermal tolerance at temperatures ranging from 30.0°C to 42.0°C. A double set of triplicate tubes was then run at target temperatures of 35.0°C, 36.0°C, and 37.0°C for one hour for each size class. After cycling, veligers from one triplicate set were immediately pipetted from the PCR tubes into wells on a ten-well glass microscope slide for microscopic observation. The second triplicate set

was held at 4°C overnight and then at room temperature for three hours prior to microscopic observation the following day, to potentially facilitate recovery.

Experiment 2: Immersion tolerance.—Short-term (i.e., 20-hour) and long-term (i.e., 5-day and 7-day) immersion experiments were conducted, each consisting of incubations of veligers in 31 µl of lake water. Plastic inserts from disposable pipet tip boxes were used to hold the PCR tubes (short-term experiment and long-term 7-day experiment) or slides (long-term 5-day experiment), which were then placed in a 4-liter Rubbermaid storage container lined with tap water-saturated paper towels to maintain high humidity. Temperature and relative humidity were measured inside the container at 15-minute intervals using a data logger (Onset HOBO Pro v2 Data Logger, Part No. U23-001).

The short-term experiment had a 20-hour end-point and constant temperatures of 5°C, 20°C, 25°C, 30°C, 35°C or 40°C using a digitally controlled incubator, applying one of the six temperatures during each 20-hour incubation. Each sample consisted of a targeted number of 30–50 veligers in 31 µl of lake water within capped 200 µl PCR tubes in triplicate for each size class.

Two long-term experiments were conducted for five and seven days at ambient laboratory temperatures ranging from 21°C to 25°C. Samples were removed and examined on a daily basis. Each daily sample consisted of twenty replicate tubes or slide wells with a targeted number of 1 to 10 veligers per replicate for each of the two size classes. The 5-day experiment utilized 31 µl droplets with veligers on 10-well microscope slide wells. On days three, four, and five of the 5-day experiment, 10 – 20 µl of unfiltered Lake Mead water was added to each droplet immediately after removal from the Rubbermaid container to prevent desiccation during observation. For the 7-day experiment 31 µl droplets were placed in capped PCR tubes, as was done for the short-term experiment. These two methods were used for the long-term studies because we reasoned that each had distinct advantages and disadvantages that could influence survival. After incubation, survival was determined by direct microscopic observation of veligers at 100× on slides (i.e., 5-day experiment) or observation after transferring veligers from tubes to slides using an Eppendorf pipettor (i.e., short-term and 7-day experiments). Daily samples were discarded after examination.

Experiment 3: Emersion thermal tolerance.—Emersion experiments were run at 4- and 20-hour end-points and constant temperatures of 5°C, 20°C, 25°C, 30°C, 35°C and 40°C in triplicate, separately for each quagga veliger size class. Emersion experimental protocol was based on the ISO-GRID membrane filtration methodology used in food microbiology applications (Entis and Lerner 1996). Veligers were pipetted in 31 µl volumes from finger bowls onto pre-moistened nylon filter paper (MAGNA, 1.2 µm, 25 mm, GE Water and Processing Technologies, Cat. No. R12SP02500) situated on a glass microanalysis vacuum filter holder (Fisherbrand 09-753E) connected to a 125 ml filtration flask. A targeted delivery of 30 – 50 veligers was delivered onto nylon filter paper in each replicate droplet. Rubber tubing was attached to the flask tube and a pipet bulb was used to gently create a vacuum, drawing veligers onto the filter paper with no visible damage. Nylon filter papers with emersed veligers were removed from the vacuum frit with tweezers and placed on top of cellulose filter paper (Whatman No. 1, 42.5 mm) moistened with 500 µl of unfiltered Lake Mead water and placed in disposable Petri dishes. Petri dishes, with the top lids removed, were placed in 4 L Rubbermaid storage containers lined with tap water-saturated paper towels. Two dishes were placed in each container, each containing three filter papers representing triplicate replication of each of the two size classes of quagga veligers. A HOBO data recorder was included in each container to monitor temperature and relative humidity at

15-minute intervals. Containers were incubated in a digitally controlled incubator for the targeted experimental times and temperatures. After incubation, filter papers were removed from the Petri dishes with tweezers and the contents rinsed into a 1.0 ml gridded Sedgewick Rafter counting chamber; each filter paper was rinsed six times with 100 μ l unfiltered Lake Mead water using an Eppendorf pipet. Subsequently, microscopic observations of veligers were made at 100 \times to determine survival. The time interval between rinsing veligers from filter papers and microscopic observation ranged from 5 to 60 minutes due to the logistics of running simultaneous experiments.

Data analysis.—Obtaining data that could be used to statistically compare the effects of temperatures, size class, or temporal trends was beyond the scope of this investigation. cursory examination of the survival data indicated high variation among triplicate replication and presenting arithmetic means was deemed inappropriate. As a result most of the data are presented graphically to reflect raw triplicate values on the proportion of veligers that showed any evidence of survival (% survival). The long-term immersion experiments used 20 replicates per treatment and for these data the means and standard errors of the means were calculated and displayed. The range and mean number of veligers examined in each experiment are noted in the figure legends.

RESULTS

Immersed veligers: acute thermal tolerance (Experiment 1).—After one hour at 35°C without a recovery period, survival of quagga mussel veligers was 87–100% and 96–100% for the small- and large-size-classes respectively (Figure 1A). At 36°C survival percentages were reduced to 50–73% and 47–60% for small and large size classes, respectively, and at 37°C there was no survival in either size class. A recovery period consisting of overnight incubation at 4°C followed by three hours at room temperature did not result in increased survival (Figure 1B).

Immersed veligers: thermal tolerance of 20-hour small-volume incubations (Experiment 2).—After 20 hours immersion in 31 μ l droplets of water in closed tubes, small size class veligers exhibited survival rates of 62% or better at 5°C, 20°C, 25°C, and 30°C and no survival at 35°C or greater (Figure 2). The large-size-class veligers appeared more sensitive to high temperature, with 0–26% survival at 25°C and 0–3% at 30°C compared to 97–100% survival at 25°C and 63–91% at 30°C for small-size-class veligers (Figure 2).

Immersed veligers: long-term survival in small volumes (Experiment 2).—Experiments were conducted to examine multiple-day survival of veligers in 31 μ l volumes using two methods: droplets on slides and droplets in closed tubes. Veligers in the small size class immersed in droplets of lake water on a microscope slide, incubated at ambient lab temperature with a relative humidity >95%, exhibited mean survival rates of \geq 14% upon examination daily over five days (Figure 3A). Veligers in the large size class under identical conditions exhibited \geq 23% mean survival rates over the five-day observation period (Figure 3A).

Veligers in the small size class immersed in 31 μ l droplets of water in closed tubes incubated at ambient lab temperature exhibited mean survival rates of at least 45% over seven days (Figure 3B). Under the same conditions the large size class veligers exhibited mean survival rates of \geq 7% over seven days including 16% on day seven (Figure 3B).

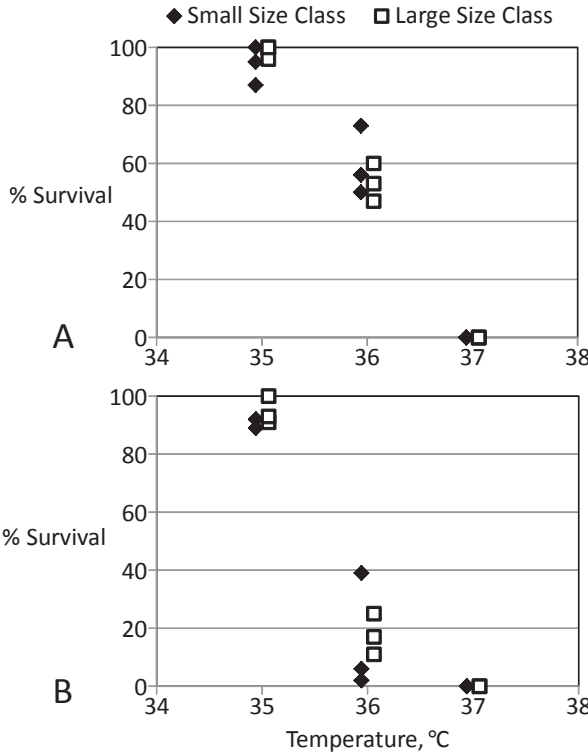


FIGURE 1.—Acute thermal tolerance of small and large size class quagga mussel (*Dreissena bugensis*) veligers in 1-hour lake water immersion exposures. Data are the percentages of survival in three replicate tubes at each temperature shown. Observations were made immediately after exposure (A) and after a recovery period ranging from 14.5 to 18.5 hours at 4°C followed by 2.5–3 hours at ambient laboratory temperature (23°C–25°C). (B). The number of veligers per replicate immediately after exposure (A) ranged from 38 to 87, with an average of 58 for the small size class and a range of 9 to 25 with an average of 16 for the large size class. The number of veligers per replicate after overnight recovery (B) ranged from 27 to 106 with an average of 56 for the small size class and a range of 9 to 28, with an average of 18 for the large size class.

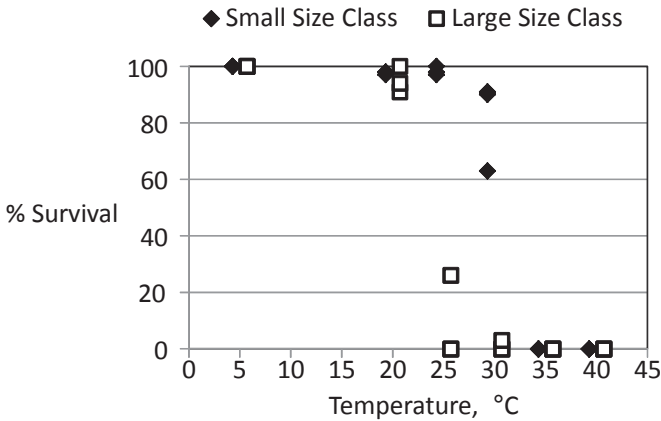


FIGURE 2.—Survival of small- and large-size class quagga mussel (*Dreissena bugensis*) veligers following 20-hour immersion in 31 µl lake water at various temperatures. Data are the percentages of survival in three replicate tubes at each temperature shown. The number of veligers per replicate ranged from 33 to 78 with an average of 51 for the small size class, and from 7 to 47 with an average of 21 for the large size class.

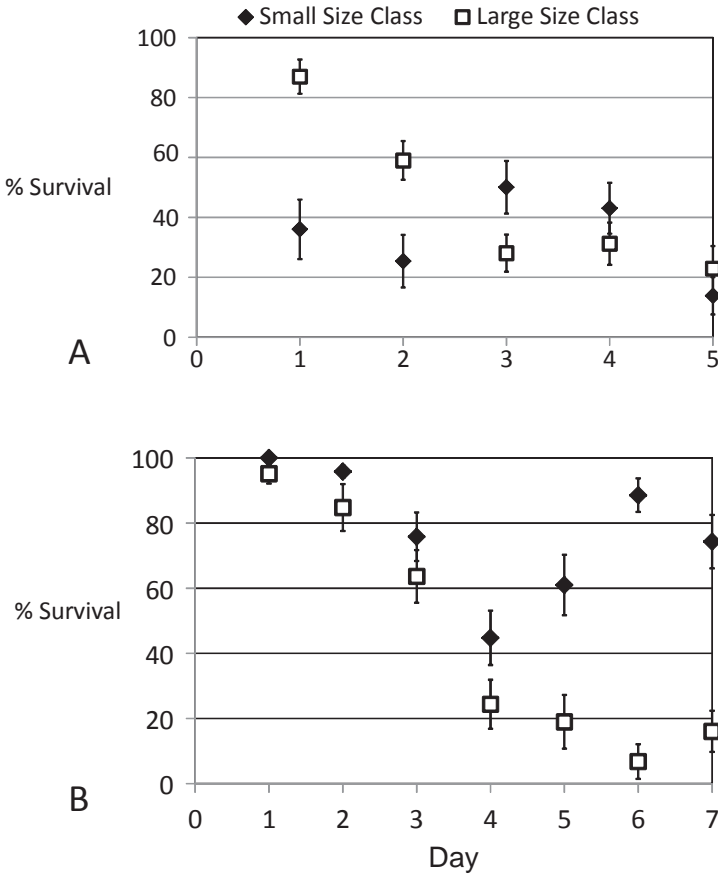


FIGURE 3.—Survival of small- and large-size class quagga mussel (*Dreissena bugensis*) veligers following immersion in 31 µl lake water droplets on glass slides (A) and in closed 200 µl PCR tubes (B) at ambient laboratory temperature ranging from 21°C to 25°C. Each data point represents the average survival in 20 replicate droplets. Bars indicate standard error of the mean. The average number of veligers per replicate in the small-size class ranged from 4.5 to 9.8 in (A) and from 7.4 to 18.6 in (B). Average numbers of veligers per replicate in the large class ranged from 3.9 to 6.2 in (A) and from 2.2 to 8.6 in (B).

Emersed veligers: thermal tolerance (Experiment 3).—After four hours emersion at a relative humidity >95%, the small-size-class veligers exhibited survival rates of ≥24% at temperatures of 5°C, 20°C, 25°C, and 30°C and no survival at 35°C or 40°C (Figure 4A). The large-size-class veligers exhibited survival rates of ≥67% at 5°C, 20°C, 25°C, and 30°C but, again, no survival at 35°C or 40°C (Figure 4A). After 20 hours of emersion the small-size-class veligers exhibited much lower and more variable survival rates than at four hours; survivors were present at 5°C, 20°C and 25°C, but not at 30°C or higher (Figure 4B). The large-size-class had survival at 5°C and in two of the three replicates at 20°C, but not at 25°C or higher (Figure 4B).

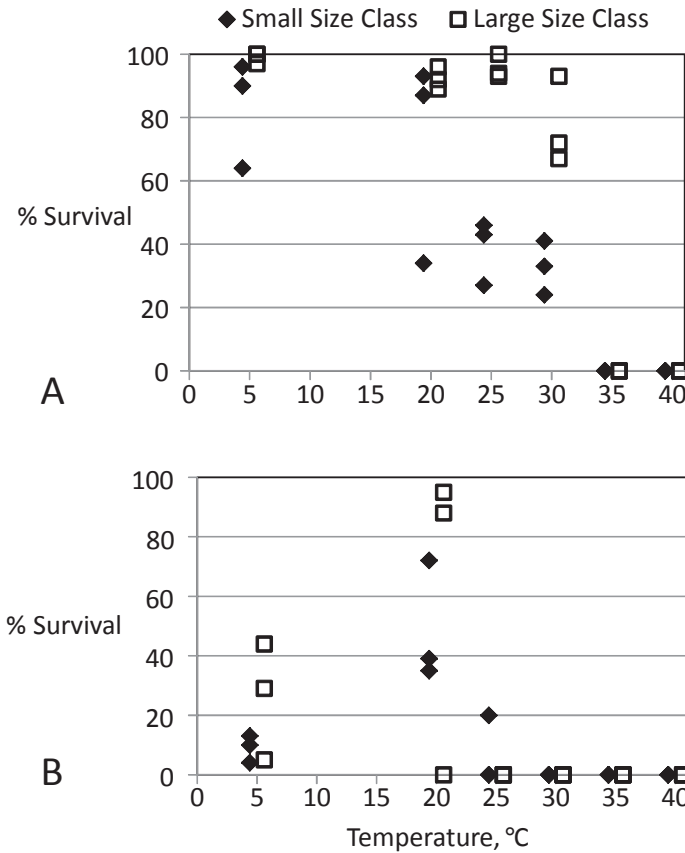


FIGURE 4.—Survival of small and large size class quagga mussel (*Dreissena bugensis*) veligers following emersion for 4 hours (A) and 20 hours (B) at various temperatures. Data points represent the percent survival for each replicate with three replicates per size class at each temperature. The number of veligers per replicate for 4-hour emersions (A) ranged from 23 to 262 with an average of 78 for the small-size class and a range of 14 to 56 with an average of 28 for the large-size class. The number of veligers per replicate following emersion for 20 hours (B) ranged from 13 to 89 with an average of 37 for the small size class and a range of 3 to 49 with an average of 13 for the large-size class. Data for 30C° are represented by only two replicates because the third replicate, with only one veliger present, was excluded.

DISCUSSION

The first phase of this study involved developing methods to determine veliger survival. Using proxy native oyster (*Ostrea lurida*, Carpenter 1864) veligers from the Bodega Marine Laboratory, Bodega Bay, California and quagga mussel veligers from Lower Otay Reservoir, California, neutral red (a living-cell inclusion stain) and trypan blue (a living-cell exclusion stain) were employed to develop a means of determining survival that is independent of behavior (Crippen and Perrier 1974, Tolnai 1975). Unfortunately, the use of stains proved to be unreliable in that results were inconsistent and difficult to

interpret. Our results were in agreement with those of Sykes (2009), who reported neutral red staining too ambiguous and variable to determine veliger survival. Ultimately, any movement of the musculature, cilia or velum was used as an indication of post-treatment survival, with the recognition that some unknown percentage of those showing movement would not be viable until and completing metamorphosis. The same approach was used in earlier studies (Sykes 2009, Craft and Myrick 2011, Choi et al. 2013). Observations of 9,700 veligers were made during eight days at Lake Mead in the study presented here, and in all but a few cases the determination of survival, as we defined it, was unambiguous. Extensive observations were made to carefully examine each veliger for movement in cases where survival was not obvious. Nevertheless, mortality could have been overestimated.

Despite sieve fractionation of plankton tows, there were unavoidable phytoplankton and zooplankton contaminants of sizes similar to those of the veligers in both size classes. These contaminants could have confounded results of the immersion trials in two distinct ways. First, these contaminants could increase biological oxygen demand and potentially result in hypoxia or other adverse environmental conditions, particularly in closed tubes. Survival of veligers in closed tubes for at least seven days suggests that this was not a critical factor, although it may have contributed to the variation observed. Second, copepods were a common contaminant in our immersion treatments and are known to prey upon the veligers of a quagga mussel congener, the zebra mussel (*Dreissena polymorpha*, Pallas 1771) (Liebig and Vanderploeg 1995). We observed copepod-veliger interactions that may have indicated a predator-prey relationship. In addition, the occurrence of serrated veliger shell fragments suggested the presence of copepod predation (J. P. Snider, California Department of Fish and Wildlife (CDFW), unpublished observations). Empty veliger shells and shell fragments that may have resulted from predation were not included in counts.

Scavenging, ciliated protozoans were observed occasionally within the valves of dead veligers. The movements of these protozoans could have been mistaken for veliger ciliary movement but careful observation was employed to account for this potentially confounding factor.

Our results demonstrate that there is a risk of transporting live immersed quagga veligers within 31 μ l droplets of water at ambient air temperatures of up to at least 25°C for at least seven days and likely longer, considering the rates of survival at the experimental endpoint. The individual water droplets in our five-day and seven-day treatments averaged between 2.2 and 18.6 veligers per replicate, which would be concentrations far exceeding those realistically encountered under field conditions given reported densities of dreissinid larvae in infested waterbody samples. For example, Gerstenberger et al. (2011) reported a density of 28.6 veligers/L in September of 2008 in the Boulder Basin of Lake Mead. At that density, the likelihood of one veliger being in a single 31 μ l droplet of water would be one (veliger) in 1,128 (droplets of water). Nevertheless it is reasonable to assume conveyance risk if standing water is present on a watercraft leaving an infested waterbody. As previously noted, Choi et al. (2013) reported that Lake Mead quagga veligers held in shaded, 15-L containers of lake water survived up to about five days under summer conditions (summer air and water temperatures ranged from 25°C to 40°C and 27°C to 36°C respectively) and 27 days under autumn conditions (autumn air and water temperatures ranged from 6°C to 18°C and 8°C to 16°C respectively). Together with the results of our studies, which included more defined, repeatable conditions, as well as exceedingly smaller water volumes, it is clear that residual water on trailered watercraft leaving infested waterbodies can pose significant risk of live veliger conveyance. Short-term immersion survival data suggest a risk for next-

day conveyance up to a temperature of 30°C. There was no evidence of immersed veligers suffering cold-related mortality, in agreement with our unpublished observations that veligers typically survive well for at least eleven days in a standard laboratory refrigerator (4°C). Our acute thermal tolerance data suggest that veligers are able to tolerate temperatures of 36°C for one hour; however, given 100% mortality at 35°C in all treatments greater than one hour, there would be no practical conveyance risk if microhabitat conditions reach this temperature for one hour or longer.

The pattern of immersed veliger survival under closed environmental conditions (e.g., 200 µl PCR tubes) was very different from that of emersed veligers or veligers in open droplets, for which the larger veligers appeared to demonstrate a higher survival rates than the smaller veligers. Higher metabolism of the larger size-class or the presence of more predators, or both, may have accounted for the lower survival rates in closed tubes. One reason we used closed tubes in addition to open microscope slides for the long-term experiments was the risk of the open slides evaporating over the multiple-day period despite our efforts to maintain high humidity. While we were able to maintain live veligers in droplets on slides for five days at high humidity, some evaporative loss on slides was observed and recorded on days three, four, and five of the five-day immersion study. Consequently, the addition of 10–20 µl of water was required to prevent desiccation during observation.

This is the only study to date that examines and documents veliger immersion survival in a small water volume and emersion survival on moist microhabitat. In preliminary, unpublished observations, we found no difference in survival between incubation volumes ranging from 31 µl to 4 ml. In conjunction with the results reported here, we hypothesize that veliger survival in un-concentrated lake water samples is independent of water volumes 31 µl or greater. Survival in such small volumes of water for at least seven days was an unanticipated result, but provided for efficient microscopic sample assessment without further concentration, sample splitting, or other manipulations.

The emersion periods we examined, 4 hours and 20 hours, were chosen to represent same-day and next-day conveyance of watercraft from infested to uninfested waterbodies. Our results suggest that there is a risk of emersed quagga veliger survival on trailered watercraft under microhabitat conditions of high relative humidity (>95%) for four hours at up to 30°C and for 20 hours at up to 25°C. A number of other observations can be gleaned from the emersion data, with the caveat that the numbers of veligers contributing to the survival data was relatively low (Figure 4A, 4B). Four-hour emersion followed a pattern where survival decreased as temperatures increased (Figure 4A). The apparent greater emersion tolerance of large veligers as compared to small veligers followed the same pattern that Ricciardi et al. (1995) described for emersion tolerance of adult quagga mussels. An unexpectedly low survival was observed at 5°C after 20 hours of emersion (Figure 4B). Evaporative stress could be a factor given that at 95% relative humidity with a standard barometric pressure of 760 mm Hg, absolute humidity at 5°C is 6 g/m³, while at 20°C the absolute humidity is 16 g/m³ (PlanetCalc Online Calculator version 2.0.1533.0). This suggests that moderately warm temperatures may actually facilitate emersion survival. There was large inter-replicate variation in 20-hour emersion survival at 5°C, 20°C and 25°C. We speculate that this may have been due to temperature and humidity variation within the holding containers, underscoring the importance of microhabitat conditions in transport risk. After 20 hours of emersion, some replicate filter papers at both 20°C and 25°C were qualitatively dry while others remained moist. This observation provides evidence of such

variation, although survival data did not seem directly related to filter moisture (S. N. Byron and J. P. Snider, CDFW, unpublished observations).

Generalizations can be made regarding watercraft access policies as they relate to veliger conveyance. If exposure temperatures reach 35°C there is no practical risk of conveying emersed veligers and at exposure temperatures $\geq 25^{\circ}\text{C}$ the risk is likely no more than one day. If immersed veligers are in water that reaches a temperature of 35°C, there is no practical risk of conveyance. This assumption can be made if the ambient temperature exceeds 35°C and the water volume is small (e.g., on the order of the 31 μl droplets we used). However for larger volumes or thermally protected microhabitat such as a hull or live well, it would be very difficult to accurately determine actual exposure temperature in a field setting. Comparisons between air temperature and relative humidity inside and outside of several different types of watercraft demonstrated variable temperatures under different exposures, and that temperatures inside watercraft cannot be reliably predicted by ambient air temperature alone (L. Corvington, CDFW, unpublished observations). At temperatures $\leq 25^{\circ}\text{C}$, any volume of water might harbor live veligers for at least one week. Therefore, under essentially any realistic thermal regime, a policy of complete draining and drying of watercraft is recommended. In comparing our data on emersed veligers with that for emersed adult quagga and zebra mussels (McMahon et al. 1993, Ricciardi et al. 1995), veligers are far less resistant to aerial exposure than adults. Thus, any evaluation standard applied to emersed adults would prevent the conveyance of emersed veligers as well.

Additional studies are required to more accurately resolve thermal immersion sensitivities between 25°C and 35°C as a function of time. A time-series study assessing survival of single veligers in droplets of water to 100% mortality (or settlement) with realistic temperature resolution would be informative. Future 'next-day' and long-term survival studies could employ thermal regimes with diel changes rather than constant temperatures, as were used in our investigations. Also, determination of emersion conveyance risk for time intervals at temperatures $< 30^{\circ}\text{C}$ will require additional studies, and further development of emersion study techniques should be explored. Lastly, studies of veliger viability through successful metamorphosis and settlement under various conveyance scenarios are needed to assess fully the actual risk of mussel establishment into new water bodies.

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Flathead catfish population estimate and assessment of population characteristics, Diamond Valley Lake, California

Quinn Granfors*

California Department of Fish and Wildlife, 33752 Newport Rd., Winchester, CA 92596, USA

**Correspondent: Quinn.Granfors@wildlife.ca.gov*

Flathead catfish (*Pylodictis olivaris*) were inadvertently introduced to Diamond Valley Lake, Riverside Co., California, where their population has become well-established. The species is highly piscivorous, extremely opportunistic, and is the least gape-limited of North American piscivores. Flathead catfish can exhibit extreme predatory pressure on existing fish populations in waters where they are introduced. Multiple mark-recapture methods were used to estimate the flathead catfish population in Diamond Valley Lake. Population characteristics including proportional stock distribution (PSD), relative stock distribution (RSD) and relative weight (W_r) were evaluated. Anchor tag retention was also evaluated. Understanding the status and characteristics of the flathead catfish populations will aid fisheries management decisions for the reservoir.

Key words: California, demography, Diamond Valley Lake, flathead catfish, piscivory, population estimate, *Pylodictis olivaris*

Flathead catfish (FCF; *Pylodictis olivaris*) is a piscivorous ictalurid catfish native to central North America that have been introduced throughout the United States (Jackson 1999). They are capable of achieving very large sizes in excess of 1.4 m and 27 kilograms (Moyle 2002). The least gape-limited of North American freshwater piscivorous fishes, evidence suggests FHC are highly opportunistic, mostly nocturnal feeders which prey upon any fish species encountered (Ashley and Buff 1988, Quinn 1988a, Eggleton and Schramm 2004, Pine et al. 2005). Flathead catfish may be detrimental for fishery management if their numbers escalate to a level where they affect other managed fish populations. For instance, many California reservoirs support salmonid or centrarchid fisheries that may be negatively impacted by introduction of this species. The expansion of FCF outside their native range has led to dramatic declines in native fish populations through predation and competition in other states (Guier et al. 1981, Quinn 1988a, Marsh and Brooks 1989, Bart et al. 1994, Marsh 1996).

Little documentation exists about the absolute predation pressure FCF exert on existing host systems, but evidence suggests that their predatory activities can decimate entire populations in a short amount of time (Guier et al. 1981, Minkley 1982, Quinn 1988a, Marsh and Brooks 1989, Marsh 1996), even when other large-bodied species are present (Bart et al. 1994). Barr and Ney (1993) estimated that FCF annually consumed 20–35% of the centrarchid population of a Virginia reservoir. FCF are the least-studied of the three predominant catfish species in North America, and very little if any information exists regarding their populations in California reservoirs. Given California regulations that mostly prohibit methods of take traditionally used in their native range (i.e., noodling, trot lines, jugging, etc.), FCF are largely unavailable to California's angling public. Without exploitation or natural predators, FCF populations may grow unchecked and can negatively affect the management of fisheries of other species in California (Moyle 2002).

Diamond Valley Lake (DVL) is a relatively new, no-body-contact reservoir in Riverside County, California, managed as a two-tiered fishery for both warm-water and cold-water species. DVL was constructed to receive water from both the Colorado River Aqueduct (CRA) and California State Water Project (CSWP) and opened to the public in October 2003. Fish species that have been stocked in the lake and are managed by the California Department of Fish and Wildlife (CDFW) include Florida-strain largemouth bass (*Micropterus salmoides floridanus*), smallmouth bass (*Micropterus dolomieu*), bluegill (*Lepomis macrochirus*), redear sunfish (*Lepomis microlophus*), channel catfish (*Ictalurus punctatus*), blue catfish (*Ictalurus furcatus*), rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*). Inadvertently introduced fish species have become established within the reservoir through the water inflows from both the CRA and CSWP, and include striped bass (*Morone saxatilis*), black crappie (*Pomoxis nigromaculatus*), Wakasagi (*Hypomesus nipponensis*), threadfin shad (*Dorosoma petenense*), inland silverside (*Menidia beryllina*), prickly sculpin (*Cottus asper*), bigscale logperch (*Percina macrolepida*) and flathead catfish. The CSWP has no recorded FCF; however FCF were introduced into the Colorado River in 1962 (Bottroff et al. 1969) and are now well-established there (Moyle 2002). Hence, the Colorado River is the likely source for introduction of FCF into DVL. The lake stopped receiving CRA water in late 2006, prior to the discovery of quagga mussels (*Dreissena bugensis*) in Lake Mead in January 2007. DVL has not received CRA water since that time to prevent the establishment of mussels in the reservoir. The first FCF was sampled in DVL during a CDFW electrofishing survey in October 2007. Since that initial sample the number of FCF sampled or observed during annual electrofishing surveys at DVL has steadily increased.

Due to a combination of restrictive fishing and access regulations, the population of FCF is subject to minimal angling pressure. With a lack of natural predation or other sources of mortality, DVL provides an ideal environment for an invasive species like FCF to expand unchecked. The present study was initiated over the concern for the potential effects of FCF on other managed species in DVL, as well as a scarcity of prior research on this species. The objectives of this study were to estimate FCF population size, population characteristics, and tag retention in Diamond Valley Lake, California.

METHODS

Study area.—Diamond Valley Lake (33° 41' N, 117° 02' W) is an off-stream storage reservoir built by Metropolitan Water District of Southern California and is located 6.4 kilometers (km) southwest of Hemet in western Riverside County, California. The mean depth at full pool (elevation 535 m) is 61 m, 49 m at the East Dam, and 79 m at the West Dam with 1,990 ha of surface area. At full pool the lake has approximately 40 km of shoreline including the three dams. DVL is subjected to annual water level fluctuations of approximately 10 m with the maximum drawdown to the emergency supply level at 27 m below full pool.

Methods.—FCF were collected from 17 July to 27 August 2014 using two electrofishing boats from 0530 to 1000. Sampling efforts were concentrated along the three earthen dams covered with rip-rap and large chunk-rock habitat where FCF were more likely to be concentrated (Hale et al. 1987, Quinn 1988b, Cunningham 1995, Daugherty and Sutton 2005, Travnicek 2011). This habitat represents 20–25% of the DVL shoreline at full pool and is where all FCF have been sampled or observed during prior electrofishing surveys. One boat was actively electrofishing moving parallel to the shoreline using low frequency, pulsed direct current (30–60 pps) in 1–3 m of water. Low frequency electrofishing provides the most efficient and precise sample for FCF compared to other gears (Cunningham 1995). A chase boat followed behind, collecting FCF outside the range and behind the active electrofishing boat. Both boats had a boat operator, two forward netters, and two personnel handling fish and recording data. To avoid resampling fish, the lead electrofishing boat applied a partial left pelvic fin clip to all FCF sampled and a red numbered Floy FD-68D tag to those larger than 350 mm total length (TL). The tags were applied just below and behind the dorsal fin (Guy et al. 1996, Buckmeier and Irwin 2000).

During the initial sampling effort all FCF were collected, measured for TL to the nearest mm, and weighed to the nearest gram. During additional sampling efforts only FCF larger than 350 mm were checked for a partial left pelvic fin clip and the presence of a red numbered Floy tag. If neither fin clip nor tag were present, they were applied and the fish was tallied as a new mark. If both fin clip and tag were present, the fish was tallied as a recapture. If the left pelvic fin clip was present but the tag absent, a different colored un-numbered Floy tag was applied to the dorsal fin and the fish was tallied as a recapture with a lost tag. All fish collected were released following measuring, marking, and tagging. Schnabel and Schumacher-Eschmeyer mark-recapture estimates with 95% confidence intervals were calculated for a closed population. Since DVL is an off-stream storage reservoir with no natural inflows and there are no fish removed by anglers, the population was considered closed.

Fish condition was calculated using relative weight (W_r). Length-specific standard weights (W_s) were derived from the standard weight equation, $\log_{10}(W_s) = -5.542 + 3.23 \log_{10} TL$ (Bister et al. 2000). Relative weight was not calculated for FCF less than 150 mm in length due to the W_s equation excluding smaller individuals that produced variance-to-mean errors larger than 0.02 (Bister et al. 2000). Proportional stock distribution (PSD) and relative stock distribution (RSD) indices were calculated based on length categories proposed by Quinn (1991) and Bister (2000).

RESULTS

A total of 1,871 FCF larger than 350 mm were captured during the four mark-recapture efforts and of those, 10% (n=187) were recaptures. The Schnabel point estimate was 6,295 stock-size FCF (5,107–8,205, $P<0.05$), and the Schumacher-Eschmeyer point estimate was 6,660 stock-size FCF (3,707–32,747, $P<0.05$). Applying the mean weight of all tagged fish (1.096 g) to the estimated number of fish yielded total biomass estimates of 6,899 kg and 7,299 kg with the Schnabel and Schumacher-Eschmeyer estimators, respectively. The Schnabel and Schumacher-Eschmeyer methods indicate 3.6 to 3.8 fish per surface hectare respectively, at the lake level when the study was initiated. Retention of the FD-68D Floy tags from the 187 FCF recaptured during the duration (41 days) of the sampling was 98.25%.

A total of 707 FCF were collected during the first sample; all FCF were collected, weighed and measured to assess population structure. Flathead catfish sizes ranged from 133 to 790 mm TL with 421 being ≥ 350 mm (i.e., stock size; Figure 1). PSD and RSD-P were both low (16 and 1, respectively), and no FCF of memorable or trophy size were captured, nullifying any ability to calculate RSD-M or RSD-T.

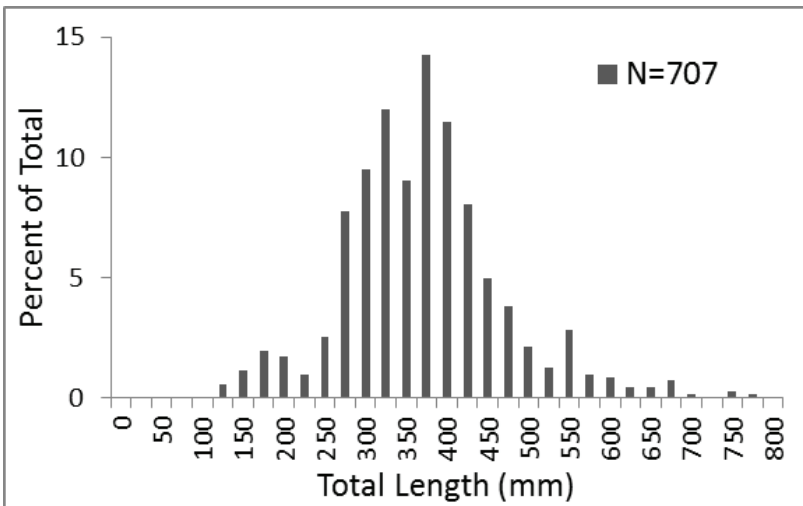


FIGURE 1.—Frequency distribution of lengths of flathead catfish (*Pylodictis olivaris*) collected from Diamond Valley Lake, Riverside County, California, during July 2014.

A length-weight regression yielded a model similar to the expected weights generated from the regression slope equation described by Bister et al. (2000; Figure 2). Relative weights were averaged across all proposed size classes, with mean values of W_r between 89 and 102 (Figure 3).

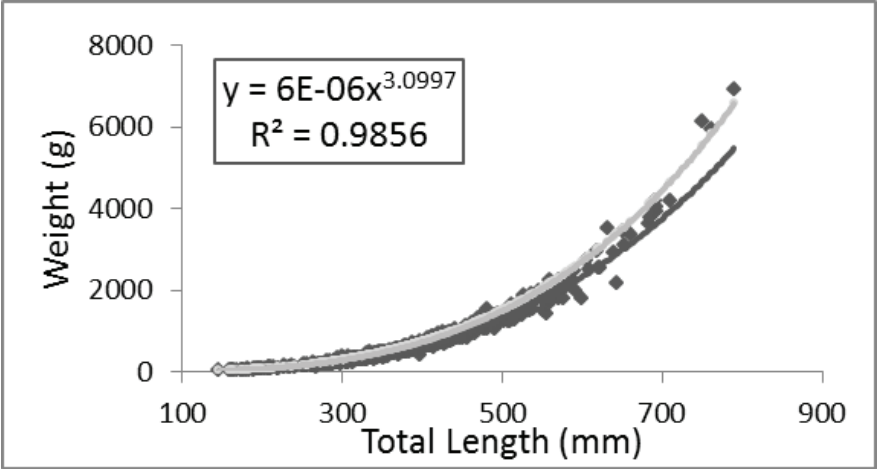


FIGURE 2.—Length-weight scatter plot with power regression line, slope equation and R^2 for flathead catfish (*Pylodictis olivaris*) collected from Diamond Valley Lake, Riverside County, California, during July 2014. The light gray line is the expected weight-at-length power regression line taken from the slope equation of Bister et al. 2000.



FIGURE 3.—Relationship between mean relative weights (W_r) and proposed stock distribution length categories for flathead catfish (*Pylodictis olivaris*) collected from Diamond Valley Lake, California, during July 2014. The shaded lines represent the range of W_r values of individuals sampled with the dark circle representing the mean W_r value.

Reservoir elevation dropped 3 m during the 41-day duration of sampling and has fallen 10.66 m since 1 January 2014. Reservoir surface water temperatures ranged from 24°C to 28°C during sampling. Stratification of the lake resulted in an epilimnion reaching a depth of only 9 m throughout sampling. Dissolved oxygen was 8 mg/liter down to 9 m, beyond which it dropped to 1 mg/liter in the metalimnion (11 m) and became anoxic throughout the hypolimnion.

DISCUSSION

Ninety-eight percent of the Floy FD-68D anchor tags applied were retained during the sampling period. Though the retention rate was high for the Floy tags, using them for a FCF mark-recapture population estimate is not recommended, as any tag loss can lead to lost marks. Lost marks violate one of the basic, yet critically important, assumptions of mark-recapture population estimators (Krebs 1999). This study utilized a partial pelvic fin clip as a secondary mark to identify FCF that would have been adequate on its own for the purpose of these population estimates.

The FCF population in Diamond Valley Lake appears to be abundant and consisting of mostly stock (350 mm) and quality (510 mm) sized fish. Typically, unexploited fish stocks are characterized by high population abundance, a low rate of annual mortality and a broad range of fish age and length classes (Clady et al. 1975, Goedde and Coble 1981). Although abundant, FCF of the larger memorable (860 mm) and trophy (1020 mm) sizes in DVL were not sampled as compared to other FCF populations in the U.S. (Bister et al. 2000). This is due to this population becoming established relatively recently, not permitting enough time for many FCF to achieve larger sizes. Since DVL is recently impounded (2000) and the FCF population is recently established, the growth rates should be high (Buck 1956, Pisano et al. 1983, Sakaris et al. 2006). Sneed et al. (1961) suggested that FCF growth is faster in reservoirs than in rivers, although more recent data suggest that growth rates in reservoirs are variable and are generally similar in range to riverine based populations (Guier et al 1984). Although age data were not available, fish captured likely ranged from 1 to 7 years old, with the majority 2–4 years old based on length-at-age data collected from the Colorado River or Coachella Canal (Pisano et al. 1983, Young and Marsh 1990). Water from the Colorado River imported into DVL is the most likely source of FCF. Also, given the geographical proximity of DVL to the Colorado River and Coachella Canal, growth rates are expected to be similar. Daugherty and Sutton (2005) suggested thermal gradient and length of growing season associated with geographic location are reliable indicators of FCF growth rates. The length-weight results from DVL appear very similar to pooled data collected (Bister et al. 2000), with DVL FCF larger than 600 mm generally weighing less (Figure 2). Although the DVL population appears to lack older fish the population appears to be growing at a typical rate and may develop higher RSD-P, RSD-M, and RSD-T over time.

There are likely larger specimens within the lake that were not sampled as part of this study, as a few have been reported caught by anglers or have been seen during other CDFW electrofishing efforts. The first recorded FCF was sampled in 2007 and was 495 mm TL, likely 3–4 years old at that time, based on work by Pisano et al. (1983). By 2014, this individual may be greater than 1000 mm (Pisano et al. 1983, Marsh and Young 1990, Moyle 2002) and able to ingest a largemouth bass up to 604 mm TL (Slaughter and Jacobson 2008). Thomas (1993) noted it took 10 years for the FCF population in the Altamaha River, Georgia to increase from relative obscurity to the dominant predator present in mainstream habitat. It appears the FCF population in DVL is progressing in a similar manner given a single FCF was sampled in 2007 and up to 2.24 fish/minute larger than stock size were sampled during this study.

The abundance of approximately 2–4 year old fish coincides with a dramatically increased, and then relatively stable, water level in DVL from 2010 to 2012, which also

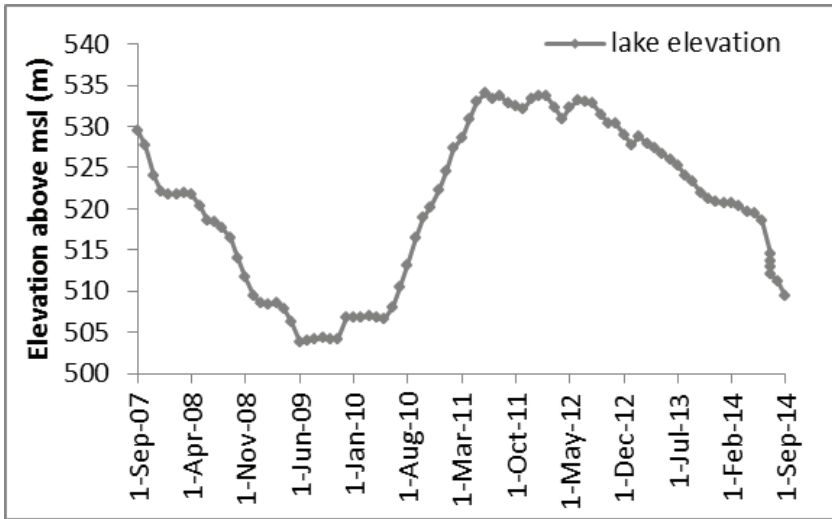


FIGURE 4.—Monthly pool elevation above mean sea level at Diamond Valley Lake, California, from September 2007 to July 2014.

increased the availability of suitable spawning and residential habitat (Figure 4). Relatively stable water levels with some spring flooding improve FCF reproduction and survival in hydropower storage reservoirs (Plosky et al 1984). Flathead catfish have habitat requirements that vary with age. Juveniles in rivers prefer riffles and runs with complex structure, and adults prefer deep pools with large rocks and woody debris in areas of strong flow (Moyle 2002). In reservoirs, the availability of rock rip-rap limits FCF populations more than the availability of suitable forage, where they use the habitat type for cover, spawning, and foraging (Layher and Boles 1980). Weller and Winter (2001) reported that FCF total abundance may be limited to the amount of rock and wood habitat available in a reservoir. Their results showed FCF used rocky substrate the majority of the time (61.4%–62.6%) in Buffalo Springs Lake, Texas, where that type of habitat accounted for only 16% of the available substrate.

The amount of habitat suitable for FCF spawning or residence within DVL is mostly limited to the rip-rap areas on the three dams and other smaller areas intermittently spaced around the inlet-outlet tower and marina at higher water levels. Areas of rip-rap with larger interstitial spaces for fish to use are also more abundant at higher water levels in DVL. Large water fluctuations cause erosion and deposition of sediments, which fills interstitial spaces at lower water levels. This was evident during the last sampling effort when the water level dropped rapidly, revealing rip-rap devoid of interstitial spaces as a result of silting. The number of fish sampled during the final effort was much lower ($n=156$) than in weeks prior ($\bar{x}=572$). In addition to the rip-rap areas, possible spawning habitat includes 2,500 PVC pipe caves that were placed on the lake shoreline prior to filling; however, all of these caves were in 8–30 m of water for the last 2–4 years of higher water, and away from the rocky areas of the lake. At this depth, FCF are unlikely to use the caves for spawning, as FCF rarely occupy deeper water during spawning (Weller and Winter 2001). Summerfelt

(1971) reported FCF in reservoirs prefer to spawn at depths of 2–5 m. The PVC spawning caves, thus, are unlikely to have contributed to FCF spawning success over the last 2–4 years.

Recruitment of juvenile FCF (114–318 mm) to adults increased in years when reservoir surface area is higher than average in spring and followed by a more extensive than usual summer drawdown (Lee and Terrel 1987). Diamond Valley Lake has been higher in the spring with drawdowns in the summer or fall over the last 2 years, which likely improved recruitment of the 2–4 year classes observed in this study.

Approximately 6.44 km of rip rap were sampled resulting in an estimated density of 977 to 1,034 fish/km for this habitat in DVL. Studies in the lower Colorado River, Arizona, and St. Joseph River, Michigan, estimated FCF densities of 155 to 229 fish/km (Marsh et al. 1988) and 145 fish/km (Daugherty and Sutton 2005), respectively. The numbers of FCF per km in DVL are much higher than those found in either river, although only the St. Joseph River investigators focused on selectively sampling preferred habitat. However, Weeks and Combs (1981) estimated the total FCF population in a 4,050 ha Oklahoma reservoir at 4.1 fish/ha, which compares to 3.6–3.8 fish/ha within DVL. Given the behavioral tendency of FCF to maintain and defend a home range and populations that are generally regulated by the availability of rip-rap habitat, it is surprising that only 10% of the FCF marked were recaptured during this study. Many investigators have documented FCF to be solitary in nature, to have a propensity for site fidelity, and to be extremely aggressive toward other FCF, which implies they are unwilling to leave their established home ranges (Funk 1957, Swingle 1964, Hackney 1965, Hart and Summerfelt 1974, Gholson 1975, Skains and Jackson 1995, Weller and Winter 2001, Gelwicks and Simmons 2011). Each sampling effort at DVL was conducted while a drastic reduction in water level occurred (3.96 m over 41 days; Figure 4) and a strong thermal stratification limited the usable habitat (i.e., conditions were anoxic below 11 m). These factors confined FCF to shallower zones in the rip-rap areas where they were more vulnerable to the electrofishing sampling method used in this study. Rip-rap habitat is fairly homogenous and the large number of unmarked fish sampled at a high catch rate may indicate FCF population saturation within the preferred habitat. Even though rip-rap habitat on the dams extends much deeper than was habitable due to the thermal stratification, the rapidly decreasing water level may have crowded FCF and forced some of them to move laterally and away from preferred habitat to find other areas in which to forage or reside, particularly during the last sampling effort when the number of FCF sampled was much less than prior efforts.

Weller and Winter (2001) also reported a reduced amount of FCF habitat availability in a reservoir due to anoxic conditions below 7.9 m. During lake stratification they reported FCF utilized rock substrates only 47% of the time, rarely in shallow (0.1–0.9 m) or deep water (6.0–7.9 m), although their findings were not affected by changing lake levels. It is possible that fish collected were displaced from their home range by physical relocation due to sampling efforts. However, radio telemetry studies have documented homing tendencies of FCF with individuals returning to the same area in as little as 1.7 days (Hart and Summerfelt 1974, Duncan and Meyers 1978, Dobbins et al. 1999, Pugh and Schramm 1999).

Due to their nocturnal nature and restrictive regulations on lake use, FCF are not generally available to anglers in DVL. Methods of take typically used for targeting FCF are not legal in Riverside County, further inhibiting an anglers' ability to target FCF. Annual creel surveys conducted at DVL showed only seven FCF caught by anglers in 2013 and four

in 2014 (Q. Granfors, CDFW, unpublished data). The fish were reported as being caught on artificial lures by anglers targeting other species, which indicates they were incidental catches.

Low fishing pressure increases the potential of the FCF population in DVL to grow unchecked and negatively affect the management of the other fisheries. Thomas (1993) reported a significant increase in CPUE for FCF by electrofishing, but without a discernable increase in the number of FCF reported to creel surveys and observed a decline in abundance of red breast sunfish (*Lepomis auritus*) and bullhead (*Ameiurus* sp.) over the same time period. Also, 50% of the FCF sampled for this study are at, or just below, the size or age of reaching sexual maturity (Turner and Summerfelt 1971, Moyle 2002), which could result in a future population eruption. Because of this potential, methods for controlling the FCF population may be needed. FCF are long-lived and have relatively low fecundity, resulting in altered population size and age structure with excessive harvest (Jenkins and Burkhead 1994, Stauffer et al. 1996, Jackson 1999). Given population estimates of 6,295-6,660 FCF, a removal similar to our first sampling effort (i.e., 1,871 FCF over 350 mm) would be equivalent to removing 28–30% of the population. Sakaris et al. (2006) predicted that an intensive electrofishing removal plan, coupled with minimal protection from anglers, would considerably reduce the biomass of FCF in the Satilla River, Georgia, where invasive FCF were introduced in the mid-1990s. Bonvechio et al. (2011) evaluated the use of intensive electrofishing to remove FCF from the Satilla River, Georgia, resulting in 65% of the total FCF biomass being removed from the river. Bonvechio et al. (2011) recommended periodic removal of FCF as a reasonable method to manage FCF where they have been introduced. Reducing the predatory and competitive potential of FCF should provide other sport-fish populations in DVL a greater opportunity to flourish, resulting in a better fishery overall for anglers in California.

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Straying of late-fall run Chinook salmon from the Coleman National Fish Hatchery into the lower American River, California

GENA R. LASKO*, ROBERT G. TITUS, JOE R. FERREIRA, AND RONALD M. COLEMAN

California Department of Fish and Wildlife, Ecosystem Restoration Program, 830 S Street, Sacramento CA 95811, USA (GRL)

California Department of Fish and Wildlife, Fisheries Branch, 8175 Alpine Avenue, Suite F, Sacramento, CA 95826, USA (RGT)

California Department of Fish and Wildlife, Fisheries Branch, 830 S Street, Sacramento, CA 95811, USA (JRF)

Department of Biological Sciences, California State University, Sacramento, 6000 J Street, Sacramento, CA 95819 USA (RMC)

*Correspondent: gena.lasko@wildlife.ca.gov

Salmon typically home to their natal streams when returning to spawn in fresh water. Straying, however, is a natural behavior for a small fraction of individuals in a population, and may have an adaptive advantage under some circumstances. In the winter of 2006–2007, tens of thousands of late-fall run Chinook salmon (*Oncorhynchus tshawytscha*) reared in the Coleman National Fish Hatchery (CNFH) were released at several downstream locations as part of a Sacramento-San Joaquin River Delta survival study. In the winter of 2008–2009, biologists observed a pulse of late-season spawners in the American River, which turned out to be stray late-fall run Chinook salmon from the CNFH, spawning where the American River fall-run Chinook salmon were completing their spawning. Late-fall run Chinook salmon have not been known to spawn in the American River and understanding the reason for this unusual behavior was the basis for this project. We used coded-wire tag inland return data to test the hypothesis that salmon released close to the mouth of the American River are more likely to stray into the river during their return spawning migration than are fish released farther from the river's mouth. Results indicated that straying increased relative to proximity of release location to the mouth of the American River and with respect to

downstream releases in general. No salmon released in the vicinity of the CNFH were recovered in the lower American River. This study indicates that release location should be carefully evaluated if future downstream releases are conducted by Sacramento River watershed hatcheries.

Key words: American River, anadromous, California, coded-wire tag, Coleman National Fish Hatchery, escapement, hatchery, homing, late-fall run Chinook salmon, *Oncorhynchus tshawytscha*, release location, straying

Nearly all species of salmon and trout (family Salmonidae) spawn in fresh water, and many have at least facultative anadromous life histories (Quinn 1997, Quinn 2005, Railsback et al. 2014). Homing, the behavior of adult salmonids returning to spawn in their natal stream, is a major part of the anadromous life history (Quinn et al. 2000, Beacham et al. 2002, Keefer et al. 2008). Homing serves to genetically isolate populations of the same species spawning in different waterways, thus allowing for eventual adaptation to local conditions (Quinn et al. 2000, Beacham et al. 2002, Keefer et al. 2008). This could include evolved compatibility to natal habitat conditions via adaptations for temperature tolerance or resistance to pathogens in the stream, as locally adapted salmonids are generally far more successful at spawning than occasional strays (Quinn 2005). Overall estimates for natal area fidelity via homing in Pacific salmon (*Oncorhynchus* spp.) are 80%–100%, based primarily on hatchery data (Quinn 1997). Imprinting, or olfactory learning, of anadromous salmonids to their natal stream appears to occur before and during the parr-smolt transformation, as well as during emigration, although to a lesser extent during earlier life stages in some Pacific salmon of hatchery origin (Dittman et al. 1994, Dittman and Quinn 1996, Quinn 1997, Dittman et al. 1996, Lema and Nevitt 2004, Yamamoto et al. 2010).

The term “straying,” as used in this paper, refers to anadromous salmonids that either intentionally or unintentionally return to and spawn in a non-natal stream. Anadromous salmonids that spawn in a river or stream other than the one of their origin exhibit the “truest” sense of straying (Quinn et al. 1991), which Keefer et al. (2008) referred to as permanent straying. It is not known why some anadromous salmonids stray and the explanation is likely complex. The tendency to home or stray may be genetically inherited, and the pattern and stability of anadromous salmonid distributions may be a reflection of ecological constraints on the fish (Quinn 2005). Straying may occur in response to environmental conditions, or in response to disturbance events that prevent the fish from reaching or spawning in their natal stream (Quinn 2005, Waples et al. 2009). Anadromous salmonids may also wander, explore new habitats for suitability, follow schools of conspecifics from other rivers, or opportunistically spawn in another stream with favorable conditions (Jonsson et al. 2003, Keefer et al. 2008). Furthermore, anadromous salmonids may be distracted by odors or flows from a river they are migrating past, or simply get lost or confused by some combination of cues that they encounter during their upriver migration. Straying can be adaptive through rapid colonization of newly available habitat after events such as landslides, forest fires, or low flows and high temperatures resulting from drought or ice melt and glacial recession (Quinn 1997, Moyle 2002, Quinn 2005, Waples et al. 2009). Straying likely results in gene flow between different populations in the system (Quinn 2005). Strays might be the only successful spawners following a major climatic or catastrophic event, such as the eruption

of Mount St. Helens which rendered natal streams inaccessible or unsuitable for spawning (Quinn 2005). In effect, straying can provide a kind of insurance in space from these types of events (Thorpe 1994).

There is great variability in salmon straying rates from year to year and between populations, by size and age (Quinn and Fresh 1984), and across species (Quinn 1997). Salmonids of hatchery origin appear to stray at a higher rate than salmonids that are of natural-origin, and straying also appears to increase with increased hatchery selection (Jonsson et al. 2003). It may be that this bias towards greater straying by salmonids of hatchery origin is due to fewer studies of straying behavior in wild populations (Quinn 1995). Straying may increase when salmonids of hatchery origin are released away from their natal hatchery, and may also increase with greater release distance from the hatchery (Newman 2008). Different rivers seem to vary in their attractiveness to Pacific salmon strays, possibly because of flow or temperature variations from year to year (Quinn et al. 1991, Carmichael 1997, Crateau 1997, Phillips et al. 2000), and strays might choose a river resembling their natal stream (Quinn et al. 1991). There also appears to be considerable variation in the amount of straying based on location, and straying can occur both upstream and downstream from an individual's natal stream. Johnson et al. (1990) found only a rough correlation between straying rate and release distance from the natal stream.

Anadromous salmonid hatcheries supplement natural populations to support fisheries and to enhance, conserve, and restore natural populations. Salmonids reared in hatcheries can quickly become adapted to their artificial environments (Araki et al. 2008). Unintended genetic changes have been documented in cultured populations as a result of historical hatchery practices, with loss of alleles through drift, artificial selection, non-random mating, and the relaxation of sexual selection (Meffe 1986, Waples 1999). An overarching effect is that fitness may be compromised (Hatchery Scientific Review Group 2009). Straying hatchery-origin salmonids can place natural populations at risk both through potential interbreeding and through ecological interactions with natural-origin spawners (Bakke 1997, Leider 1997). They also have the potential to disrupt the genetic composition of natural populations, and beneficial genes in locally adapted natural-origin salmonids may become diluted by mating with hatchery-origin individuals. The greatest risk is if the hatchery fish have been selected for domestication or are from a non-native stock (Keefer et al. 2008).

Release strategies for Chinook salmon produced in hatcheries in the Sacramento River system in California's Central Valley (Figure 1) include releases at downstream locations, as well as from the hatchery itself. The rationale behind downstream releases is that by being released closer to the ocean, Chinook salmon smolts avoid potential sources of mortality that they would otherwise encounter in the rivers in route to the ocean. Mortality may be either direct or indirect from sources that include impaired rearing and migratory habitat, predation by both native and introduced piscivorous species, and entrainment into water diversions). The goal of downstream releases is to increase survival of Chinook salmon produced in the hatcheries, and hence increase the number of fish available to fisheries and returning to the hatcheries for spawning. Coded-wire tag recoveries provide evidence that this goal is being attained; results of 2010 and 2012 Chinook salmon ocean harvest and spawner escapement surveys showed that downstream net-pen releases in the San Francisco Bay made significant contributions to ocean fisheries. In some instances, these contributions were greater compared to upstream releases (Kormos et al. 2012, Palmer-Zwahlen and Kormos 2013).

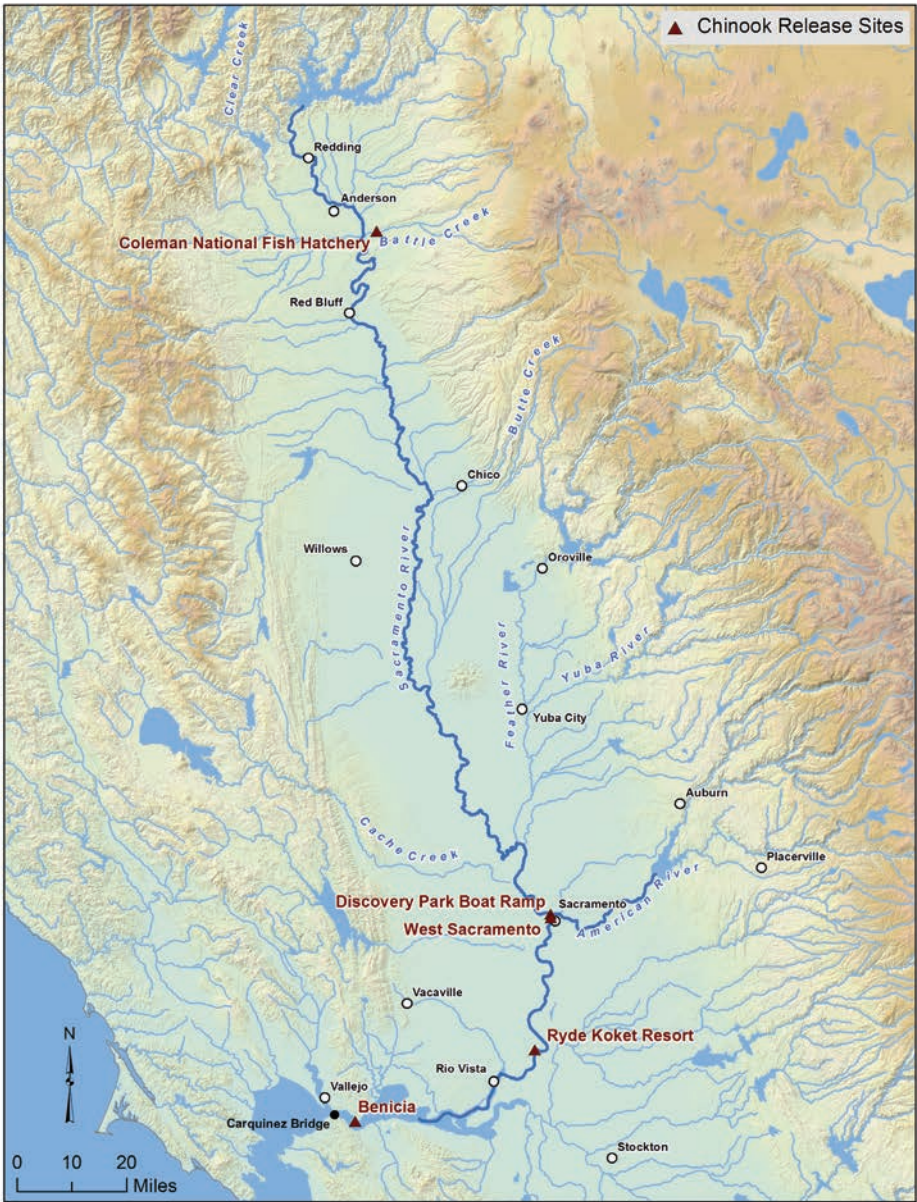


FIGURE 1.—Map of the Sacramento River watershed including Coleman National Fish Hatchery and release sites at Discovery Park, West Sacramento, Ryde Koket Resort, and Benicia during winter, 2006-2007. Map created by Daniel Rankin, California Department of Fish and Wildlife.

Release location of hatchery-origin Chinook salmon may affect their straying rates (Quinn 1997), perhaps because the fish released away from the hatchery do not acquire the sequence of cues that fish released at the hatchery acquire as they migrate downstream. Both the distance between release site and the hatchery facility, and location of the release site within the watershed, can affect homing (Quinn 1997). Downstream releases may result

in improved survival, but at the cost of impaired homing (McCabe et al. 1983). However, Pacific salmonids released long distances of 100 km or more (Ebel 1980) from their rearing site still may return to the hatchery (Ebel et al. 1973, Slatick et al. 1975).

In January 2009, a late pulse of fresh-run adult Chinook salmon appeared in the lower American River, a major tributary to the lower Sacramento River (Figure 1). These fish were observed at what would have normally been the end of the 2008–2009 fall-run Chinook salmon spawner escapement survey on the lower American River. To determine the origin of these fish, the spawner escapement survey was extended through February 2009. Most of the late arriving Chinook salmon were adipose fin-clipped and coded-wire tagged, which indicated that they were of hatchery origin. Through recovery of the coded-wire tags, these fish were determined to be strays of 2006 brood-year releases of late-fall run Chinook salmon that had been produced at Coleman National Fish Hatchery (CNFH) in the upper Sacramento River system (Figure 1). These late-fall run Chinook salmon came from downstream, experimental release groups and were part of a juvenile Chinook salmon survivorship study conducted in the Sacramento-San Joaquin River Delta during the winter of 2006–2007 (P. Brandes, U.S. Fish and Wildlife Service, personal communication, 2011).

Fall-run Chinook salmon (Table 1) are currently the only naturally extant run of Chinook salmon on the lower American River (Williams 2001). They are produced at the Nimbus Fish Hatchery (at river km 36) and also occur as a natural population that spawns in the river. Late-fall run Chinook salmon (Table 1) arriving in January could negatively impact fall-run Chinook salmon production on the American River by competing for spawning space, or by excavating or superimposing their redds on those of fall-run Chinook salmon that had already spawned in the river. The presence of a significant number of late-fall run Chinook salmon in the American River posed itself as a management concern to the California Department of Fish and Wildlife (CDFW), thus warranting further study and analysis.

TABLE 1.—Timing and characteristics of Central Valley Chinook salmon (*Oncorhynchus tshawytscha*) runs (from Fisher 1994).

Central Valley Chinook salmon run	Migration period	Spawning period	Fork length at ocean entry
Late-fall run	October–April	Early January–early April	160 mm
Winter run	December–July	Late April–early August	120 mm
Spring run	March–July	Late August–early October	80 mm
Fall run	June–December	Late September–December	80 mm

The purpose of this study was to determine the relationship between straying by adult late-fall run Chinook salmon into the American River, and downstream release locations of juvenile CNFH late-fall run Chinook salmon from the 2006 brood year. This information will inform fishery managers about the relative risk of straying into Sacramento River tributaries, such as the American River, when making decisions about downstream release locations for hatchery-produced Chinook salmon. Because of the variability in straying between river systems and populations, this research is most pertinent to the lower American River, but may have application to other river systems with hatcheries and naturally spawning populations.

Three primary hypotheses about late-fall run Chinook salmon straying were tested in this study: (1) late-fall run Chinook salmon produced at CNFH and released downstream are more likely to stray than those released at or in close proximity to CNFH; (2) downstream releases of late-fall run Chinook salmon increase the net straying rate into the American River; and (3) salmon released in close proximity to the mouth of the American River are more likely to stray into the American River than those released farther from the river's mouth.

MATERIALS AND METHODS

Data collection.—Coded-wire tag release and return data from CNFH's 2006 brood-year of late-fall run Chinook salmon were analyzed in this study. One hundred percent of the 2006 brood-year late-fall run Chinook salmon were coded-wire tagged and adipose fin clipped. Release data by coded-wire tag number included brood year, release location and date, and the number of fish tagged in each release group. Coded-wire tag return data for this cohort were recovered in the American River and other inland spawning locations and hatcheries in the Sacramento River Basin (see Results for locations). This included recoveries by the United States Fish and Wildlife Service (USFWS) and CDFW. This allowed for the comparison of the number of fish found to have strayed into the lower American River or elsewhere in the watershed to the number of fish that homed to CNFH. The 2006 brood-year return data included corresponding return data over several years from winters 2007–2008 through 2010–2011 (capturing 2–5 year-old fish). All coded-wire tag data used in this study were obtained from the Regional Mark Processing Center (RMPC), where coded-wire tag release and recovery data are uploaded. These data are available for use at www.rmhc.org.

Escapement surveys.—Field surveys were conducted as an extension of the lower American River escapement surveys conducted by CDFW in survey years 2008–2009 through 2009–2010 (two season span). Surveys also occurred in 2010–2011 but were limited because of high river flows and turbidity. When river conditions allowed, the Chinook salmon carcass surveys were conducted weekly in the spawning reaches of the lower American River, primarily from a short distance below Nimbus Dam down to the crossing of the Sunrise Bridge in Sacramento, CA (approximately 4 km). All carcasses encountered during these surveys were collected and evaluated for the presence of a coded-wire tag; carcasses were selected by the absence of the adipose fin. If the adipose fin was absent, therefore indicating the possible presence of a coded-wire tag, the head was removed by machete, labeled, and retained for tag recovery. Recovery of the coded-wire tag data (reading and recording tag information) collected in the lower American River was conducted by CDFW. For more information regarding CDFW's lower American River escapement surveys, see Vincik and Mamola (2010).

Data analysis.—Three hypotheses were tested using a Chi-square test for independence to compare: (1) the total number of recovered fish from the 2006 brood year found to have strayed or not strayed by release location (hatchery or downstream release); (2) the percent of returning fish that strayed into the American River from the 2006 cohort that were released at the hatchery to the percent that strayed into the American River from the downstream release groups; and (3) the observed counts of fish that were recovered in the American River (strayed) to the counts of fish recovered at the CNFH (not strayed) based on the release location (distance) from the American River. The Chi-square tests assessed

whether the tendency to stray was associated with release location. Note that $df=1$ when observations from all downstream release locations were grouped together in comparisons with observations associated with releases made upstream at CNFH.

The Spearman Rank correlation coefficient was used to determine the strength of the relationship between distance of release site to the mouth of the American River, to the percentage of individuals from each release site that were observed straying into the lower American River.

The relationship between survival and release site for 2006 brood-year late-fall run Chinook salmon was assessed using Chi-square tests for independence to determine if there was a difference in survival between fish released from CNFH compared to fish released at downstream sites. In this analysis, adult return rates were used as an index of survival.

We also assessed other coded-wire tag recoveries of 2006 brood-year late-fall run Chinook salmon that were not related to straying, including smolt recoveries made in the Sacramento-San Joaquin Delta (Delta). This investigation was conducted to determine if smolt entrainment at the Central Valley Project (CVP) or the State Water Project (SWP) pumping facilities may have impacted adult recovery statistics. In addition, Chi-square tests for independence were used to determine if release location contributed to either ocean or freshwater fisheries returns, and if spawning returns differed with the inclusion of fisheries returns. In all statistical tests used in this study, $\alpha=0.05$.

RESULTS

Overall results.—USFWS released 1,070,896 coded-wire tagged juvenile late-fall run Chinook salmon from the 2006 brood-year raised at CNFH. Of these, 854,496 were released in close proximity to the hatchery and 216,400 were released at downstream locations that included: Discovery Park; Sacramento River at West Sacramento; Sacramento River at Ryde Koket Resort; Georgiana Slough, and Benicia (Table 2, Figure 1). The nearest downstream location to the American River was Discovery Park, over 300 river km south of Battle Creek. Direct counts of recorded recoveries (returns) of 2–5 year-old adults, excluding the ocean and freshwater fisheries, from the 2006 brood-year were made from 2007–2008 through 2010–2011. A total of 6,487 adults returned to spawn in the Sacramento River watershed, and 6,103 (94%) of those Chinook salmon homed back to CNFH, while 384 (6%) strayed to other locations within the watershed. Of the strays, 279 (73%) were recovered in the lower American River, captured either at Nimbus Hatchery or in the river during the Chinook salmon carcass surveys. The percentage of returning late-fall run Chinook salmon that were released proximate to CNFH and homed was 99.3%, while the percentage released downstream that homed back to CNFH was 34.0%. Of the Chinook salmon released at the hatchery, 0.70% survived to be captured in the watershed, while 0.24% of the fish released downstream survived (Table 2).

Hypothesis 1: Hatchery produced late-fall run Chinook salmon released at downstream locations are more likely to stray than those released at or in close proximity to the CNFH.—The overall stray rate of late-fall run Chinook salmon released at downstream locations was 66.0%, while that for late-fall run Chinook salmon released near CNFH was 0.06% (Table 3), and this difference was highly significant ($X^2_1=3624$, $P<0.001$). Therefore, for brood-year 2006, the stray rate was significantly higher for downstream releases than it was for releases made at or in close proximity to CNFH.

TABLE 2.—Summary of coded-wire tag data releases and adult returns for late-fall run Chinook salmon (*Oncorhynchus tshawytscha*), 2006 brood-year, Coleman National Fish Hatchery, California.

Total juveniles released	1,070,896
Total juveniles released at hatchery	854,496
Total juveniles released downstream	216,400
 Total adults returned	6,487 (0.61%)
Total adults returned that were released at hatchery	5,970
Total adults returned that were released at downstream locations	517
 Total homed to natal hatchery	6,103
Total strayed	384 (6%)
Total homed released at natal hatchery	5,927
Total strayed released at natal hatchery	43 (0.07%)
Total homed released at downstream locations	176
 Total downstream releases that strayed	341 (66%)
Released downstream & strayed into American River	279 (73%)
Released downstream & strayed elsewhere	62
 Percent homed of all returns that were released at hatchery	99.3
Percent homed of all returns that were released downstream	34.0
Percent returned/survived released at hatchery	0.70
Percent returned/survived released downstream	0.24

TABLE 3.—Late-fall run Chinook salmon (*Oncorhynchus tshawytscha*), brood-year 2006, that strayed or homed as a function of release location, California.

Release Location	Total Returns	Homed	Strayed	Percent Strayed	Standard Error (95% CI)
Hatchery	5,970	5,927	43	0.07%	0.1%
Downstream	517	176	341	66.0%	2.1%

Hypothesis 2: Downstream releases of 2006 brood-year late-fall run Chinook salmon increased straying into the American River.—Among releases of 2006 brood-year late-fall run Chinook salmon, 54% of those released at downstream locations strayed into the American River, while 0% of those released in close proximity to CNFH did so (Table 4), and this difference was highly significant ($X^2_1=3786, P<0.001$).

Hypothesis 3: Salmon released in close proximity to the mouth of the American River are more likely to stray into the American River than those released farther from the river's mouth.—Releases of late-fall run Chinook salmon at West Sacramento had the highest stray rate (88%), followed by Discovery Park (64%), Ryde Koket (42%), Benecia

TABLE 4.—Number of late-fall run Chinook salmon (*Oncorhynchus tshawytscha*) that strayed into the American River that were released at the Coleman National Fish Hatchery or downstream locations in the Sacramento River and Sacramento-San Joaquin Delta, California.

Release Location	Total Returns	Homed	Strayed into American River (n)	Strayed into American River (%)	Standard Error (95% CI)
Hatchery	5,970	5,927	0	0.0	0.0%
Downstream	517	176	279	54.0	2.2%

TABLE 5.—Brood-year 2006 late-fall run Chinook salmon (*Oncorhynchus tshawytscha*) from the Coleman National Fish Hatchery that strayed into the American River or returned elsewhere in the watershed, as a function of release site. Approximate distance from mouth of the American River: Discovery Park, 0 km; West Sacramento, 2 km; Ryde Koket, 48 km; Benecia, 113 km; Coleman National Fish Hatchery (NHF), 322 km.

Location	Total Returns	Returned Elsewhere	Number Strayed ^a	Percent Strayed ^a
Discovery Park	122	44	78	63.9
West Sacramento	139	17	122	87.8
Ryde Koket	162	94	68	42.0
Benecia	94	83	11	11.7
Coleman NFH	5970	5970	0	0
Total	6487	6208	279	4.3

^a Strays that entered the American River

(12%), and lastly CNFH, which had no observed strays into the American River (Table 5). These observed differences in frequencies of straying relative to proximity of release point to the American River were highly significant ($X^2_4=4246$, $P<0.001$). Generally, the fidelity of returning adult late-fall run Chinook salmon to their release locations was high, relative to their returns elsewhere in the Sacramento River system, including CNFH (Figure 2).

Correlation analysis.—The Spearman Rank Correlation coefficient indicated a strong negative relationship between stray rate and distance of release location from the American River ($r_s = -0.90$, $P=0.037$). Generally, stray rates decreased with increasing release location distance from the lower American River (Figure 3).

Survival by release location.—There was a significant difference in adult return rates between releases of 2006 brood-year late-fall run Chinook salmon made at CNFH and those made at downstream release locations ($X^2_4=616.8$, $P<0.001$). Return rates were highest in association with releases made at CNFH (0.70%) followed by those made at Benecia (0.39%). The other three downstream release locations had similar return rates to one another (0.21%–0.23%; Table 6 and Figure 4).

Smolt recoveries.—We summarized coded-wire tag recovery data for CNFH 2006 brood-year late-fall run Chinook salmon smolts that were recovered at various locations in the Delta, including at the SWP and CVP pumping facilities (Table 7). There were so few smolt recoveries made relative to their corresponding release numbers (0.06% of total

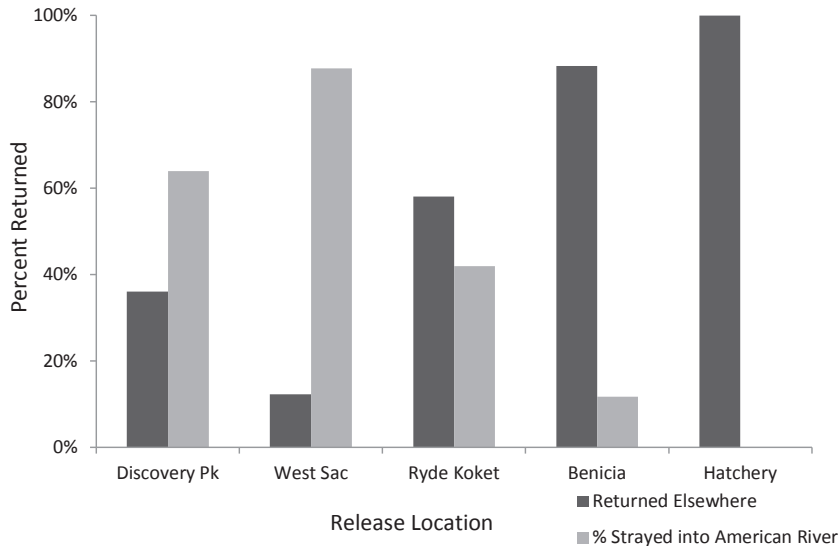


FIGURE 2. —Percent of adult 2006 brood-year late-fall-run Chinook salmon (*Oncorhynchus tshawytscha*) from the Coleman National Fish Hatchery that strayed into the American River compared to percent adult returns released at Discovery Park, West Sacramento, Ryde Koket Resort, Benicia, and hatchery collected between 2008 and 2011.

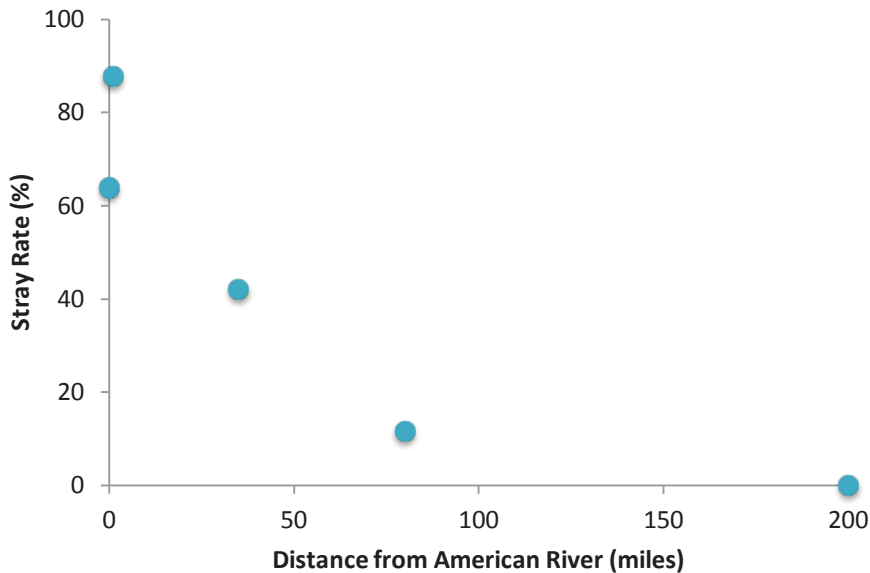


FIGURE 3. —Stray rate of adult 2006 brood-year late-fall-run Chinook salmon (*Oncorhynchus tshawytscha*) from the Coleman National Fish Hatchery into the American River versus distance of release site from the American River collected between 2008 and 2011. There is a significant negative relationship; stray rates decrease as distance of release site from the American River increases.

TABLE 6.—Brood-year 2006 total count of inland late-fall run Chinook salmon (*Oncorhynchus tshawytscha*) adult returns, excluding fish caught in the fresh water fishery. NFH = National Fish Hatchery.

Location	Number Released	Number Returned	Percent Returned
Discovery Park	52,948	122	0.23
West Sacramento	67,500	139	0.21
Ryde Koket	71,853	162	0.23
Benicia	24,099	94	0.39
Coleman NFH	854,496	5,970	0.70
Total	1,070,896	6,487	0.61

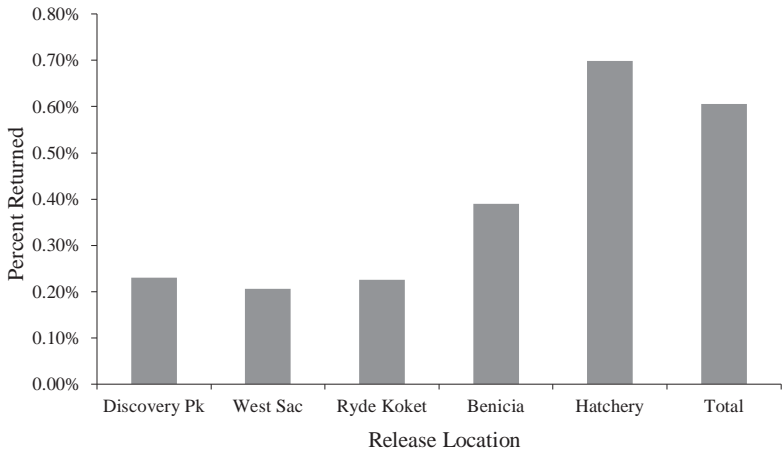


FIGURE 4.—Percent survival of 2006 brood-year late-fall run adult Chinook salmon (*Oncorhynchus tshawytscha*) in total and that returned by release location collected between 2008 and 2011.

TABLE 7.—Chinook salmon (*Oncorhynchus tshawytscha*) recoveries of brood-year 2006 smolts at the Central Valley Project (CVP), State Water Project (SWP), or other locations combined (Chippis Island, Sherwood Harbor, and others); NFH = National Fish Hatchery.

Recovery Location	Release location					Total
	Ryde Koket	Discovery Park	West Sacramento	Benecia	Coleman NFH	
CVP	6	10	3	0	63	82
SWP	14	16	2	0	63	95
Other	21	137	46	2	236	1442
Total	41	163	51	2	362	619

releases) that it is unlikely that loss of these tags would have a significant impact on adult return statistics in this study.

Fisheries returns.—Late-fall run Chinook salmon released at CNFH contributed proportionately more to the freshwater fishery in the Sacramento River than individuals from all downstream release groups combined ($X^2_1=9.1$, $P=0.0025$). Conversely, releases made at CNFH did not contribute proportionately more to the ocean fisheries ($X^2_1=0.038$, $P=0.85$). Releases made at CNFH and at downstream release locations contributed equally to the ocean fishery (Table 8).

TABLE 8.—Summary of 2006 brood-year ocean and freshwater late-fall run Chinook salmon (*Oncorhynchus tshawytscha*) adult catches. Percent catch is based on returns divided by total released downstream or at the Coleman National Fish Hatchery.

Release Location	Ocean Fishery	Freshwater Fishery	Percent of Catch	
			Ocean	Freshwater
Downstream	4	13	0.0018%	0.0060%
Hatchery	15	124	0.0018%	0.0145%
Total	137	19		

Finally, we found that there was no significant difference in percentage of adult returns for 2006 brood-year late-fall run Chinook salmon when comparing returns used for this straying study (adults intercepted in river escapement surveys and hatcheries only) to adult returns that included fishery returns by release location or as total returns (for all cases, $X^2_1\leq1.8$, $P\geq0.17$).

DISCUSSION

Homing and straying are natural behaviors in anadromous salmonids (Quinn 1984, Kaitala 1990, Quinn 2005). Salmonids from different watersheds stray at different rates, and different rivers have varying levels of attractiveness to returning fish (Quinn et al. 1991). Also, hatchery fish tend to stray at generally higher rates than those of natural origin (Jonsson et al. 2003). The results of this study suggest that when late-fall run Chinook salmon reared at CNFH are released at downstream locations, straying of these fish increases in the Sacramento River system, including into the lower American River. Additionally, the results suggest that the closer juvenile releases are to the mouth of the American River, the more likely they are to stray into the river as adults. It is worth noting in this regard that although releases made at Discovery Park had a lower stray rate than those made at West Sacramento, the proximity of the two locations is very close (within about 1.6 river km). Thus, the distance between these two locations may not have been a significant factor in the difference between their corresponding stray rates (Table 5).

Fish released near the mouths of other Sacramento River tributaries might also be more likely to stray into those rivers as well; however, there were no data to test this assumption. Because this study did not include downstream release locations between CNFH and the American River, it is unknown if stray rates would be similarly high in such instances. There is possibly a release distance upstream at which stray rates into the American River would also be high before dropping off and approaching stray rates associated with releases made at CNFH, unless this distance puts the fish in proximity of other potentially attractive tributaries.

Notably, none of the 2006 brood-year late-fall run Chinook salmon strays detected in the American River originated from releases made in proximity to CNFH. It is possible that some may have strayed there, but were undetected. However, given that adult returns from releases made at CNFH numbered more than 11 times the number of adult returns from releases made at downstream locations, the data strongly support the hypothesis that downstream releases increase straying of late-fall run Chinook salmon into the American River.

Downstream releases of hatchery-origin Chinook salmon will likely continue, to some extent or another, in the Sacramento River system, particularly if downstream net-pen releases yield high returns for ocean fisheries (Kormos et al. 2012), and in light of degraded water quality and habitat conditions, including drought effects, water diversions, and predation by introduced species, in the Sacramento-San Joaquin River and Delta system. As these practices continue in future, we recommend that releases not occur near the mouth of the American River. Results from this study suggest that releases should be made some distance, to be determined by further study, above the American River and away from other tributaries, or farther downstream in the system, as is feasible. We also recommend that all individuals in downstream release groups be marked and tagged, and that adult returns be scrutinized based on release location. This approach would be consistent with current CDFW protocols for releases of hatchery-produced fall-run Chinook salmon used for enhancement purposes or field experiments; 100% of fall-run Chinook salmon are uniquely tagged from Feather River and Mokelumne River Hatchery enhancement programs (K. Shaffer, California Department of Fish and Wildlife, personal communication). If only a portion of the downstream releases of late-fall run Chinook salmon observed in this study had been marked and coded-wire tagged, stray rates would likely have been greatly underestimated, or these strays may have gone undetected entirely.

Results of this study do not support the use of downstream releases to increase escapement of late-fall run Chinook salmon. Escapement did not increase for late-fall run Chinook salmon from any of the downstream release groups. The absence of increased survival among Chinook salmon released downstream as compared to those released at CNFH could have been due to several factors such as environmental conditions, handling and release methods, trucking practices, holding pens or practices, release locations, water quality conditions, entrainment, and predation. Associated smolt recoveries were very low in the Delta. Recoveries at the State and Federal water project facilities were also low for this particular cohort. However, it is not known what percentage of coded-wire tags from fishes as small as late-fall run Chinook salmon smolts are not recoverable at these facilities.

There are indications that the reduction of intraspecific competition between juveniles of hatchery and natural origin could reduce the impact of hatchery stock on natural-origin Pacific salmon (Nickelson 2003, Reese et al. 2009). With this in mind, there may be

some value to using downstream releases to spatially and temporally minimize interactions between hatchery- and natural-origin juvenile Chinook salmon. Also, coded-wire tag results for fall-run Chinook salmon suggest that net-pen releases into the San Francisco Bay estuary can enhance ocean fisheries (Kormos et al. 2012, Palmer-Zwahlen and Kormos 2013).

Challenges encountered.—The greatest challenge with this study was that the extended escapement surveys on the lower American River covered only a single cohort, brood-year 2006, which had multiple downstream release groups as smolts. Downstream releases of late-fall run Chinook salmon were conducted in other years, but carcass surveys on the lower American River were not extended to recover those strays in all corresponding return years. Also, during the winter of 2010–2011, high flows washed out nearly the entire carcass survey season for both fall and late-fall runs on the American River. If the carcass survey had been successfully conducted during that additional recovery season for the 2006 brood year, it is foreseeable that the overall stray rate of 2006 brood-year late-fall run Chinook salmon into the American River might have been higher than was estimated in this study.

Another challenge, and indication for further study, is that the 2006 brood-year may have been an outlier, as evidenced by the fact that the brood year's returns to the American River attracted attention that apparently other brood year returns, from which downstream releases were conducted, did not. The 2007 brood-year of late-fall run Chinook salmon at CNFH also had downstream releases, and some strayed into the lower American River, but did not instigate extended carcass surveys by CDFW. High stray rates may have also been due to conditions in the river. Timing of releases and river conditions could have caused smolts to imprint unusually strongly to the American River, or strong attraction flows could have affected adult immigration when upstream migrating Chinook salmon adults were passing the American River.

Methods used during the extended period of the escapement surveys on the lower American River were another challenge encountered in this study. Standardized protocols of mark-and-recapture for abundance estimation were used. Heads were collected for coded-wire tag recovery, but carcasses were not marked for recapture later. Consequently, it was not possible to apply any of the expansion models that are generally used to estimate escapement from mark-and-recapture carcass survey data (Bergman et al. 2012) to estimate the total number of 2006 brood-year late-fall run Chinook salmon that strayed into the lower American River. Instead, we were relegated to using only the actual, raw return numbers, which underrepresent the number of strays in the lower American River.

Implications of increased straying into the lower American River.—There are potential problems with late-fall run Chinook salmon spawning in the American River. Strays may excavate or superimpose their redds on redds of fall-run Chinook salmon that have completed spawning. There may also be competition between the juveniles of each run in the river (Reese et al. 2009), although late-fall run juveniles would be smaller and theoretically less competitive than the older and larger fall-run juveniles. Spatial separation between the runs could possibly exist if the entire historical spawning habitat for Chinook salmon on the American River was still available. However, it is not known if there was an historic late-fall run of Chinook salmon on the American River (Williams 2001).

Some potential problems of straying might be mitigated by factors related to the life history of the two runs. Isolation between the runs occurring during the juvenile life stages could reduce potential impacts of late-fall run Chinook salmon on fall-run Chinook salmon. Fall-run Chinook salmon in the Central Valley are considered ocean-type, which

rear in the river for a short time (days to a few months) before emigrating to the Pacific Ocean, and late-fall run Chinook salmon are considered river or stream-type, and rear in the freshwater environment for a longer period of time (up to one year) before emigration (Fisher 1994, Burke 2004). Late-fall run Chinook salmon may not persist, because summer water temperatures in the lower American River typically exceed the thermal preference of juvenile Chinook salmon. Therefore, juvenile late-fall run Chinook salmon survival might be very low (R. Titus, California Department of Fish and Wildlife, personal communication). If juveniles of both runs are present together, they may or may not be competing for resources but may be occupying slightly different niches in the habitat. Smaller late-fall run Chinook salmon might even deflect predation from juvenile fall-run Chinook salmon (Reese et al. 2009).

Additional recommendations.—Analysis of late-fall run Chinook salmon spawning returns from past downstream releases of different brood years should be conducted. All future downstream release groups should be monitored for adult returns to Central Valley anadromous salmonid hatcheries. In-river surveys for late-fall run Chinook salmon should be conducted on the lower American River to determine if the 2006 brood year was an outlier, and to gain a better idea of straying patterns in the river and across the Sacramento River Basin.

Late spawning season surveys should be conducted on the American River for spawned, unmarked late-fall run Chinook salmon. Data collected should include tissue samples for genetic analysis to help determine stock origin, scales for aging, and otoliths for aging and micro-chemical analysis that may yield watershed origin and migratory history of the fish. These data would provide information on stock composition of Chinook salmon spawning in the American River, including if there are offspring or spawning adults of late-fall run Chinook salmon from CNFH.

Even if 2006 was an unusual brood year, this study documented straying trends and advises fisheries management to be cautious when using downstream release programs for late-fall or other runs of hatchery-produced Chinook salmon in the Sacramento River Basin, particularly keeping in mind potential effects upon the fall-run Chinook salmon that spawn in the American River.

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Investigation of the relationship between physical habitat and salmonid abundance in two coastal northern California streams

SEAN P. GALLAGHER*, JOE FERREIRA, EMILY LANG, WENDY HOLLOWAY, AND DAVID W. WRIGHT

California Department of Fish and Wildlife, 32330 North Harbor Drive, Fort Bragg, CA 95437, USA (SPG)

California Department of Fish and Wildlife, 830 S Street, Sacramento, CA 95822, USA (JF)

Campbell Global Management, LLC, PO Box 1228, Fort Bragg, CA 95437, USA (DWW, EL)

Pacific States Marine Fisheries Commission, 32330 North Harbor Drive, Fort Bragg, CA 95437, USA (WH)

**Correspondent: sean.gallagher@wildlife.ca.gov*

Understanding the relationship between fish abundance and stream habitat variables is critical to designing and implementing effective freshwater habitat restoration projects for coho salmon (*Oncorhynchus kisutch*) and other anadromous salmonids. In this study, we investigated the relationship between summer coho salmon and steelhead trout (*O. mykiss*) parr abundance and physical stream habitat variables in Caspar and Pudding creeks in Mendocino County, California. Relationships between summer habitat and juvenile abundance were investigated using a stratified random experimental design. Our hypothesis was that one or more of the habitat unit types and variables examined would be associated with salmonid abundance. Habitat differences were examined between the two streams, and we tested our hypotheses regarding habitat variables and salmonid abundance using a variety of statistical tools that included two-way ANOVA, factor analysis, and negative binomial regression modeling. The results indicated that juvenile coho salmon abundance was positively (proportionally) associated with slow water, water volume, and dry large-wood abundance, and negatively associated with fast-water habitat variables. Young-of-the-year steelhead trout were positively associated with water volume and dry large-wood and negatively (or inversely) associated with overhead vegetation and fast water habitats. Older age steelhead abundance was positively associated with slow water, water

volume; cover habitat formed by wet and dry wood, and undercut banks. We discuss our findings relative to the use of large wood in anadromous salmonid habitat recovery programs in California coastal watersheds.

Key words: Coho salmon, habitat relationships, large wood, *Oncorhynchus kisutch*, *Oncorhynchus mykiss*, restoration, steelhead trout

Understanding relationships between fish abundance and stream habitat is important for designing and implementing freshwater habitat restoration projects that improve conditions for fish (Roni and Beechie 2013, Bennett et al. in press). A great deal of study has been directed at understanding habitat requirements for salmonids (Bjorn and Reiser 1991), especially those related to depth and velocity for stream flow evaluations (Bovee 1986). Early works directed at understanding fish habitat requirements were primarily observational (Chapman and Bjorn 1969, Fausch 1993). A number of studies have found correlations between habitat classifications (unit types) and salmonid abundance (Swales et al. 1986, Bisson et al. 1988, Nickelson et al. 1992, Lau 1994, Kruzic et al. 2001, Sharma and Hilborn 2001, CDWR 2004), while others have shown correlations between fish abundance and differing levels of depth, velocity, and complex instream and riparian cover (Butler and Hawthorne 1968, Everest and Chapman 1972, Shrivel 1990, Sutton and Soto 2010). These observations have been supported by field and laboratory experimentation (Bustard and Narver 1972, McMahon and Hartman 1989, Fausch 1993, Kruzic et al. 2001). Few studies have attempted to determine if individual habitat variables are related to fish abundance using multivariate approaches (Kratzer and Warren 2013).

Introducing large wood to improve instream habitat for Pacific salmon (*Oncorhynchus* spp.) and steelhead trout (*O. mykiss*) was suggested as part of the recovery strategy for California's coastal coho salmon (*O. kisutch*) (CDFG 2004; NMFS 2013a, b) and steelhead trout (NMFS 2007, 2013c). Following intensive logging, road building, and instream disturbance (Burns 1971, 1972), both Caspar and Pudding creeks experienced large wood removal during the 1970's and 1980's (Allan Grass, CDFW, personal communication). For these reasons, large wood density and abundance is low throughout the Mendocino coast region (Carah et al. 2014).

Solazzi et al. (2000) provided evidence that addition of large wood significantly increased steelhead trout habitat and abundance during summer in two coastal streams in Oregon. Johnson et al. (2005) found that addition of large wood significantly increased coho salmon summer habitat and freshwater survival in Tenmile Creek, a coastal tributary in Oregon. There is also evidence suggesting that a lack of winter habitat may limit coho salmon production in coastal streams (Nickelson et al. 1992). Overwinter habitat limits coho salmon survival in Pudding and Caspar creeks (Gallagher et al. 2012) and summer is the lowest growth season for salmonids in Pudding Creek (Wright et al. 2012).

During the summer of 2013, physical habitat and salmonid abundance data were collected as part of a multiyear before-after-control-impact experiment (Stewart-Oaten et al. 1986) designed to determine if adding large wood to over 80% of the spawning and rearing habitat of a treatment stream (i.e., Pudding Creek) will increase summer and winter stream habitat and improve abundance, growth, and survival of salmonids relative to a control stream (i.e., Caspar Creek). Summer habitat and salmon abundance data were collected in a

stratified random experimental design for the purpose of investigating habitat differences and similarities between the two study streams, estimating salmonid abundance, and examining relationships between salmonid abundance and freshwater habitat variables. This is the first study of its kind in California, similar to studies conducted in Oregon, to evaluate the effect of appreciably increasing instream wood to improve habitat condition (i.e., over-summering and over-wintering habitat) and fish abundance.

The purpose of this study was to evaluate relationships between coho salmon and steelhead summer parr abundance and physical stream habitat variables in Caspar and Pudding creeks, Mendocino County, California using multivariate analyses. We hypothesized that one or more of the nine habitat unit types (e.g., cascade, dam pool, plunge pool, riffle, etc.) and some assortment of the 29 habitat variables (e.g., water depth, unit area, percent cover or substrate, etc.) examined would be associated with salmonid abundance (Table 1). We tested our hypothesis that some collection of habitat variables would be associated with salmonid abundance with two-way Analysis of Variance (ANOVA) and negative binomial regression modeling. We conclude with a discussion of whether or not our findings support the supposition that salmonid abundance will increase by addition of large wood to streams.

TABLE 1.—Detailed habitat variables collected in each selected unit in Caspar and Pudding creeks, Mendocino County, California, during summer 2013.

Habitat Unit Type	Percent Fish Cover	Substrate Composition	Measured Unit Variables	Calculated Unit Variables
Cascade ^a	Aquatic Vegetation	Bedrock	Mean Depth	Residual Pool Depth ^b
Dam Pool ^a	Artificial Structures ^a	Boulders	Bankfull Width	Residual Pool Volume ^b
Dry Units ^a	Dead Woody Debris	Cobbles	Length	Unit Surface Area
Falls ^a	Live Overhanging Vegetation	Course Gravel	Maximum Depth ^b	Unit Volume
Non-turbulent	No Cover	Fine Gravel	Tail Crest Depth ^b	Dry LWD ^c Abundance
Off Channel	Undercut Banks	Fines	Width	Wet LWD Abundance
Plunge Pool		Sand		Dry LWD Density
Rapid ^a		Fines< 2 mm ^b		Wet LWD Density
Riffle		Fines 2-6 mm ^b		
Scour pool				

^aFew or none encountered; ^bPools only; ^cLarge Woody Debris

MATERIALS AND METHODS

Physical habitat.—A habitat survey was conducted in July 2013 throughout the anadromous fish habitat in both Pudding and Caspar creeks. During the survey, field staff classified all mesohabitat unit types and collected detailed information on habitat attributes in association with individual units (Table 1). Habitat data were collected in accordance with the Columbia Habitat Monitoring Protocol (CHaMP) (Bouwes et al. 2012), as modified by Holloway et al. (2013). Habitat attributes included unit type, fish cover, substrate composition, depth, wetted length and width, volume, area, and large wood abundance (Table 1). Bouwes et al. (2012) fully describes habitat attributes collected in this study. Due to logistical constraints, all physical habitat variables could not be collected in every unit.

Basic dimensions were measured in every habitat unit, and a systematic sample within the habitat census was used to select habitat units in which additional measures were collected in both streams. These detailed attributes were collected in the first and every 10th habitat unit for each of nine types (Bouwes et al. 2012). To further assess differences between the study streams we evaluated gradient, sinuosity, alkalinity, and stream flow data generated from more detailed CHuMP surveys conducted in August of 2013 in five randomly selected sites in Pudding Creek and four sites in Caspar Creek.

Salmonid abundance.—Salmonid abundance surveys were conducted in a spatially balanced, systematic sample of the units selected for additional measures during the survey. An existing Generalized Random Tessellation Sampling (GRTS) design, developed for regional spawning ground surveys (Gallagher et al. 2013), was employed. Salmonid sampling was conducted in five GRTS reaches in Caspar Creek and eight GRTS reaches in Pudding Creek (Figure 1). Three small gulches, one in Caspar Creek and two in Pudding Creek, were not included due to intermittent summer stream flows. To achieve a balanced design for evaluating fish-habitat relationships, 10 samples of each of the five primary habitat unit types (scour pool, plunge pool, riffle, non-turbulent, and off-channel) were selected in each stream. Dam pools, cascades, and rapid unit types were not included due to their rarity in both streams. Salmonid sampling was conducted in the 10th additional attribute unit of each unit type in each GRTS reach. To achieve the desired number of units, the 30th unit was also sampled in all five GRTS reaches in Caspar Creek and in two randomly selected GRTS reaches in Pudding Creek. Because selecting each 10th unit would not provide the desired 10 plunge pools or off-channel units in either stream, we randomly selected 10 of each of these unit types from the collection of all plunge pools and off-channel units in each stream.

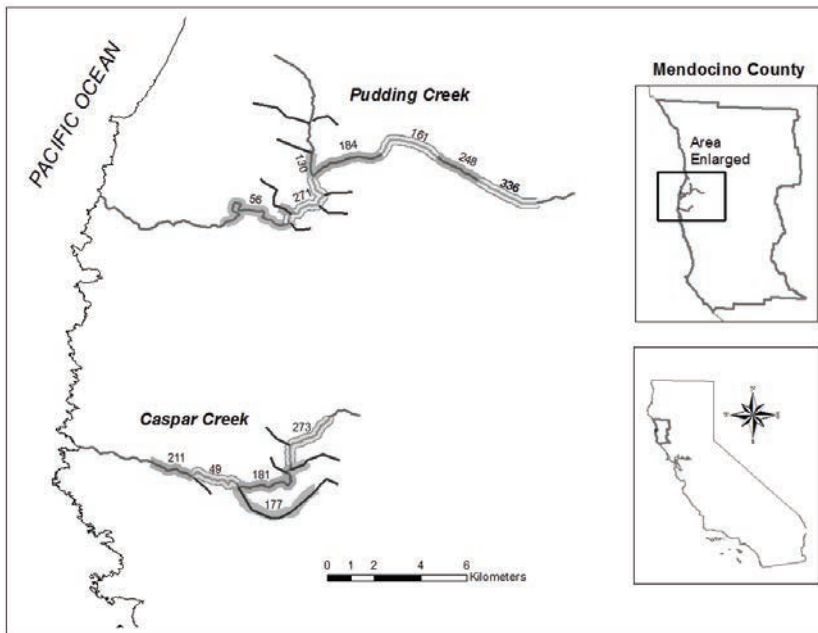


FIGURE 1.—Location of Caspar Creek and Pudding Creek in Mendocino County, California. Numbers are Generalized Random Tessellation Stratified reaches. The thin lines are stream areas that were not sampled.

Juvenile salmon abundance was estimated by depletion electrofishing in all units less than 1.2 m deep, and by snorkeling in units that exceeded 1.2 m of depth. All selected units were surveyed in July (summer) and again in October (fall) of 2013. Abundance estimates were generated for both summer and fall coho salmon juvenile (parr) and steelhead trout young-of-the-year (YoY), year old (Y+), and two-year and older fish (Y++) in each selected habitat unit (Holloway and Gallagher 2013). Steelhead trout age classes were based on fork length; fish <70 mm were considered YoY, fish between 70 mm and 120 mm were considered Y+, and fish > 120 mm were considered Y++ (Neillands 2003). All captured fish were anesthetized using tricaine methane sulfonate (MS-222), examined for previous marks, weighed, and measured.

Statistical analysis.—The habitat sampling in each selected unit resulted in 23 variables, of which 6 were calculated (Table 1). In pool habitats, we collected data for an additional four, and calculated another two, variables. Bouwes et al. (2012) directs collection of large wood data in a detailed matrix of 32 wet and dry large wood categories. For this analysis, all wood >0.1 m diameter and >3 m length was combined into total dry and total wet large wood for each unit. Unit length, width, and depth measurements were made during the habitat census and again on the day the units were sampled for fish abundance. These measurements were used to calculate unit area, volume, large wood density, residual pool depth, and residual pool volume. The habitat data from 20 replicates of the five predominate unit types in each stream were tested using a two-way Analysis of Variance (ANOVA) design to examine differences in fish habitat variables between streams (factor one) and habitat unit type (factor two). We calculated Shannon's index (H') of habitat diversity in the two creeks following Brower and Zar (1984).

Coho salmon and steelhead trout abundance was estimated in each selected unit from depletion electrofishing using the jackknife estimator (Pollock and Otto 1983). For snorkeled units, we used the method of bounded counts to estimate salmonid abundance (Regier and Robson 1967). Unit abundance and total length of stream was then used to estimate total abundance for each stream (Sarndal et al. 1992). Fish density was computed using unit length, width, and depth measurements collected during salmonid abundance surveys. Similar to the habitat evaluation, a balanced two-factor ANOVA was used to examine differences in habitat variables (Table 1), fish density and abundance between habitat unit types and streams. Significant differences found via the ANOVA tests were followed with post-hoc test based on Tukey's all pairwise comparisons to identify specific significant differences at $p < 0.05$ (Glantz 1997).

A negative binomial regression approach was conducted to evaluate relationships between fish abundance and physical habitat variables (Zuur et al. 2009). The habitat data in Table 1 included a large number of variables that were found to be highly correlated. While not an explicit, required assumption of regression, collinearity in multiple regression is a problem because regression evaluates the importance of each variable based on its marginal (or unique) contributions to the dependent variable. When variables are highly collinear, this implies that they are somewhat redundant and thus can cause the coefficients to be unstable, this can create a cancellation effect leading to the variables incorrectly being found insignificant. The first action to address the high correlation among the independent variables was to remove measured variables and substitute them with their corresponding calculated variables. Thus, the original data set was reduced from 28 to 17 variables (Table 2). This reduced variable set indicated that multi-collinearity remained present.

TABLE 2.—Reduced data set of variables used in factor analysis to evaluate relationships between salmonid abundance and physical stream habitat in Caspar and Pudding creeks, Mendocino County, California, summer 2013.

Unit	Stream	Fish Cover	Substrate ^a	Large Woody Debris
Abundance ^b	Caspar Creek	Aquatic Vegetation	Bedrock	Abundance of Dry
Type	Pudding Creek	Live Overhead Vegetation	Boulder	Abundance of Wet
Volume		No Cover	Course Gravels	
		Overhead Dead Wood	Fine Gravels	
		Undercut Banks	Sand	
			Fines	

^aPercent; ^bDependent variable: Coho Salmon, Steelhead YOY, Steelhead Y+, or Steelhead Y++

Factor analysis (FA) is one strategy that can help address multi-collinearity (Williams et al. 2010). FA is a dimension reducing scheme that finds linear combinations of the independent variables representing latent (i.e. underlying) factors. A benefit of FA is that it does not eliminate variables, but results in a variable set with lower dimensions. This produces a reduced data set for use in linear modeling that still contains the original components. Furthermore, by choosing a varimax rotation, FA finds factors that are independent of each other, thereby reducing the multi-collinearity effect (Abdi 2003). Formally, varimax searches for a rotation (i.e., a linear combination) of the original factors such that the variance of the loadings is maximized. In other words, the FA retains all the variables but compresses them into common chunks that yield independent component factor scores necessary for negative binomial regression modeling. The optimum number of factors was determined as those factors that explained $\geq 70\%$ of the variation in the original variable set, based on principle components analysis. In FA, the factors represent constructs (linear combinations) of all the variables with the highest loadings (absolute correlations between the factors and the variables) helping to define the factors. An absolute correlation (or loading) threshold of 0.3 was selected to identify the variables defining each factor. Studying the variable loadings for the factors helped derive meaningful names for each factor. These factors, along with the original response, became the new basis to determine relationships between habitat (independent variables) and fish abundance (response or dependent variable).

The final FA results found that the total variation explained was low. Furthermore, the factors yielded asymmetrical distributions. To address this new issue, we had to conduct an additional statistical revision; the factors were natural log transformed. Since zeros were present, prior to log transforming, a constant was added to the factors. The transformations were found to improve the amount of total variation explained (Chi-square > 0.10). Since the response variable (i.e., fish abundance) was a count type variable, Poisson regression was used to gain understanding of the relationship between abundance and habitat factors. However, due to excessive zeroes in the response variable, a negative binomial regression approach was used to understand the relationship between abundance and the habitat factors (Zuur et al. 2009). Excessive zeros inflate the variance and the negative binomial is one approach than can deal with such a situation. All statistical analyses were performed in program R (R Development Core Team, <http://www.r-project.org/>). Statistical significance was accepted at $P < 0.05$.

RESULTS

Physical habitat.—We observed only two cascade and dam pool units in Caspar Creek, and three rapid units in Caspar Creek and one in Pudding Creek. There were a total of 34 dry units in both streams in summer 2013. These unit types were not sampled for fish density or included in further analysis. In both streams, the predominant habitat types were scour pools, riffles, and non-turbulent units (e.g. runs). The frequency of habitat types was not different between the two streams (Figure 2); both streams had similarly low proportions of off-channel and plunge pool habitat types. Habitat diversity in Caspar Creek ($H' = 0.50$) was nearly identical to that of Pudding Creek ($H' = 0.51$).

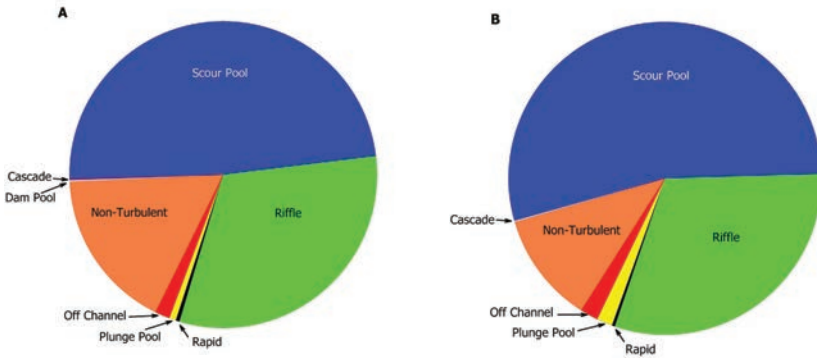


FIGURE 2.—Proportion of habitat unit types in (a) Caspar Creek (a) and (b) Pudding Creek, Mendocino County, California, during the summer of 2013. There were no dam pools in Pudding Creek.

As we expected, many of the habitat variables differed among habitat units (Table 3). Generally, units associated with moving water had higher percentages of coarse stream substrate than units associated with slow water. The percentage of fine sediment was highest in slow water units and decreased with higher velocity unit types (e.g. off channel > pool > non-turbulent > riffle). Slow water units generally had more overhead fish cover than did fast water units. Both plunge pools and scour pools had more undercut banks than other unit types. Plunge pools were deeper, had more volume, and had higher percentages of pool tail fine substrate than scour pools. And pools were deeper and had more volume than off channel units, which were deeper and had more volume than non-turbulent units. Riffles were the shallowest units with the lowest volume.

Eleven of the 29 (38%) variables we examined with ANOVA were significantly different between the two creeks in summer 2013 (Table 3). Notably, cover variables and large wood density and large wood abundance were not different between the two streams. The number of pieces of wet large wood/100 m averaged 21.76 ($SE = 5.06$) in Caspar Creek and averaged 28.17 ($SE = 6.90$) in Pudding Creek. Pudding Creek is a longer stream with a larger drainage area than Caspar Creek and, thus, had more surface area and volume of fish habitat. Pools were deeper and had more residual pool volume in Pudding Creek than they did in Caspar Creek. Caspar Creek had more boulder and cobble substrate than Pudding Creek; whereas Pudding Creek had more coarse gravel and sand substrate and more fines in pool tails than did Caspar Creek.

TABLE 3.—Results of two-factor ANOVA between stream, habitat unit type, and habitat variables. NS = not significant; NT = non-turbulent; OC = off channel; PP = plunge pool; RI = riffle; and SP = scour pool. Degrees of freedom for the *F* statistic are 4, 401. Caspar and Pudding creeks, Mendocino County, California, summer 2013.

Habitat Category	Variable	Significant Differences			Group Differences	
		Unit	Stream	Interaction	Habitat Types	Stream Differences ^f
Substrate	Bedrock	NS	NS	NS	None	None
	Boulders	NS	<i>F</i> =10.4; <i>P</i> =0.002	NS	None	C=3.2, <i>P</i> =1.3
	Cobbles	<i>F</i> =3.6; <i>P</i> =0.007	<i>F</i> =27.3; <i>P</i> <0.001	NS	RI>OC, PP, SP	C=12.3, <i>P</i> =4.4
	Course Gravel	<i>F</i> =46.1; <i>P</i> <0.001	<i>F</i> =27.3; <i>P</i> <0.001	NS	RI>PP,SP,NT; NT>SP,PP,OC	C=26.1, <i>P</i> =32.9
	Fine Gravel	<i>F</i> =3.1; <i>P</i> =0.02	NS	NS	NT>OC	None
	Sand	<i>F</i> =20.04; <i>P</i> <0.001	<i>F</i> =6.07; <i>P</i> =0.01	NS	OC,SP>RI	C=21.2, <i>P</i> =25.1
	Fines	<i>F</i> =16.8; <i>P</i> <0.001	NS	NS	OC>PP>SP>NT>RI	None
	Fines<2 mm ^a	<i>F</i> =202; <i>P</i> <0.001	<i>F</i> =6.46; <i>P</i> =0.001	NS	PP>SP	C=13.2, <i>P</i> =22.3
	Fines 2-6 mm ^a	<i>F</i> =2.13; <i>P</i> =0.09	NS	NS	PP>SP	None
	Artificial Structure	Not observed	Not observed	Not observed	Not observed	Not observed
	Aquatic Veg.	NS	NS	NS	None	None
	DWD ^b	<i>F</i> =12.1; <i>P</i> <0.001	NS	NS	OC>NT,PP,RI,SP	None
	LOV ^c	<i>F</i> =5.67; <i>P</i> <0.001	NS	NS	OC>NT,PP,RI	None
Fish cover	No Cover	<i>F</i> =19.19; <i>P</i> <0.001	NS	NS	RI>NT,PP; OC>NT,PP,RI,SP	None
	Undercut Banks	<i>F</i> =6.86; <i>P</i> <0.001	NS	NS	SP,PP>RI,NT	None
	Average Depth	<i>F</i> =63.3; <i>P</i> <0.001	NS	NS	PP>SP>OC>NT>RI	None
Measured metrics	Bankfull Width	<i>F</i> =20.35; <i>P</i> <0.001	NS	NS	OC>NT,PP,RI,SP	C=5.7m, <i>P</i> =5.2 m
	Max. Depth ^d	NS	<i>F</i> =6.26; <i>P</i> =0.007	NS	None	C=53.3 cm, <i>P</i> =59.1 cm
	Tail Crest Depth ^e	NS	NS	NS	None	None
Calculated metrics	Residual Depth ^a	<i>F</i> =3.09; <i>P</i> =0.05	<i>F</i> =10.34; <i>P</i> =0.001	NS	PP>SP	C=40.0 cm, <i>P</i> =55.0 cm
	Residual Volume ^a	<i>F</i> =5.29; <i>P</i> <0.001	<i>F</i> =9.52; <i>P</i> =0.002	NS	PP>SP	C=16.2 m ³ , <i>P</i> =24.7 m ³
	DLWD ^d Abund.	NS	NS	NS	None	None
	WLWD ^d Abund.	<i>F</i> =4.8; <i>P</i> <0.001	NS	NS	PP,SP>NT,RI	None
	DLWD ^d Density	NS	NS	NS	None	None
	WLWD ^d Density	<i>F</i> =8.65; <i>P</i> <0.001	NS	NS	OC>NT,RI,PP; PP>NT,RI,SP	None
	Unit Volume	<i>F</i> =13.22; <i>P</i> <0.001	<i>F</i> =8.13; <i>P</i> =0.004	<i>F</i> =3.43; <i>P</i> =0.008	SP>OC,NT,PP,RI	C=4.2 m ³ , <i>P</i> =9.4 m ³
	Unit Surface	<i>F</i> =12.56; <i>P</i> <0.001	<i>F</i> =9.00; <i>P</i> <0.001	<i>F</i> =2.58; <i>P</i> =0.04	SP>NT,OC,PP,RI	C=28.8 m ³ , <i>P</i> =49.9 m ³

^aMeasured or calculated only in pool units; ^bDWD=Dead Woody Debris; ^cLOV=Live Overhanging Vegetation; ^dDLWD=Dry Large Woody Debris; ^eWLWD= Wet Large Woody Debris; ^fC=Caspar Creek, *P*=Pudding Creek

The average gradient of Caspar Creek (0.40, *SE* = 0.13) was not significantly different from that of Pudding Creek (0.69, *SE* = 0.15). Caspar Creek’s average sinuosity (1.16, *SE* = 0.02) was not different than Pudding Creek’s (1.38, *SE* = 0.20). Both streams had average summer daily mean water temperatures between 11°C and 16°C. Caspar Creek’s average alkalinity of 167 (*SE* = 6.3) and average conductivity of 52 (*SE* = 10.4) was similar to Pudding Creek (250, *SE* =55.2, 64 *SE* = 9.8, respectively). Stream flows during summer 2013 were less than 1 cfs in both streams.

Salmonid abundance.—Coho salmon abundance differed among habitat units in both summer and fall and was significantly higher in pools than in off-channel units and riffles (Table 4) and not different among the other unit types examined. Steelhead trout YoY were more abundant in non-turbulent units and scour pools (fall only) than in plunge pools and off-channel units in both summer and fall. Similarly, steelhead trout Y+ abundance was significantly higher in scour pools than in the other unit types during summer and fall. Older steelhead trout (Y++) abundance was significantly higher in scour pools and plunge pools (fall only) than in the other unit types during both seasons.

Coho salmon and steelhead trout YoY and Y+ density was not significantly different among habitat unit types in summer 2013. In fall, coho salmon density was significantly higher in plunge pools than it was in riffles and off channels, whereas steelhead trout YoY and Y+ density was not different among unit types during fall 2013. The density of steelhead trout Y++ was significantly higher in plunge pools than in all other unit types during both summer and fall 2013.

TABLE 4.—Results of two-factor ANOVA between stream, habitat unit type, and salmonid abundance and density. NS = not significant; NT = non-turbulent; OC = off channel; PP = plunge pool; RI = riffle; SP = scour pool. Degrees of freedom for the *F* statistic are 4, 401. Caspar and Pudding creeks, Mendocino County, California, summer 2013.

Season	Abundance ^a or Density ^b of Salmonids	Significant Differences			Group Differences	
		Unit	Stream	Interaction	Habitat Types	Stream ^d
Summer	Coho Salmon Parr Abu.	$F = 4.53; P = 0.0002$	$F = 18.73; P < 0.001$	$F = 2.47; P = 0.049$	SP> PP,OC,RI	C = 4.78, P = 42.82
	Coho Salmon Parr Den.	NS	$F = 38.54; P < 0.001$	NS	None	C = 0.09 m ² , P = 1.22 m ²
	Steelhead YoY Abu.	$F = 3.26; P = 0.01$	$F = 9.44; P = 0.003$	NS	NT> PP,OC ^c	C = 5.64, P = 20.18
	Steelhead YoY Den.	NS	$F = 15.37; P < 0.001$	NS	None	C = 0.14 m ² , P = 0.60 m ²
	Steelhead Y+ Abu.	$F = 6.36; P < 0.001$	NS	NS	SP> NT,PP,OC,RI	None
	Steelhead Y+ Den.	NS	$F = 5.06; P = 0.02$	NS	None	C = 0.04m ² , P = 0.08 m ²
	Steelhead Y++ Abu.	$F = 8.31; P < 0.001$	NS	NS	PP, SP> NT,OC,RI	None
	Steelhead Y++ Den.	$F = 4.92; P < 0.001$	NS	NS	PP> NT,OC,SP,RI	None
Fall	Coho Salmon Parr Abu.	$F = 4.79; P = 0.001$	$F = 16.65; P < 0.001$	$F = 2.69; P = 0.03$	SP>OC,RI	C = 3.60, P = 28.90
	Coho Salmon Parr Den.	$F = 5.62; P < 0.001$	$F = 122.1; P < 0.001$	$F = 3.33; P = 0.006$	PP>OC,RI	C = 0.06m ² , P = 0.70 m ²
	Steelhead YoY Abu.	$F = 4.01; P = 0.005$	NS	NS	NT,SP>PP,OC.	None
	Steelhead YoY Den.	NS	NS	NS	None	None
	Steelhead Y+ Abu.	$F = 7.64; P < 0.001$	NS	NS	SP> NT,PP,OC,RI	None
	Steelhead Y+ Den.	NS	NS	NS	None	None
	Steelhead Y++ Abu.	$F = 5.35; P < 0.001$	NS	NS	SP> NT,OC,RI	None
	Steelhead Y++ Den.	$F = 5.72; P < 0.001$	NS	NS	PP> NT,OC,SP,RI	None

^aAbu.=Abundance; ^bDen.=Density; ^cTukeys pairwise comparison $p < 0.10$; ^dC=Caspar Creek, P=Pudding Creek

In summer and fall 2013, coho salmon abundance and density were higher in Pudding Creek than in Caspar Creek (Table 4). Steelhead trout YoY abundance and density were also significantly higher in Pudding Creek than in Caspar Creek during summer, but not in fall 2013. Older age steelhead trout abundance was not different between the two streams in either season. However, steelhead trout Y+ density was significantly higher in Pudding Creek than in Caspar Creek during summer 2013. The ANOVAs indicated a significant interaction between stream and habitat type for summer and fall coho abundance and fall coho salmon density (Table 4). The interaction for coho salmon abundance and density was due to differences in riffles between the two streams (Figure 3). During summer and fall 2013, only a few coho salmon were captured in two riffles in Caspar Creek, whereas coho salmon were captured in all riffles in Pudding Creek.

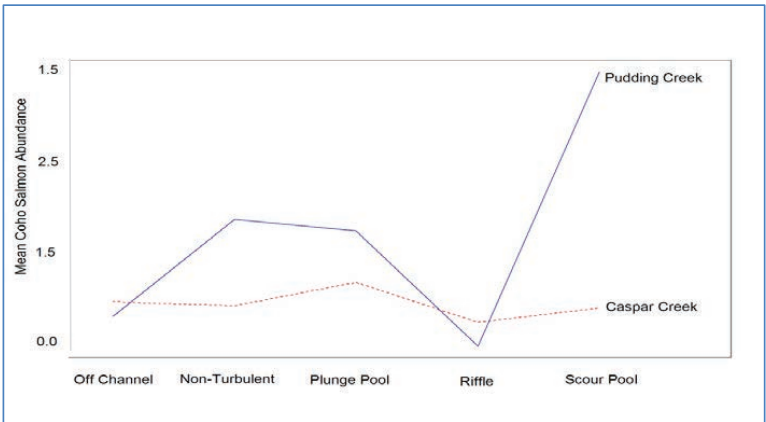


FIGURE 3.—Interaction plot of mean coho salmon (*Oncorhynchus kisutch*) abundance and habitat unit type in Caspar Creek and Pudding Creek, Mendocino County, California summer 2013.

Coho salmon and steelhead trout total abundance was significantly higher in Pudding Creek than in Caspar Creek during summer and fall 2013. The estimated summer coho salmon abundance in Pudding Creek of 83,306 (95% *CI*, 57,452-107,161) was 13.2 times higher than the 6,306 (95% *CI*, 2,635-9,975) estimated in Caspar Creek. The large difference in stream abundance was similar (13.9 times higher) between the two creeks in fall. In Pudding Creek, we estimated 61,353 (95% *CI*, 43,301-79,905), and in Caspar Creek, we estimated 4,393 (95% *CI*, 960-7,825) coho salmon. During summer 2013, there were five times as many steelhead trout YoY in Pudding Creek (42,335: 95% *CI*, 27,445-57,275) than the estimate of 8,471 (95% *CI*, 4,675-12,267) in Caspar Creek. In fall 2013, there were twice as many steelhead trout YoY in Pudding Creek, where we estimated 10,454 (95% *CI*, 6,709-14,200) steelhead trout YoY versus 5,145 (95% *CI*, 2,879-7,412) in Caspar Creek. Steelhead trout Y+ and Y++ were between 1.7-2.6 times more abundant in Pudding Creek than in Caspar Creek during summer and fall 2013, respectively.

Relationships between salmonid parr abundance and fish habitat.—Factor analysis on 17 salmonid habitat variables (Table 2, excluding unit abundance) revealed seven significant factors (Chi-square 42.24, *df* = 38, *P*=0.29) accounting for >56% of the variation in the data set (Table 5). Based on examination of the variables that were highly correlated (*r*>0.30) to each of the factor loadings (these define the factors), two factors were associated with cover, two were associated with volume, three were associated with wood, one was related to slow water, and two were related to fast water. Three of the 17 habitat variables (i.e., aquatic vegetation cover, percent bedrock, and unit type) were not found to have significant loadings in any of the seven factors. All of the 14 habitat variables, that were important loadings for the seven factors, contributed significantly to one or more of the factors (Table 5).

TABLE 5.—Factor names, factor loadings (variables), and loading coefficients (>0.30) resulting from factor analysis of 17 salmon stream habitat variables. Bold font indicates statistically significant loading coefficients for each factor. Caspar and Pudding creeks, Mendocino County, California, summer 2013.

Variable	Factor Names						
	VDLW ^a	Wood	OV ^b	TWSDLW ^c	SWV ^d	FW ^e	UB ^f
Bedrock				0.59			
Boulders				0.89			
Cobbles							
Coarse Gravels					-0.38	-0.74	
Fine Gravels						-0.46	
Sand					0.96		
Fines						0.64	
Large Wood Wet		0.75					
Large Wood Dry	0.31	0.47		0.34			
Overhead Vegetation Cover			0.76				
Overhead Wood Cover		0.72					
Aquatic Vegetation Cover							
Undercut Banks							0.98
No Cover		-0.43	-0.86				
Unit Type							
Unit Volume	0.79				0.32		
Stream				0.33			

^aVolume and dry large wood; ^bOverhead vegetation; ^cTurbulent water stream and large dry wood; ^dSlow water volume; ^eFast water; ^fUndercut banks

The negative binomial regression modeling of the scores of the seven habitat factors and coho salmon unit abundance revealed that three factors were significant for predicting coho salmon abundance ($z>3.12$, $P<0.001$). Coho salmon were positively associated with volume, slow water, and dry large wood, and negatively associated with fast water (Tables 5 and 6). Overhead vegetation cover, undercut banks, and wood were not important factors for predicting coho salmon abundance. Steelhead trout abundance was significantly associated with all seven factors ($z>2.17$, $P<0.03$). Steelhead trout YoY were associated two of the same factors as coho salmon and also were associated negatively with overhead vegetation cover and turbulent water. Like coho salmon, older age steelhead trout were positively associated with volume and dry large wood. Steelhead trout Y+ and Y++ were positively associated with the factor wood. Steelhead trout Y+ were positively associated with slow water, volume, and undercut banks, and negatively associated with fast water and overhead vegetation, while steelhead trout Y++ did not have these positive or negative associations.

TABLE 6.—Habitat factors associated with salmonid abundance. Positive and negative refer to the sign of the regression coefficient for each factor that was significant for predicting salmonid abundance. NS = not significant. Caspar and Pudding creeks, Mendocino County, California, summer 2013.

Salmonid Abundance	Factor Names						
	VDLW ^a	Wood	OV ^b	TWSDLW ^c	SWV ^d	FW ^e	UB ^f
Coho Salmon	+	NS	NS	NS	+	–	NS
Steelhed YOY	+	NS	–	–	NS	–	NS
Steelhead Y+	+	+	–	–	+	–	+
Steelhead Y++	+	+	NS	NS	NS	–	NS

^aVolume and dry large wood; ^bOverhead vegetation; ^cTurbulent water stream and large dry wood; ^dSlow water volume; ^eFast water; ^fUndercut banks

DISCUSSION

The differences among habitat units fit the hydraulic and geomorphic theories underpinning the classification scheme from which they were derived. As such, it is not surprising that we found differences in physical habitat variables among unit types. Units associated with moving water had higher percentages of coarse substrate than those associated with slow water. Off-channel units had higher percent overhead cover and the least amount of “no cover” when compared to other units because they are in the riparian zone of the stream. Scour pools had the most undercut banks because the substrate degradation processes that form them are the same that create undercut banks. Pools are, by definition, deeper than the other unit types and plunge pools are deeper than scour pools because of the geomorphic and hydraulic forces that form them. Dry large wood abundance and density were not different among unit types, probably because large wood is rare in coastal California streams (Carah et al. 2014). The reason the number of pieces of large wood in the water was higher in both pool types than in riffles and non-turbulent units is likely due

to the fact that large wood is generally responsible for forming and maintaining pools, but not riffles and non-turbulent units.

Salmonid freshwater habitat was similar in Caspar and Pudding creeks during summer 2013. The percentage of habitat unit types in both streams was not different, and habitat diversity indices were nearly identical. Both streams had few dam pool, off-channel, and plunge pool units. The gradient, sinuosity, alkalinity, and conductivity of the two streams were not different, and while stream flows were very low, stream flow and water temperatures were not appreciably different. Of the physical habitat variables we examined, 38% differed among the two streams. None of the fish cover or large wood variables was different between the two streams. This is probably because overhead fish cover and large wood abundance was similar in both streams. Average total fish cover was 22.6% ($SE = 3.25\%$) in Caspar Creek and 20.3% ($SE = 3.08\%$) in Pudding Creek. Cover percentages in our study streams were higher than Justice (2007), who estimated cover values between 5%-14% in two coastal California coho salmon streams in Humboldt County. Large wood abundance averaged 21.7 ($SE = 5.05$) pieces per 100 m in Caspar Creek and 28.2 ($SE = 6.88$) pieces per 100 m in Pudding Creek. These values are much lower than the 100-800 pieces of large wood per 100 m, reported by Bilby and Ward (1989) for undisturbed streams of variable sizes in western Washington.

Of the variables that differed between the streams, many are likely not biologically meaningful and others were within our measurement error. For example, the five substrate categories differed by less than 10% (two differed by less than 5%). These categories were estimated in the field in 5% increments such that a difference of <5% may be an artifact of our field methods. The reason Caspar Creek had higher percentages of boulder and cobble substrate than Pudding Creek may be because the sediment dams in the north and south forks of Caspar Creek have been removing fine sediment as part of the State Experimental Forest's studies on sediment and logging for over 50 years (Cafferata et al. 2011). Our results suggest that Pudding Creek had more spawning substrate (i.e., coarse gravel), and that the creek may be a slower stream, as indicated by the higher percent finer substrate materials compared to Caspar Creek. It is clear that Pudding Creek was deeper and had more surface area and volume of salmonid habitat than Caspar Creek. An average difference of 15 cm in residual pool depth and 8.5 m³ in residual pool volume suggests that Pudding Creek provides a great deal more pool habitat than does Caspar Creek. These differences may help explain why Pudding Creek produces more coho salmon smolts than Caspar Creek (Gallagher et al. 2012).

It is not surprising that coho salmon were more abundant in pool habitats than in riffles and off-channel units, because it is well known that coho salmon prefer pools in summer (Bisson et al. 1988). Nickelson et al. (1992) found that coho salmon were more abundant in pools than other unit types in coastal Oregon streams during summer. Sharma and Hilborn (2001) found that watershed pool density was a good predictor of smolt density; a greater number of pools was associated with higher smolt production. Coho salmon density was not significantly associated with any habitat type in summer. In fall, as stream flows dropped and fish became more concentrated, coho salmon density was significantly higher in plunge pools than in riffles. Similar to our results, Lau (1994) found summer coho salmon density was significantly higher in pools than in riffles in Caspar Creek.

Unlike other studies (Everest and Chapman 1972, Bisson et al. 1988, CDWR 2004) that found steelhead trout prefer riffles and other high velocity areas over pools, we found that steelhead trout were significantly more abundant in pools in both summer and fall

2013 than in the other units we examined. This may be because we report fish abundance by age-class, whereas other researchers did not. Also, both Caspar and Pudding creeks are small streams with little stream flow in summer; riffles, although having moving water, did not have “high” velocities (i.e., riffle velocities were $< 0.10\text{m/s}$). In addition, 2013 was a drought year with very low summer flows. The density of YoY and Y+ steelhead trout was not different among habitat units in both summer and fall 2013. This finding corresponds with Lau (1994) who found no significant difference in steelhead trout density among habitat types in Caspar Creek. However, we found steelhead trout Y++ density was significantly higher in plunge pools than in the other unit types during both summer and fall 2013.

Coho salmon abundance and density were higher in Pudding Creek than in Caspar Creek in both summer and fall. While Pudding Creek was 25% longer than Caspar Creek and had deeper pools and more volume of stream habitat, it produced 13 times more parr. This difference is probably attributable to the fact that adult Coho salmon escapement was approximately 28.3 (95% *CI*, 14.4–53.3) times higher in Pudding Creek than in Caspar Creek during winter 2013 (Gallagher et al. 2013). Stream flows in the winter and spring of 2013 were low, so it is likely that redd scour was correspondingly low resulting in high egg-to-emergence survival in both streams. This could explain why coho salmon parr abundance in Pudding Creek during fall 2013 was well above the 2006–2013 average, even though adult escapement in 2013 (i.e., 248 coho salmon) was well below the 12 year average of 462 spawners (Gallagher et al. 2013). In Caspar Creek, escapement of coho salmon and resultant parr abundance in fall 2013 were both below the 12 year average. The magnitude of difference between proportion of spawners (28.3 times higher) and that of parr (13 times higher) in Pudding Creek is likely a result of density-dependent factors (Gallagher et al. 2012). Therefore, the difference in abundance between the two streams may be a synergy of differences in parental spawner abundance, habitat differences, and low winter and spring streamflow conditions during 2013. The difference in abundance between the two streams was also a result of the interaction of stream and habitat abundance (Figure 3), there were few coho salmon captured in riffles in Caspar Creek, whereas many riffles in Pudding Creek supported coho salmon.

Steelhead trout YoY abundance was significantly different between Caspar and Pudding creeks in summer but not during fall 2013. In contrast, steelhead trout YoY density was not significantly different between streams in either season. The reasons for the observed difference in abundance are likely similar to our explanation for coho salmon. There were approximately 4.85 times more steelhead trout adults in Pudding Creek than in Caspar Creek during winter 2013. This is similar to the difference we found between the two streams in summer steelhead trout YoY abundance. There was no difference in steelhead trout YoY abundance in fall 2013 between the two streams, as Pudding Creek only had approximately 1.8 times more fish than Caspar Creek. Apparent summer-to-fall survival of steelhead YoY was different between the two streams; it was much lower in Pudding Creek than in Caspar Creek (e.g., 0.25 vs 0.68, respectively). Steelhead trout mortality may have been due to competition with, and/or predation by, the high density of coho salmon in Pudding Creek during summer and fall 2013. That older age steelhead abundance was not different between the two streams is likely due to a lack of difference in adult escapement in the two streams in earlier years. From 2009 to 2012, steelhead trout escapement and redd estimates were not different between the two streams (Gallagher et al. 2013).

Our approach to understanding relationships between physical stream habitat and salmonid abundance differs from many previous studies in that we used a balanced sampling

design and multivariate analyses. Factor analysis and negative binomial regression modeling allowed us to evaluate 17 variables commonly collected during stream habitat evaluations (Ropper et al. 2010, Bouwes et al. 2012) and reduce them into seven composite factors. Previous studies of relationships between physical stream habitat and salmonid abundance primarily used habitat classifications as sample units and correlation for determining significant relationships. These studies all suggest that coho salmon prefer pools, and steelhead trout prefer riffles (Swales et al 1986, Bisson et al. 1988, Nickelson et al. 1992, Lau 1994, Kruzic et al. 2001, CDWR 2004). Our ANOVA results, which also used unit type as the sampling unit, support these findings for coho salmon but not for steelhead. However, factor analysis did not indicate unit type as an important variable in any of the factors.

The factor names are generalizations of the combinations of variables comprising the loadings of the factors (Table 5). In other words, all the factors are a linear combination (i.e., construct) of all the variables, but some variables within the construct are more influential. The focus is on the most influential variables within a factor. Thus, factors are latent (un-observed) that define an underlying concept made up of phenomena that we are able to measure. Three of the 17 variables did not play a significant role in any of the factors: aquatic vegetation, percent bedrock, and unit type. Because previous research identified differences in abundance and density between habitat types (discussed above), we expected habitat unit type to be an important variable loading in the factors and to be associated with salmonid abundance. Unit type was probably not important, because most of the other variables were found in all unit types and pools were deeper, more voluminous, and contained more wet large wood than other unit types. Both bedrock and aquatic vegetation cover were not important because they were rare in both streams. Of the 100 units we sampled, only three had either bedrock or aquatic vegetation.

In the field, the variable dry large wood was defined as either single pieces of wood in the bankfull channel or dry log jams within and above the channel. The factor we called *volume and dry-large-wood* was made up primarily of log jams, which generally cause scour during winter; thus, the association between volume and large wood. The factor *wood* is composed of both wet and dry large wood and overhead wood cover, whereas the factor called *overhead vegetation* is made up of vegetation within 1 m of the water surface (Bouwes et al. 2012). Shrivel (1990) defined cover objects as things that provide fish protection or shelter and cover habitat as preferred levels of velocity, depth, light intensity, reduced social interaction, and reduced predation. The factor we called *wood* potentially contains all these elements of cover habitat, the factor *overhead vegetation* only provides reduced light intensity to the aquatic habitat. Increased light intensity is thought to increase predation risk (Shrivel 1990).

We interpreted the inclusion of fines and cobbles (negative coefficient) to indicate slow water in the factor we called *slow-water volume*. Similarly, we interpreted the loadings of fines and coarse and fine gravel to indicate *fast-water* in that factor. The association between fast water and dry wood in the factor *turbulent-stream and dry large wood* is likely due to the boulder and cobble variables being significantly different between the two streams. Consequently, we assumed the large wood component is related to bankfull wood deposited during high flows in faster water areas. The use of turbulent is slightly misleading, because both streams are low gradient and had drought-caused, very low stream flows in summer 2013.

The negative binomial regression modeling showed coho salmon abundance was positively associated with factors generally attributed to pools (i.e., *slow-water volume*) and

negatively associated with factors related to riffles (i.e., *fast-water*) (Tables 5-6). Using correlation analysis, Bisson et al. (1988) found that coho salmon selected pools (i.e., deep, slow moving areas) over riffles (i.e., fast water). Kruzic et al. (2001) used multivariate analysis to show that coho salmon growth was significantly higher in pools than in riffles, which they primarily attributed to difference in water depth, a component of volume in our factor analysis. In our study, unit type was not statistically significant in any of the factors associated with coho salmon abundance, but unit volume was, probably because we found coho salmon in all habitat unit types and they were more abundant in pools compared to riffles (Table 4). Sutton and Soto (2010) found that coho salmon were congregated in cold-slow-water habitat with abundant, complex cover. Similar to Fausch (1993), we found that coho salmon were not associated with cover habitat. Young (2004) found that coho salmon occupied low-velocity pools and displaced steelhead trout into high-velocity riffles. Our results suggest that YoY steelhead trout prefer deep water areas with dry large wood and were negatively associated with fast water and overhead vegetation cover. Contrarily, Fausch (1993) found that age-0 (YoY) steelhead trout preferred areas of overhead cover. Steelhead YoY might select low-velocity areas due to metabolic needs if temperatures are high and food input limited by to low flows. However, water temperatures in both streams were below 16°C, so high temperature is not likely why YoY steelhead selected low-velocity, high-volume areas in our study.

Older age steelhead trout were associated with the factors *volume and dry-large-wood* and *wood* (Table 6). They were either not associated with (Y++), or negatively associated with (Y+), the factors *fast-water* and *turbulent-stream and dry large wood*, which are factors related to riffle habitats. Steelhead trout Y+ were also positively associated with slow water. As discussed above, this differs from other studies that found steelhead trout were primarily associated with riffles. The difference may be related to stream size; in larger streams and rivers, riffles have deeper water and larger substrate in which steelhead trout hide (Everest and Chapman 1972). Both Caspar and Pudding creeks are small streams with relatively shallow riffles. Bisson et al. (1988) found that steelhead trout preferred riffles but also used deep pools with high velocities in the center of the channel. Consistent with this finding, our results showed that steelhead trout abundance was significantly higher in pools than other unit types (Table 4), and they were associated with the factor *volume and dry-large-wood*. Unlike coho salmon and steelhead trout YoY, older steelhead trout were positively associated with the factor *wood*, and Y+ were positively associated with the factor *undercut banks*. These findings are consistent with other studies that found steelhead trout preferred both overhead and velocity cover (Butler and Hawthorne 1968, Fausch 1993).

Our results suggest that increasing low-velocity, high-volume habitat areas and decreasing high-velocity areas should provide more preferred habitat for coho salmon and steelhead trout in small coastal streams such as Pudding and Caspar creeks. It should be noted that 2013 was a drought year, conducting this study over multiple years might help elucidate if drought conditions influence habitat use by coastal salmonids. In particular, we found that plunge pools were, although rare, important for salmonids as streams dried in fall. These unit types are formed by large wood, and we anticipate an increase in this unit type resulting from large wood additions. Addition of large wood has increased habitat for salmonids and increased smolt production in most of the places it has been evaluated. Kratzer and Warren (2013) found that trout biomass could be expected to increase with increasing wood habitat in Vermont. Solazzi et al. (2000) increased salmonid habitat and smolt production by adding large wood to a coastal Oregon stream. Similarly, Johnson et

al. (2005) found increases in habitat and salmonid abundance resulting from the addition of large wood. Treating a large portion of a salmonid stream by adding large wood (Roni et al. 2010) significantly increased the low-velocity, high-volume salmonid habitats (Jones et al. 2014) in coastal Oregon. These were the habitats that we found were preferred by salmonids in coastal California. We have shown that habitat associated with, or created and maintained by, large wood had higher abundance of salmonids in Caspar and Pudding creeks. In particular, we expect large wood additions to create more low-velocity-high volume areas for coho salmon, reduce fast water areas for both species, and provide more wood and undercut bank cover for steelhead trout.

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Morphometric variation among four distinct population segments of California steelhead trout

FARHAT S. BAJJALIYA*, ROBERT G. TITUS, JOE R. FERREIRA, AND RONALD M. COLEMAN

California Department of Fish and Wildlife, Fisheries Branch, 830 S Street, Sacramento, CA 95811, USA (FSB, JRF)

California Department of Fish and Wildlife, Fisheries Branch, 8175 Alpine Avenue, Suite F, Sacramento, CA 95826, USA (RGT)

California State University, Sacramento, Department of Biological Sciences, 6000 J Street, Sacramento, CA 95819 (RGT, RMC)

**Correspondent: Farhat.Bajjaliya@wildlife.ca.gov*

Salmonid morphology can vary due to many factors including phenotypic expression in response to immediate environment, anthropogenic influences such as artificial propagation, and difficulty and distance of spawning migration. Because reproductive homing minimizes genetic interchange and promotes the maintenance of local adaptations, morphology of adult steelhead trout (*Oncorhynchus mykiss*) should be distinguishable between geographically isolated populations. The objective of this study was to compare adult steelhead trout morphometrics among four distinct population segments in California, including both coastal and inland populations groups. This study is the first to examine morphometric variation on a regional scale in California. We predicted that means of each morphometric response variable—body depth, fork length, and weight—would differ statistically by distinct population segment, sex, origin, and by the interactions of these factors. Adult steelhead trout were sampled at 11 locations in four distinct population segments over two sampling seasons, yielding a sample size of 4,986 steelhead trout. We found significant trends among distinct population segments, including a clear morphological distinction between coastal and inland populations where, on average, steelhead trout in coastal populations were significantly larger and morphologically more robust than those in inland populations. The Nimbus Hatchery stock within the Central Valley Distinct Population Segment was a notable exception that included, on average, the largest and most robust steelhead trout observed in this study. It is important to understand how adult steelhead trout morphology not

only varies among and within geographically isolated populations, but also how morphology functions as a locally adapted life history trait, which will aid fishery managers in establishing instream flow requirements that accommodate passage of larger bodied individuals, and may also aid in the successful replacement of out-of-basin broodstocks with others exhibiting morphological traits in agreement with local environmental conditions.

Key words: California, distinct population segment, instream flow, morphometrics, Nimbus Hatchery, *Oncorhynchus mykiss*, steelhead trout

In biology, morphology is defined as the quantitative description, analysis, and interpretation of shape and shape variation (Rohlf 1990). Morphometric methods can be utilized when it is necessary to describe and compare shapes of individual organisms within and among conspecific populations (Rohlf and Marcus 1993). Morphology of individuals can vary due to many factors, including geographic origin, phenotypic expression in response to immediate environment, sexual dimorphism, and anthropogenic influence such as artificial propagation (Beacham and Murray 1985, Fleming and Gross 1994, Hard et al. 2000).

Previous studies involving members of the family Salmonidae have shown that environmental conditions, such as difficulty and distance of spawning migration, influence the distribution of morphometrics between geographically isolated populations (Beacham and Murray 1987, Fleming and Gross 1989, Quinn et al. 2001, Kinnison et al. 2003, Quinn 2005, Doctor and Quinn 2009). The countervailing pressures associated with extensive spawning migrations lead to phenotypic selection favoring reductions of body depth, fecundity, and secondary sexual characteristics in both male and female salmonids (Fleming and Gross 1989, Taylor 1991, Kinnison et al. 2003). Because extensive migrations are often arduous, it is plausible that selection for a smaller, streamlined body may benefit locomotion and efficiency of migration over longer distances (Kinnison et al. 2003, Wilson et al. 2003).

Reproductive homing in anadromous salmonids minimizes genetic interchange among geographically isolated populations and promotes the maintenance of heritable genetic adaptations to local environments (Scheer 1939, Horrall 1981, McIsaac and Quinn 1988, Fleming and Gross 1989, Taylor 1991, Quinn et al. 2000, Doctor and Quinn 2009). Reproductively isolated populations exposed to little or no gene flow from other populations may experience phenotypic differentiation (Carvalho 1993). Consequently, locally adapted and maintained traits, such as morphology, should be distinguishable between geographically isolated populations of anadromous salmonids, including steelhead trout (*Oncorhynchus mykiss*) in California (Fleming 1986, Fleming and Gross 1989, Taylor 1991).

Within California, coastal populations of steelhead trout generally migrate shorter distances to spawn as compared to inland populations, which experience difficult migrations over great distances. Given the differences in migratory conditions, we would predict that morphological characteristics will be distinct between coastal and inland populations of California steelhead trout, where coastal steelhead trout may exhibit larger, deeper bodies, and inland steelhead may exhibit smaller, narrower bodies, based on previous observations with Coho salmon (*O. kisutch*) by Fleming and Gross (1989).

Although steelhead trout are widespread in California (Moyle 2002), most populations are in decline. In response to precipitous decline, the National Marine Fisheries

Service (NMFS) (Busby et al. 1996) delineated six genetically Distinct Population Segments (DPS) of steelhead trout in California (Figure 1), and subsequently listed five of them under the United States Endangered Species Act (ESA). The Northern California (Federal Register 2000), Central California Coast (Federal Register 1997), Central Valley (Federal Register 1998), and South-Central California Coast (Federal Register 1997) DPSs are listed as threatened, and the Southern California DPS is listed as endangered (Federal Register 1997). The Klamath Mountains Province DPS is the only steelhead trout DPS in California that is not federally-listed (Federal Register 2006). Distinct Population Segments are described as representing evolutionary significant units of the species that are substantially reproductively isolated from other conspecific population units and also represent an important component in the evolutionary legacy of the species (Federal Register 1991). Morphological variation of adult California steelhead trout among DPSs remains undocumented, and gaining a better understanding of how selective forces influence steelhead trout morphometrics may contribute to our ability to manage and recover the species.

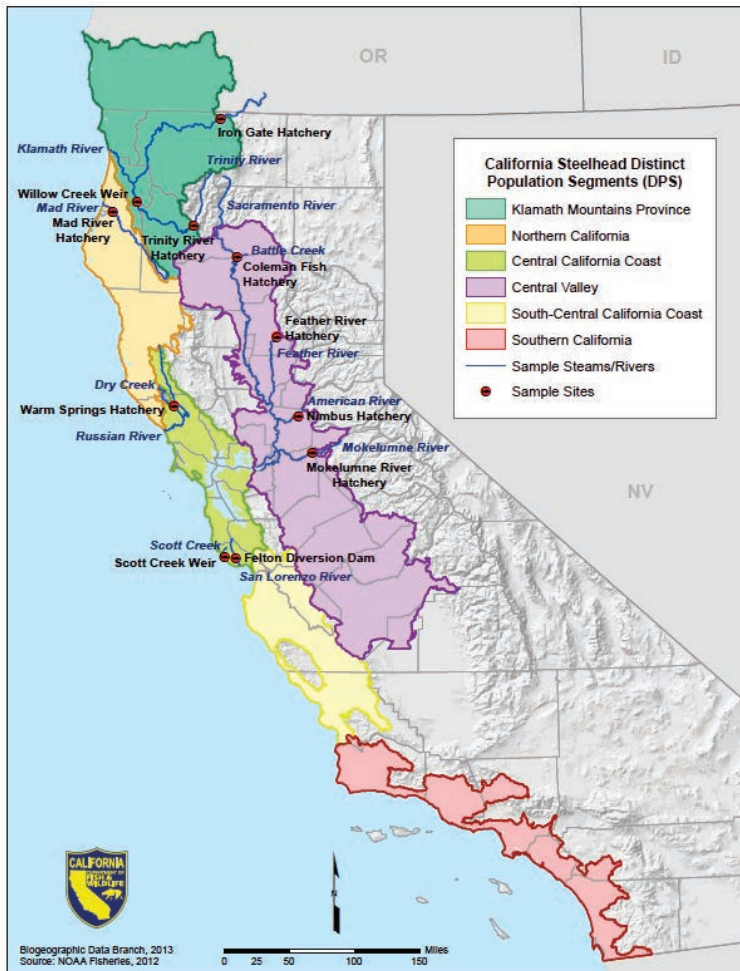


FIGURE 1.—The six steelhead trout District Population Segments and locations of study sampling sites in California.

Conservation of both coastal and inland steelhead trout populations is often associated with artificial propagation programs (Fleming and Petersson 2001, Morita et al. 2006, McClure et al. 2008, Chilcote et al. 2011). The founding broodstock used for hatchery propagation in California is usually established with individuals taken from within the same basin in which the hatchery is located. However, in some cases, hatcheries have obtained their broodstock from inter-basin transfers, which are often from a distinctly different biogeographic region than the hatchery location. Once established, the out-of-basin lineage is maintained through hatchery-produced adults returning to their hatchery of origin to be spawned (Chilcote et al. 2011).

An example in the current study is Nimbus Hatchery, located on the American River in the Central Valley DPS (Figure 1). The broodstock propagated at Nimbus Hatchery as mitigation for the Folsom Dam Project is a combination of steelhead trout native to the American River and a variety of other introduced stocks including fish from the Sacramento, Russian, and Eel rivers in California; the Washougal River in Washington; and the Siletz River in Oregon (Figure 2; McEwan and Nelson 1991, McEwan and Jackson 1996, McEwan 2001, Myrick and Cech 2005). Recent phylogeographic analysis suggests that the Nimbus Hatchery broodstock is most closely related to Eel River steelhead trout, which occurs in the Northern California DPS (Garza and Pearse 2008). What remains undocumented is to what extent the Nimbus Hatchery stock differs in morphology from the steelhead trout population that occurred in the American River prior to construction of Folsom Dam, and from other steelhead trout populations in the Central Valley DPS.



FIGURE 2.—Locations of the six Pacific coast rivers that provided stock used to develop steelhead trout broodstock at the Nimbus Hatchery on the American River, California.

Instream flow characteristics are another management concern related to morphometrics of steelhead trout. Like other Pacific salmonids, steelhead trout require sufficient stream discharge for maintenance of their freshwater habitat, including for migration, spawning, and juvenile rearing (Vadas 2000), and previous studies have shown that variance in stream flow between populations may play a role in local adaptation (Taylor 1991). For instance, variance in stream velocities influences prolonged swimming performance and holding ability among populations of juvenile salmonids (Riddell and Leggett 1981, Taylor and McPhail 1985b, Taylor 1991). In adult salmonids, variance in stream velocities affects morphology, where salmonids in faster flowing or headwater streams exhibit more streamlined bodies when compared to individuals in slower streams or those closer to the ocean (Riddell and Leggett 1981, Taylor and McPhail 1985a, Taylor 1991).

There is also evidence that stream discharge is associated with the abundance of returning adult salmonids, and may also affect selection for body size given that less-than-optimal flows interfere with returns of larger-bodied individuals (Mitchell and Cunjack 2007). Many steelhead trout streams in California are over-appropriated for instream water resources and, while provisions exist to protect instream flows (McEwan and Jackson 1996), the science that informs implementation of these provisions is often inadequate (Castleberry et al. 1996). Gaining a clear understanding of how adult steelhead trout morphometrics differ among DPSs will provide information for determining adequate instream flows for upstream passage of adult steelhead trout on their spawning migrations in both coastal and inland watersheds.

The objective of this study was to compare morphometrics of adult steelhead trout among four DPSs in California. Sampling locations (Figure 1) focused on hatcheries and weirs where adult steelhead trout, of both hatchery and natural origin, are intercepted annually in fishery management activities. Several factors may affect steelhead trout morphometrics, but three were chosen for analysis: geographic location, including the Klamath Mountains Province, Northern California, Central California Coast, and Central Valley DPSs; sex; and reproductive origin (natural or hatchery origin). Morphometric response variables were body depth, weight, and fork length. Fork length and weight serve as independent indices of the overall size of steelhead trout, whereas the interaction between fork length and weight, along with body depth, provide indices of body robustness (Anderson and Neumann 1996, Jones et al. 1999).

We tested the hypotheses that (1) there is a significant difference in mean body depth, fork length, and weight of adult steelhead trout among DPSs; (2) there is a significant difference in mean body depth, fork length, and weight between adult male and female steelhead trout; (3) there is a significant difference in mean body depth, fork length, and weight between adult steelhead trout of natural and hatchery origin; and (4) there are significant interactions between DPS, sex, and origin that influence the mean of each morphometric response variable: fork length, weight, and body depth.

MATERIALS AND METHODS

Sampling locations.—Adult steelhead trout were sampled at 11 locations in the Klamath Mountains Province, Northern California, Central California Coast, and Central Valley DPSs. Sampling in the Klamath Mountains Province DPS occurred at Iron Gate Hatchery on the Klamath River (Siskiyou County), Trinity River Hatchery (Trinity County), and Willow Creek Weir on the lower Trinity River (Humboldt County). Sampling in the

Northern California DPS occurred at Mad River Hatchery in Humboldt County. In the Central California Coast DPS, steelhead trout were sampled at Warm Springs Hatchery on the Russian River (Sonoma County), and Scott Creek Weir and Felton Dam on the San Lorenzo River (Santa Cruz County). Sampling in the Central Valley DPS occurred at Coleman National Fish Hatchery on Battle Creek (Shasta County) in the upper Sacramento River Basin, Feather River Hatchery on the Feather River (Butte County), Nimbus Hatchery on the American River (Sacramento County), and Mokelumne River Hatchery on the Mokelumne River (San Joaquin County).

Sampling occurred over two steelhead trout spawning seasons (December 2010 to March 2011 and December 2011 to March 2012) to obtain an adequate sample size for each location (Table 1). Willow Creek Weir was added during the second field season to supplement the Klamath Mountains Province DPS dataset with additional steelhead trout of natural origin. Adults encountered at Willow Creek Weir were marked with a spaghetti tag and were not resampled if encountered at the Trinity River Hatchery in the upper basin. Felton Dam and Scott Creek Weir were added as sampling locations during the second season to provide data on steelhead trout in the southernmost portion of the Central California Coast DPS.

TABLE 1.—Sample size of adult steelhead trout for each sampling location, by Distinct Population Segment.

Distinct Population Segment	Sampling Location	Hatchery Origin Steelhead Trout (<i>n</i>)		Natural Origin Steelhead Trout (<i>n</i>)		Total for Location
		Male	Female	Male	Female	
Klamath Mountains Province	Iron Gate Hatchery	59	53	1	3	116
	Trinity River Hatchery	311	421	6	8	746
	Willow Creek Weir	1	1	55	41	98
	DPS Totals	371	475	62	52	960
Northern California	Mad River Hatchery	268	488	15	33	804
	DPS Totals	268	488	15	33	804
Central Valley	Coleman National Fish Hatchery	283	317	103	141	844
	Feather River Hatchery	247	251	46	12	556
	Nimbus Hatchery	276	274	58	35	643
	Mokelumne River	193	192	5	1	391
	DPS Totals	999	1034	212	189	2434
Central California Coast	Warm Springs Hatchery	358	178	16	6	558
	Felton Diversion Dam	43	44	34	55	176
	Scott Creek Weir	4	3	13	21	41
	DPS Totals	405	225	63	82	775

Data collection.—Adult steelhead trout were measured for body depth (mm), fork length (FL, mm), and weight (0.01 kg). Body depth was measured using a large caliper while holding the fish vertically by the tail. The measurement was made from the anterior insertion of the dorsal fin to the ventral surface of the fish, along an axis perpendicular to the lateral line.

With the exception of Warm Springs Hatchery, measurements at all locations were taken from both male and female steelhead trout in pre-spawned condition. This was not possible at Warm Springs Hatchery due to the hatchery protocol specific to this location. At Warm Springs Hatchery, measurements were taken from pre-spawned females and post-

spawned males. We assumed the difference in weight and body depth between pre- and post-spawned males was negligible.

The sex (i.e., male or female) and origin (i.e., hatchery or natural) were also recorded for each steelhead trout from which morphometric data were collected. Sex was determined primarily through the expression of milt and eggs from males and females, respectively, but also by secondary sexual characteristics, such as a hooked kype in males. Origin was determined by the presence or absence of the adipose fin, given that all steelhead trout produced in hatcheries in California receive an adipose fin clip as pre-smolts prior to release.

Sample size.—A total of 2,182 adult steelhead trout was sampled during the 2010–2011 spawning season, and 2,804 were sampled during the 2011–2012 spawning season, yielding a total sample size of 4,986 steelhead trout (Table 1). Sampling occurred every other week at most hatcheries in both years, and on a continuous basis at Willow Creek, Felton Dam, and Scott Creek. Resampling was avoided by marking each fish with a caudal fin clip as they entered a hatchery or were trapped at a weir or dam. Iron Gate Hatchery was not sampled in 2011–2012 due to a lack of returning steelhead trout.

Statistical analysis.—Factorial Analysis of Variance (ANOVA) was used to analyze the various factor and morphometric response variables examined in this study. Assumptions of normality and homogeneity of variances were not always met with the data. However, Factorial ANOVA is robust and can adequately address departures from these assumptions when sample sizes are large (Table 1) because of the asymptotic properties of the central limit theorem (Zar 1999). Factorial ANOVA results were then corroborated with non-parametric resampling methods, the details of which are reported in Bajjaliya (2014, Appendix B).

We ran the Factorial ANOVAs to include both main factor effects and factor interactions. Because we found that there were significant interactions between factors (i.e., DPS, sex, and origin) for each morphometric response variable (i.e., body depth, fork length, and weight), we conducted a series of pairwise *t*-tests as post hoc analysis to determine where specific differences occurred. Pairwise *t*-tests were corrected for type I errors to preserve the overall alpha of $P \leq 0.05$. The Sidak adjustment method (Sokal and Rohlf 2012) was chosen, because only a subset of all pairwise comparisons was tested. To visually ascertain the dependency relationships between factors, two- and three-way interaction plots were generated (Figures 3–5). Interaction plots included the relationship between origin, DPS, and sex for each morphometric response variable.

We conducted a series of one-way ANOVAs to gain a better understanding of how morphometrics of Nimbus Hatchery steelhead trout compared to the morphometrics of steelhead trout in the Northern California DPS, their DPS of origin, as well as the rest of the Central Valley DPS, to which they were introduced. The ANOVAs compared the mean of each morphometric response variable (i.e., body depth, fork length, and weight) among these groups. When ANOVA results led to rejection of the null hypothesis that the means of a response variable were equal among these groups, post hoc pairwise *t*-tests using the Sidak adjustment were used to determine where the differences existed. Graphical analysis was also used to assess differences using dot plots of mean body depth, weight, and fork length with 95% CIs for each location.

RESULTS

An overview of summary statistics for body depth, weight, and fork length suggested that there were significant differences in morphometric response variables between DPSs

TABLE 2.—Summary table of statistics for each steelhead trout response variable (body depth, weight, fork length) by Distinct Population Segment (DPS).

Response Variable	DPS	Origin	Sex	<i>n</i>	Average	<i>SD</i>	Min	Max	Skewness	<i>CV</i>
Body Depth (mm)	Northern California	Hatchery	Female	488	134.6	10.5	98	180	0.11	0.08
			Male	268	141.6	12.7	104	184	0.17	0.09
		Natural	Female	33	135.7	17.7	67	188	-0.99	0.13
			Male	15	145.3	14.1	125	173	0.32	0.10
	Central California Coast	Hatchery	Female	225	137.6	13.1	98	169	-0.53	0.10
			Male	405	129.9	20.6	82	187	0.03	0.16
		Natural	Female	82	115.3	19.0	65	153	-0.07	0.16
			Male	63	122.4	26.3	52	178	-0.10	0.21
	Klamath Mountains Province	Hatchery	Female	474	112.1	11.6	83	153	0.38	0.10
			Male	370	121.3	14.1	84	161	0.15	0.12
		Natural	Female	52	114.8	13.3	89	148	0.60	0.12
			Male	61	118.6	13.0	87	149	0.41	0.11
	Central Valley	Hatchery	Female	1032	116.1	18.3	78	167	0.39	0.16
			Male	994	125.0	19.7	80	191	0.58	0.16
		Natural	Female	189	101.9	22.3	56	183	0.96	0.22
			Male	210	114.5	25.8	69	181	0.45	0.23
Weight (kg)	Northern California	Hatchery	Female	488	3.4	0.7	1.5	7.6	0.90	0.20
			Male	267	3.5	0.8	1.6	6.7	0.64	0.22
		Natural	Female	33	3.6	1.0	0.5	7.3	0.59	0.28
			Male	15	3.7	1.0	2.5	6.1	0.97	0.27
	Central California Coast	Hatchery	Female	225	3.7	0.8	1.2	5.5	-0.40	0.22
			Male	404	3.0	1.2	0.8	7.4	0.28	0.40
		Natural	Female	80	2.4	1.2	0.7	7.0	0.99	0.48
			Male	63	2.5	1.5	0.3	6.6	0.53	0.57
	Klamath Mountains Province	Hatchery	Female	475	2.2	0.6	0.8	4.6	0.76	0.28
			Male	370	2.4	0.8	0.8	4.9	0.57	0.32
		Natural	Female	52	2.4	0.8	1.0	4.2	0.75	0.31
			Male	62	2.6	0.8	1.1	5.8	1.28	0.32
	Central Valley	Hatchery	Female	1019	2.1	1.1	0.5	5.5	0.95	0.51
			Male	975	2.3	1.3	0.6	8.1	1.23	0.56
		Natural	Female	188	1.5	1.1	0.1	6.3	1.98	0.73
			Male	210	1.8	1.3	0.5	6.5	1.44	0.73
Fork Length (mm)	Northern California	Hatchery	Female	488	668.8	40.7	512	890	0.45	0.06
			Male	267	692.9	51.0	523	898	0.24	0.07
		Natural	Female	33	679.5	71.5	370	857	-1.92	0.11
			Male	15	699.1	54.8	613	813	0.36	0.08
	Central California Coast	Hatchery	Female	225	687.1	51.1	480	781	-0.98	0.07
			Male	405	643.7	89.0	462	898	-0.10	0.14
		Natural	Female	82	594.5	91.4	340	870	-0.05	0.15
			Male	63	610.1	118.7	280	860	-0.34	0.19
	Klamath Mountains Province	Hatchery	Female	474	596.7	63.3	398	764	-0.41	0.11
			Male	368	617.6	75.7	395	805	-0.67	0.12
		Natural	Female	52	594.5	59.5	462	728	-0.01	0.10
			Male	62	617.2	64.8	451	784	0.18	0.11
	Central Valley	Hatchery	Female	1030	546.7	94.2	375	882	0.79	0.17
			Male	997	566.6	106.8	398	915	0.89	0.19
		Natural	Female	189	459.5	100.8	230	770	0.82	0.22
			Male	212	505.1	121.4	330	895	0.98	0.24

(Table 2). Significant, high-order interactions were detected between the factors in the analysis for each morphometric response variable. The three-way factor interaction for both body depth ($F_{3,4888}=5.27$) and for weight ($F_{3,4888}=4.26$) were significant (both $P<0.001$; Table 2). Several two-way interactions were significant for fork length. These included interactions between DPS and sex ($F_{3,4888}=21.07$); DPS and origin ($F_{3,4888}=27.67$); and sex

and origin ($F_{1,4888}=10.50$) (all $P<0.001$; Table 3). Detection of significant factor interactions implied that response values for one factor were dependent on the values of other factors. Therefore, the factors had to be interpreted simultaneously, and not individually, during post hoc analysis.

TABLE 3.—Factorial Analysis of Variance summary table for each steelhead trout response variable (body depth, weight, fork length).

Morphometric Response Variable	<i>df</i>	Sum Sq	Mean Sq	<i>F</i>	<i>P</i> -value
Fork Length (mm)					
DPS	3	13807773	4602591	620.6	< 0.001
Sex	1	259702	259702	35.0	< 0.001
Origin	1	1572454	1572454	212.0	< 0.001
DPS:Sex	3	468943	156314	21.1	< 0.001
DPS:Origin	3	615810	205270	27.7	< 0.001
Sex:Origin	1	77927	77927	10.5	0.001
DPS:Sex:Origin	3	43712	14571	2.0	0.117
Residuals	4888	36251052	7416		
Body Depth (mm)					
DPS	3	291700	97233	318.9	< 0.001
Sex	1	55944	55944	183.5	< 0.001
Origin	1	48843	48843	160.2	< 0.001
DPS:Sex	3	28205	9402	30.8	< 0.001
DPS:Origin	3	20341	6780	22.2	< 0.001
Sex:Origin	1	2261	2261	7.4	0.007
DPS:Sex:Origin	3	4824	1608	5.3	0.001
Residuals	4888	1490486	305		
Weight (kg)					
DPS	3	1412.1	470.7	449.3	< 0.001
Sex	1	5	5.0	4.8	< 0.001
Origin	1	102.5	102.5	97.9	< 0.001
DPS:Sex	3	63.2	21.1	20.1	< 0.001
DPS:Origin	3	83.2	27.7	26.5	< 0.001
Sex:Origin	1	10.5	10.5	10.0	0.001
DPS:Sex:Origin	3	13.4	4.5	4.5	0.005
Residuals	4888	5120.6	1.1		

Body depth.—Within the Central California Coast DPS, hatchery-origin females had significantly deeper body depths than hatchery-origin males ($t=5.7$, $df=617$, $P<0.001$). In contrast, hatchery-origin males had significantly deeper body depths than hatchery-origin females in the Central Valley ($t=-10.6$, $df=2007$, $P<0.001$), Klamath Mountains Province ($t=-10.2$, $df=712$, $P<0.001$), and Northern California ($t=-7.6$, $df=469$, $P<0.001$) DPSs (Figure 3A).

Within the Central Valley DPS, natural-origin males had significantly deeper body depths than natural-origin females ($t=-5.2$, $df=399$, $P<0.001$) (Figure 3B). Hatchery-origin

females had significantly deeper body depths than natural-origin females within the Central California Coast ($t=9.8$, $df=111$, $P<0.001$) and Central Valley ($t=8.3$, $df=236$, $P<0.001$) DPSs (Figure 3C). Within the Central Valley DPS, hatchery-origin males had significantly deeper body depths than natural-origin males ($t=5.6$, $df=266$, $P<0.001$) (Figure 3D). In summary, body depth varied significantly between DPSs. However, results for body depth were not consistent for the other factor variables, sex and origin.

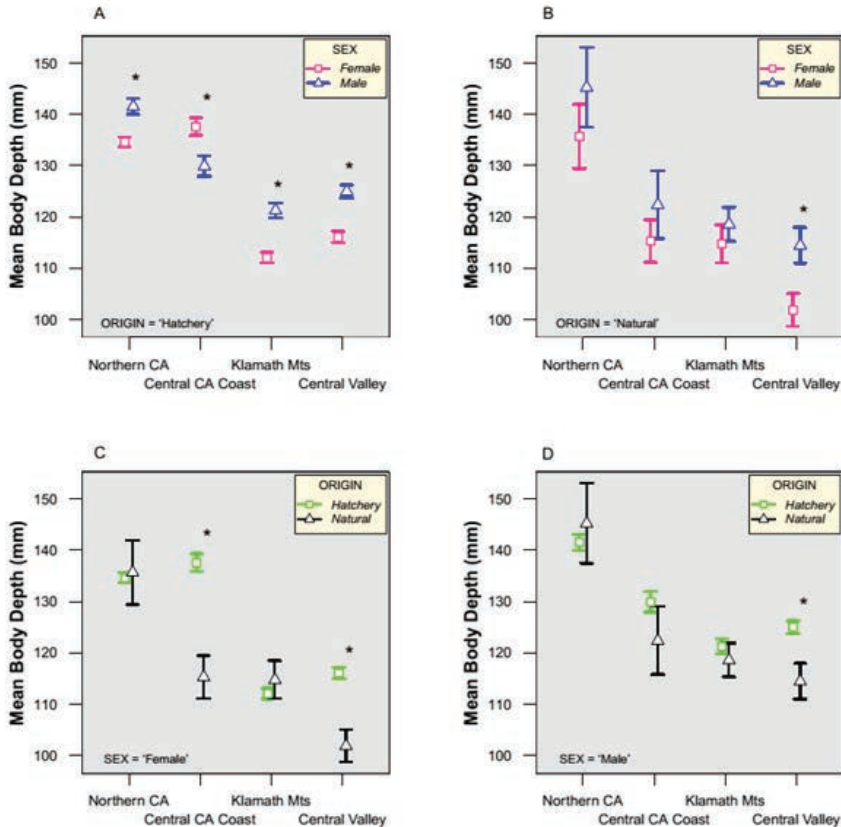


FIGURE 3.—Three-way factor interaction post-hoc results for body depth. An asterisk indicates a significant difference between the two groups at $\alpha=0.05$. (A) Distribution of mean body depth between hatchery-origin steelhead trout females and hatchery-origin males by Distinct Population Segments (DPS). (B) Distribution of mean body depth between natural-origin females and natural-origin males by DPS. (C) Distribution of mean body depth between hatchery-origin females and natural-origin females by DPS. (D) Distribution of mean body depth between hatchery-origin males and natural-origin males by DPS.

Weight.—Within the Central California Coast DPS, hatchery-origin females were significantly heavier than hatchery-origin males ($t=8.6$, $df=603$, $P<0.001$). In contrast, hatchery-origin males were significantly heavier than hatchery-origin females within the Klamath Mountains Province DPS ($t=-4.0$, $df=706$, $P<0.001$) (Figure 4A). Within the Central California Coast ($t=9.3$, $df=112$, $P\leq 0.001$) and Central Valley ($t=7.9$, $df=263$, $P\leq 0.001$) DPSs, hatchery-origin females were significantly heavier than natural-origin females (Figure 4C). Within the Central Valley DPS, hatchery-origin males were significantly heavier than

natural-origin males ($t=4.6$, $df=300$, $P<0.001$) (Figure 4D). In summary, as with body depth, weight varied significantly between DPSs. However, results for weight were not consistent for the other factor variables, sex and origin.

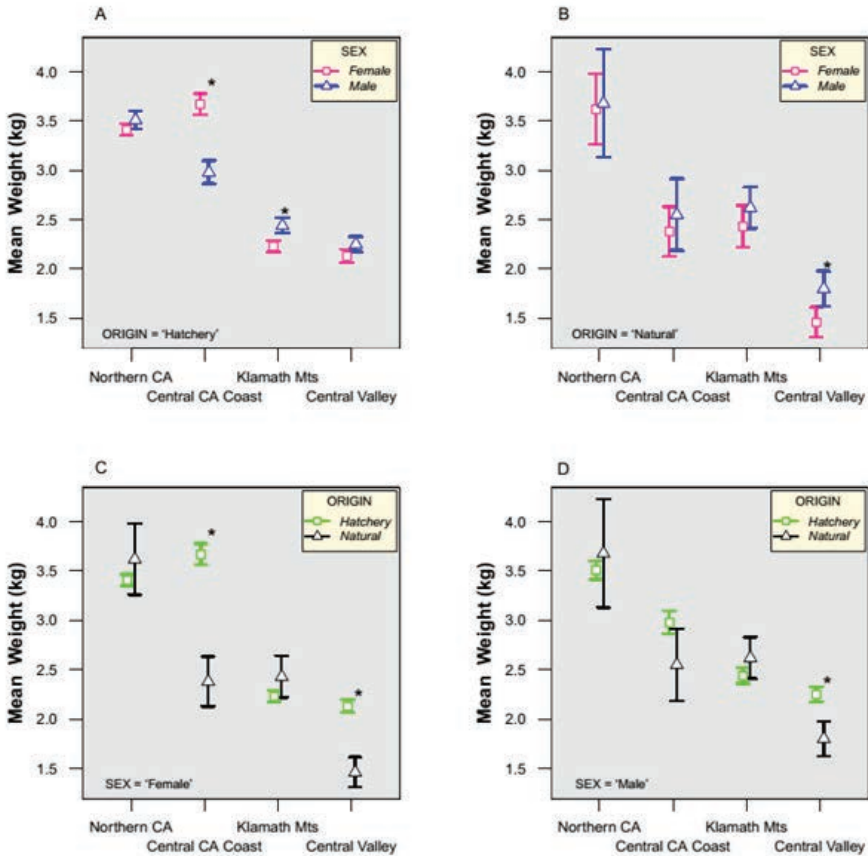


FIGURE 4.—Three-way factor interaction post-hoc results for weight. An asterisk indicates a significant difference between the two groups at $\alpha=0.05$. (A) Distribution of mean weight between hatchery-origin steelhead trout females and hatchery-origin males by Distinct Population Segments (DPS). (B) Distribution of mean weight between natural-origin females and natural-origin males by DPS. (C) Distribution of mean weight between hatchery-origin females and natural-origin females by DPS. (D) Distribution of mean weight between hatchery-origin males and natural-origin males by DPS.

Fork length.—Within the Central California Coast ($t=7.3$, $df=773$, $P<0.001$) and Central Valley ($t=15.4$, $df=2,434$, $P<0.001$) DPSs, hatchery-origin steelhead had significantly greater fork lengths than natural-origin steelhead (Figure 5A). Within the Central California Coast DPS, females had significantly greater fork lengths than males ($t=3.66$, $df=773$, $P<0.001$). In contrast, males had significantly greater fork lengths than females within the Central Valley ($t=-6.44$, $df=2,439$, $P<0.001$), Klamath Mountains Province ($t=-3.75$, $df=959$, $P<0.001$), and Northern California ($t=-3.72$, $df=802$, $P<0.001$) DPSs (Figure 5B).

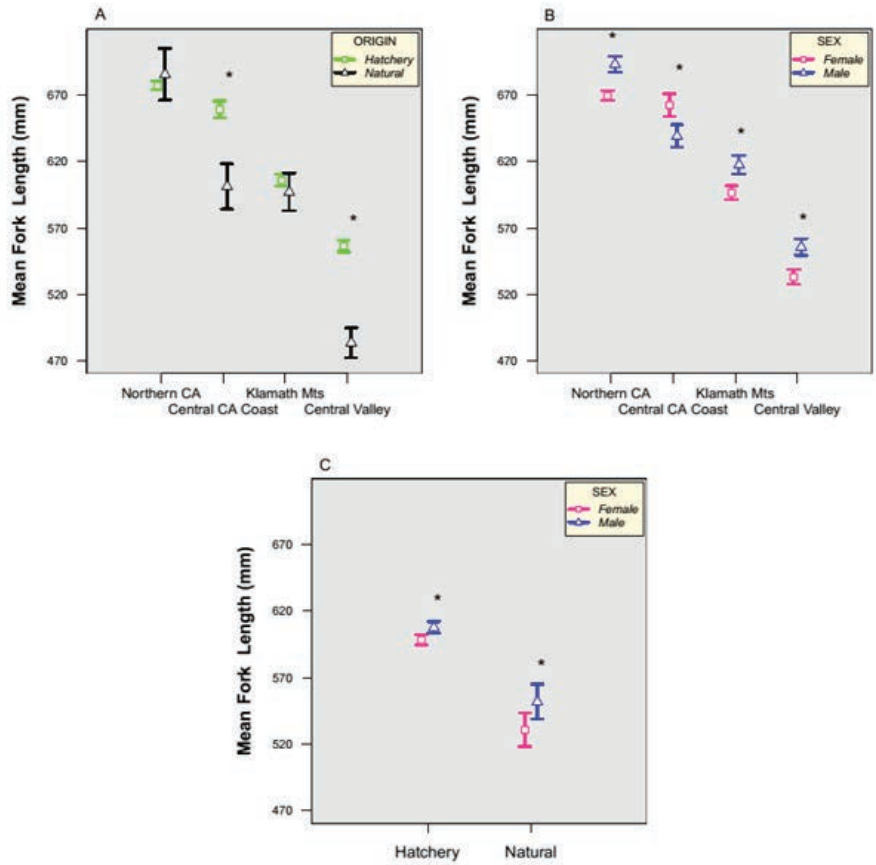


FIGURE 5.—Two-way factor interaction post-hoc results for fork length. An asterisk indicates a significant mean difference between the two groups at alpha=0.05. (A) Distribution of mean fork length between origin and Distinct Population Segment (DPS). (B) Distribution of mean fork length between sex and DPS. (C) Distribution of mean fork length between sex and origin.

Hatchery-origin males had significantly greater fork lengths than hatchery-origin females ($t=-3.5$, $df=4,263$, $P<0.001$). Natural-origin males had significantly greater fork lengths than natural-origin females ($t=-3.3$, $df=706$, $P<0.001$) (Figure 5C). In summary, as with body depth and weight, fork length varied significantly between DPSs. However, results for fork length were not consistent for the other factor variables, sex and origin.

In the comparative analysis of morphometrics that split out Nimbus Hatchery steelhead trout, there were significant differences in body depth ($F_{2, 3233}=1,710$), weight ($F_{2, 3199}=4,051$), and fork length ($F_{2, 3234}=5,057$) between the Nimbus Hatchery group, the Northern California DPS, and the Central Valley DPS excluding Nimbus Hatchery (all $P<0.001$). Post hoc analysis (Table 4; Figure 6) indicated that Nimbus Hatchery steelhead trout were significantly larger than steelhead trout within the Northern California DPS

TABLE 4.—Factorial Analysis of Variance summary table for each steelhead trout response variable (body depth, weight, fork length). Data used were from the Central Valley Distinct Population Segment excluding the Nimbus Hatchery stock, the Northern California Distinct Population Segment, and the Nimbus Hatchery stock exclusively.

Morphometric Response Variable	Group One	Group Two	Group One (\bar{x})	Group Two (\bar{x})	Diff. Avg.	LCL	UCL	<i>t</i>	<i>DF</i>	<i>P</i> -value
Body Depth	Northern CA	Nimbus	137.2	143.1	-5.9	-7.6	-4.2	-8.2	1243	<0.001
Body Depth	CV-No Nimbus	Nimbus	109.6	143.1	-33.4	-35.0	-31.8	-49.3	1164	<0.001
Body Depth	CV-No Nimbus	Northern CA	109.6	137.2	-27.5	-28.9	-26.2	-49.3	1893	<0.001
Weight	Northern CA	Nimbus	3.5	3.8	-0.3	-0.4	-0.2	-7.0	1219	<0.001
Weight	CV-No Nimbus	Nimbus	1.5	3.8	-2.3	-2.4	-2.2	-60.3	789	<0.001
Weight	CV-No Nimbus	Northern CA	1.5	3.5	-2.0	-2.0	-1.9	-69.1	1160	<0.001
Fork Length	Northern CA	Nimbus	677.8	695.1	-17.3	-24.7	-9.9	-5.6	1130	<0.001
Fork Length	CV-No Nimbus	Nimbus	490.4	695.1	-204.6	-212.0	-198.0	-70.9	967	<0.001
Fork Length	CV-No Nimbus	Northern CA	490.4	677.8	-187.3	-192.0	-182.0	-89.0	1738	<0.001

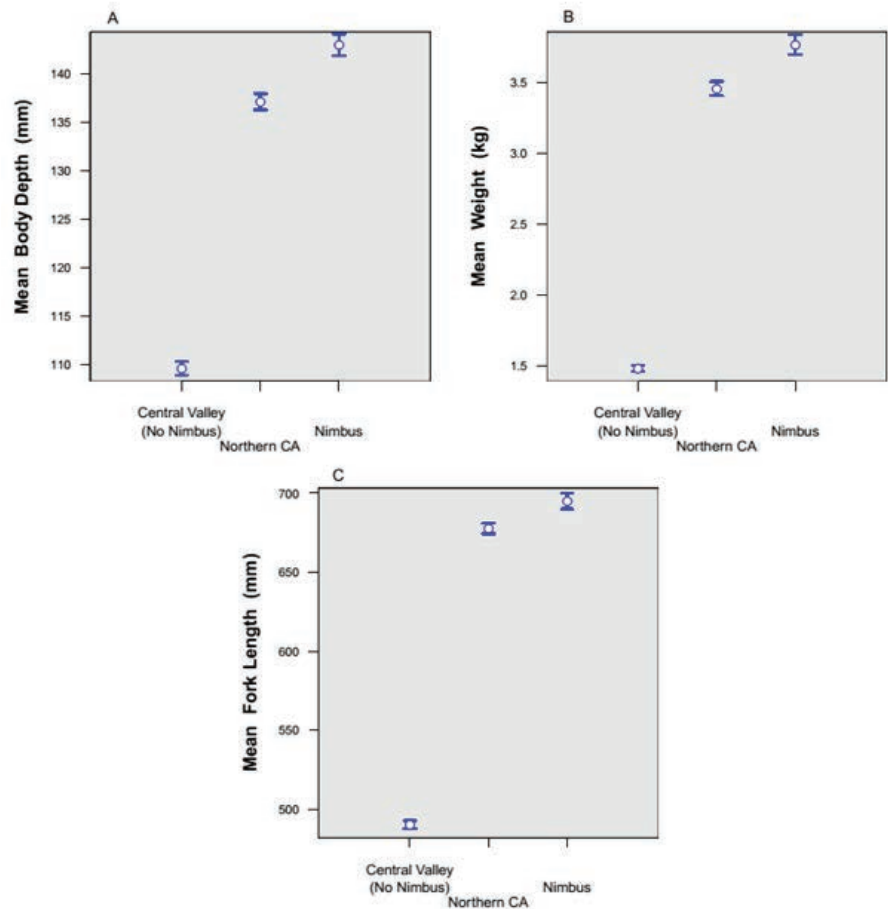


FIGURE 6.—Post-hoc 95% confidence intervals for the mean response by Central Valley and Nimbus Hatchery sampling locations. (A) Distribution of mean body depth. (B) Distribution of mean weight. (C) Distribution of mean fork length.

in terms of body depth ($t=-8.2$, $df=1243$, $P<0.001$), weight ($t=-7.0$, $df=1219$, $P<0.001$), and fork length ($t=-5.6$, $df=1130$, $P<0.001$). This same pattern existed between Nimbus Hatchery and Central Valley DPS steelhead trout in terms of body depth ($t=-49.3$, $df=1164$, $P<0.001$), weight ($t=-60.3$, $df=789$, $P<0.001$), and fork length ($t=-70.9$, $df=967$, $P<0.001$). Lastly, steelhead trout within the Northern California DPS were significantly larger than steelhead trout within the Central Valley DPS excluding Nimbus Hatchery, again in all three morphometric response variables: body depth ($t=-49.3$, $df=1893$, $P<0.001$), weight ($t=-69.1$, $df=1160$, $P<0.001$), and fork length ($t=-89.0$, $df=1738$, $P<0.001$).

DISCUSSION

While population genetic structure of steelhead trout has been assessed on a regional scale in California (e.g., Garza and Pearse 2008, Clemento et al. 2009), the current study is the first to examine morphometric variation of California steelhead trout on a similar geographic scale. We found that measurement of just a few, simple morphological features provided a basis for distinguishing among geographically isolated populations of steelhead trout.

For example, the largest adult steelhead trout, on average, occurred in the Northern California DPS, followed by those in the Central California Coast, Klamath Mountains Province, and Central Valley DPSs. We also found an overall distinction in size between hatchery and natural-origin steelhead trout, where hatchery-origin steelhead trout were longer on average than natural-origin steelhead trout (Figure 5).

Our results also provided evidence of significant trends between coastal and inland population groups. The distance migrated from the ocean to each sampling location was considered when defining the two population groups. The shortest distance migrated from the ocean was 1 km to the Scott Creek Weir and the longest was 529 km to Coleman National Fish Hatchery. Adult steelhead trout sampled at Willow Creek Weir traveled 105 km from the ocean; however, Willow Creek Weir was considered an intermediate sampling location used to sample natural-origin steelhead trout migrating to either the upper Trinity River system or to Trinity River Hatchery. There was a 10-fold difference in mean distance traveled between coastal (28 km) and inland (278 km) sampling locations.

For the purpose of this study, coastal populations were considered those in which adult steelhead trout migrated less than 160 km to where they were sampled, while inland populations were considered those in which adult steelhead trout migrated over 160 km to where they were sampled, 160 km being the approximate midpoint between the mean distances of our coastal and inland groups of sampling locations. Based on these parameters, steelhead trout sampled from the Northern California and Central California Coast DPSs were considered to be of coastal origin, while steelhead trout sampled from the Klamath Mountains Province and Central Valley DPSs were considered to be of inland origin. Our results indicated distinct morphological differences between coastal and inland adult steelhead trout where, on average, coastal populations had greater body depths, weights, and lengths than steelhead trout in inland populations (Figures 3–5, respectively).

The Central Valley DPS allowed for the opportunity to compare morphometric variation of adult steelhead trout of both inland and coastal origin within a single DPS. Nimbus Hatchery, on the lower American River, is unique in that its broodstock is an amalgamation of many intra- and inter-basin transfers made over time. However, recent phylogeographic analysis suggests that the Nimbus Hatchery broodstock is most closely

related to Eel River steelhead trout, which occurs in the Northern California DPS (Garza and Pearse 2008). What remains undocumented is to what extent the Nimbus Hatchery stock differs in morphology from the steelhead trout population that occurred in the American River prior to construction of Folsom Dam, and from other steelhead trout populations in the Central Valley DPS.

In an analysis of morphometric variation between Nimbus Hatchery steelhead trout, the Northern California DPS, and the remainder of the Central Valley DPS, we found the greatest differences in all three measures of size occurred between Nimbus Hatchery, which had the largest steelhead trout, and the Central Valley DPS, which had the smallest steelhead trout (Figure 6). Morphometrics in the Northern California DPS were intermediate in size; however, these fish grouped very closely in size with those sampled at Nimbus Hatchery (Figure 6).

One possible explanation for the very robust body morphology of Nimbus Hatchery steelhead trout may be historic selection by hatchery personnel of only the largest fish for spawning, which could have imposed strong directional selection on these fish over time (Garza and Pearse 2008). There are, however, other possible explanations as to why steelhead trout propagated at Nimbus Hatchery clearly differ in morphometric traits when compared to those comprising other populations sampled within the Central Valley DPS.

Steelhead trout life history evolution is influenced by an interacting frame network of bioenergetic constraints including growth rate, asymptotic size achieved within the riverine environment, freshwater survival, and survival to adulthood (Satterthwaite et al. 2009). It is possible that the morphometrics of Nimbus Hatchery steelhead trout are influenced, in part, by a combination of phenotypic and genotypic life history responses to the highly altered environmental conditions of the lower American River, or that these fish are pre-adapted to respond to their new environment in such a way that promotes near optimal behavior (Satterthwaite et al. 2009).

The Folsom Dam Project has blocked access to historic spawning habitat, altered historic flow regimes, and modified downstream habitat for steelhead trout in the lower American River. The alteration of historic environmental conditions has affected seasonal water temperatures and food availability, which influence growth rates of steelhead trout (Satterthwaite et al. 2009). Food availability on the lower American River is high during summer months (June-August) and results, in part, in rapid juvenile growth, early smolting, and seaward migration at age 1 (Satterthwaite et al. 2009).

Steelhead trout in the lower American River also exhibit a highly anadromous life history, which is contrary to most populations within the Central Valley DPS (Satterthwaite et al. 2009). Although few studies have been conducted, it appears that many populations of steelhead trout in the Central Valley, and elsewhere, have diverged substantially from their historic life history strategies and now include a greater proportion of fish expressing residency in response to habitat conditions that are less supportive of anadromy (Lindley et al. 2007, McClure et al. 2008, Satterthwaite et al. 2009).

It is possible that phenotypic and genotypic responses to environmental conditions in the lower American River, favoring rapid growth, early emigration, and a high degree of anadromy also influence growth potential in the marine environment and successful return of adult steelhead trout to the riverine environment to spawn. Smolt size at ocean entry influences survivability (Ward et al. 1989, Satterthwaite et al. 2009), and there is a fecundity advantage achieved by large anadromous salmonids (Scott and Crossman 1989,

Beacham and Murray 1993, Wilson et al. 2003, Quinn 2005, Satterthwaite et al. 2009). Thus, the large size and robust morphology of Nimbus Hatchery steelhead trout may not only be a consequence of the genetic linkage to the Eel River stock, but also that the Eel River stock introduction was a good match for the novel anadromous environment that the lower American River represented following construction of Folsom Dam.

The Nimbus Hatchery steelhead stock is the focal point of a fishery management and conservation dilemma. This stock supports a very successful hatchery program and recreational steelhead trout fishery, the latter of which is also supported by natural reproduction in the American River. The lower American River flows through the city of Sacramento and for steelhead trout is the fifth most fished river in California (California Department of Fish and Wildlife (CDFW), Steelhead Report and Restoration Card, unpublished data), presumably due in part to extensive public access along the American River Parkway, and because of typically reliable returns of a desirable resource. The lower American River is also unique in that it provides the angler the opportunity to catch an inland stock exhibiting larger morphometrics typically found only in coastal populations. However, NMFS considers maintaining a stock of steelhead trout with known out-of-basin derived genetics for the lower American River to be in direct conflict with recovery of the Central Valley DPS (CDFG and NMFS 2001). Moreover, Garza and Pearse (2008) suggested that the Nimbus Hatchery stock may be an impediment to recovery of Central Valley DPS steelhead trout because of its potential influence on the genetic integrity of other populations in the Central Valley DPS as the result of straying.

To address this issue, fishery managers are considering supplanting the Nimbus Hatchery stock. Replacement of the Nimbus Hatchery stock could include either introduction of steelhead trout from an extant native population within the Central Valley DPS, or reintroduction of upper American River Basin *O. mykiss* that may be genetically more similar to historic lower American River steelhead trout.

Presumably, the morphometrics of steelhead trout selected as replacement stock would reflect the smaller morphometrics of steelhead trout native to the Central Valley DPS. Steelhead trout ancestors in the upper American River Basin, while potentially having a genetic fidelity with historic lower river steelhead trout, are also likely to have adapted to local, above-barrier conditions following more than 60 years of geographic and reproductive isolation from the lower river ecosystem. Environmental conditions in the upper watershed that could contribute to local adaptation include seasonal hydrologic and temperature regimes that select against anadromy (NMFS 2014), and low stream productivity, which may preclude the growth needed to achieve the dimensions of anadromous steelhead trout (Satterthwaite et al. 2009), and perform as well in the anadromous environment of the lower American River as the extant Nimbus Hatchery stock.

The importance of locally adapted traits should be considered before attempting to supplant existing populations of steelhead trout (Taylor 1991). Previous studies have demonstrated that translocations of salmonids often fail because they are ill-suited to environmental conditions in the watershed in which they are being established (Taylor 1991). Studies have also shown adaptive variation among populations can affect swimming ability (Taylor and McPhail 1985b), homing ability (Bams 1976), and disease resistance (Gjedrem and Aulstad 1974, Taylor 1991). Thus, there is a wide range of factors, in addition to morphometrics, that needs to be taken into consideration when embarking on a steelhead trout stock supplantation.

Instream flow considerations.—The persistence of adult steelhead trout in a given locale is critically linked to their ability to successfully make the upstream migration in their natal stream to spawn. A high frequency of lower-than-normal flow conditions in a stream can directionally select for a reduction in morphological characteristics, thereby selecting against a population inclusive of larger-bodied individuals (Beacham and Murray 1987, Quinn et al. 2001, Mitchell and Cunjak 2007). If larger bodied individuals are not able to access natal streams due to low stream discharge, they either spawn in less-than-desirable habitat, leave to spawn in other waters, or refrain from spawning altogether. Spawning area limitations due to low flows may result in decreased opportunities for segregation between natural-origin steelhead trout and strays of hatchery origin, thus enhancing the chance of genetic introgression between the two types (Jonsson et al. 1990).

Morphometrics of adult steelhead trout could be of importance when establishing instream flow requirements that accommodate upstream passage of larger bodied individuals, both in regulated and unregulated streams. Until the 1970s, minimum instream passage flows were based on professional judgment rather than on quantified relationships between stream discharge and fish passage parameters, and were often a fixed percentage of average annual stream flow (Fraser 1972, Petts 2009). Minimum stream flows could be insufficient, as they may only accommodate individuals of average size or less within the population. Thus, knowledge of the specific morphological characteristics of the target steelhead trout population should protect the broadest range of sizes in the population by providing optimum passage flows.

The California State Water Resources Control Board (SWRCB 2010) described minimum upstream passage flows for migrating adult steelhead trout as “the flow that is protective of adult fish passage in the most limiting stream sites.” Sites most often limiting passage are shallow riffles or other shallow points, such as low-head weirs or dams. The ability of adult steelhead trout to navigate past these potential barriers is determined using the Thompson Method. Application of that method determines the threshold flow at which passage of anadromous salmonids will occur by providing suitable depths and velocities in at least 25% of the total width of a critically shallow passage point, 10% of which must be contiguous (Thompson 1972, Vadas 2000). A minimum depth criterion is used on a species-specific basis.

In 2010, SWRCB proposed state-wide passage criteria, which would require the provision of flows necessary to allow passage of adult steelhead trout at critically shallow points in a stream. Specifically, the criteria would provide a minimum depth of 0.21 m in at least 25% of the total-width of the stream channel, with 10% of it contiguous at such points (SWRCB 2010). Based on body depths measured in this study, the maximum of which was 0.19 m (Table 2), this depth criterion may provide minimal, suitable passage under the majority of circumstances. Our study provides evidence that body morphology of adult steelhead trout differs significantly among the DPSs sampled. The variation in body size of adult fish was most apparent between coastal and inland populations. For this reason, determining passage criteria specific to DPSs may be more appropriate than applying state-wide criteria. In some cases, passage criteria may need to be stream-specific to meet requirements of a specific population that departs morphologically from the DPS. The lower American River is an example, where steelhead trout of Nimbus Hatchery stock origin are much larger than the average for the Central Valley DPS.

Knowledge of morphological characteristics of adult steelhead trout populations throughout California could improve development of instream flow criteria for the species. Stream flow criteria for steelhead trout passage have been prescribed by SWRCB (2010), but the ability of migrating adults to pass critically low passage points per these criteria has not been substantiated in the field. Instream flow evaluations, in conjunction with morphological analysis of steelhead trout among DPSs, should be conducted to determine suitable regional, watershed, or stream-specific stream flow criteria.

Recommendations for future work.—This study represented a broad-brush assessment of the morphometrics of steelhead trout across northern and central California. It included steelhead trout of both hatchery and natural origin that were sampled to varying degrees at both terminal hatcheries and natural habitat areas (i.e., at weirs and dams along upstream migration routes) within each DPS. We detected general patterns in morphometrics on a geographic basis, perhaps most distinctly between what we described as coastal and inland population groups. For example, we found that, on average, steelhead trout in coastal populations (Northern California and Central California Coast DPSs) had greater body depths, weights, and lengths than steelhead trout in inland populations (Klamath Mountains Province and Central Valley DPSs).

While the data we collected infer broadscale differences in morphometrics among DPSs, we recommend that future work more thoroughly assess morphometric variation within DPSs. For example, we found that, on average, steelhead trout in the Central Valley DPS tended to be the smallest overall among steelhead trout sampled in four DPSs. However, we also found that, within the Central Valley DPS, the Nimbus Hatchery stock of steelhead trout were the largest observed in this study, even larger than those in the Northern California DPS, which were otherwise the largest observed in this study on a DPS basis. Thus, even though the coastal California origin of the Nimbus Hatchery stock may still be the primary factor behind the large size of these steelhead trout, they nonetheless represent an extreme variation on a within-DPS basis. Had sampling in the Central Valley DPS not included Nimbus Hatchery, or possibly the lower American River, this element of morphometric diversity within the DPS likely would not have been detected.

Another notable example where significant within-DPS variation should be accounted for is in the Klamath Mountains Province DPS. With the sampling sites for this DPS located well inland on the Klamath-Trinity rivers system, we classified this DPS as inland. We found that, like the Central Valley DPS, which we also classified as inland, steelhead trout in the Klamath Mountains Province DPS tended to be smaller in morphometric response variables than in the coastal Northern California and Central California Coast DPSs. Yet, the Klamath Mountains Province DPS extends all the way to the Pacific Ocean and includes coastal drainages that would be classified as “coastal,” per the provisional migration distance criterion of <160 km that we used.

The Smith River (Del Norte County) is among the coastal drainages found in the Klamath Mountains Province DPS. Based on sport fishery results, this stream is widely known for its large steelhead trout, with the California state record (12.4 kg) caught in the Smith River in 1976. Although we were unable to locate morphometric data for steelhead trout on the Smith River, these fish, by all accounts, seem to be in-line with the larger coastal phenotype observed in this study, as opposed to the smaller inland type. Thus, while DPSs generally seem relevant in distinguishing different population groups based on their genetic history (e.g., Garza and Pearce 2008, Clemente et al. 2009), the coastal type-inland type

model may be more applicable with respect to morphometrics. Nevertheless, we recommend that more within-DPS variation of steelhead trout morphometrics be covered in future efforts aimed at testing this model.

From a sampling design perspective, efforts should strive to include more balanced sampling between hatcheries and natural habitat areas. Doing so would alleviate concerns about potential biases that could arise from the systematic exclusion of steelhead trout—of either hatchery or natural origin—that have a behavioral aversion to ascending a fish ladder into a hatchery. Our study relied heavily upon anadromous salmonid hatcheries with successful steelhead trout propagation programs to acquire statistically robust samples, in part because of the expense and uncertainty associated with sampling adult winter steelhead trout in natural habitat areas. We did, however, take advantage of opportunities to collect data from natural-origin steelhead trout through existing monitoring programs in natural stream areas at Willow Creek Weir on the Trinity River, Scott Creek Weir, and at Felton Diversion Dam on the San Lorenzo River.

We also recommend that future work on morphometric variation in California steelhead trout include the South-Central California Coast and Southern California DPSs. Nominally, these population groups of steelhead trout would be coastal type. However, the historic population structure in these southerly DPSs may have included complements of both the large, coastal type such as those still observed in the Carmel and Big Sur rivers in Monterey County (R. Titus, CDFW, unpublished age-and-growth data), as well as smaller, inland-type steelhead trout that may have prevailed in interior drainages, and that have been especially impacted by water diversions and other habitat limitations (Busby et al. 1996). Adult steelhead trout would have made relatively extensive migrations to reach reproduction areas in many of these interior drainages, per our provisional definition of inland-type steelhead trout. Such drainages include the Salinas River (San Luis Obispo and Monterey counties), Santa Maria and Santa Ynez rivers (Santa Barbara County), and the Santa Clara River (Ventura and Los Angeles counties), and other drainages through the southernmost distribution of steelhead trout in the eastern Pacific.

Our final recommendation for future work is to further refine the terms that we used in this study to define coastal and inland type steelhead trout. The mean migration distance inland to our sampling locations differed by an order of magnitude, averaging 28 km at coastal DPS sampling locations, and 278 km at inland DPS sampling locations. We selected 160 km as the general benchmark distance for distinguishing between coastal and inland migrations, given that it is the approximate midpoint between the mean distances of our coastal and inland groups of sampling locations. While provisional, future work should be based on an appropriate sampling design to determine distributions of migration distances to putative coastal and inland steelhead trout reproduction areas in all six California DPSs. Other environmental factors that may influence the selection of morphometrics in various population groups of steelhead trout could also be included, to develop a more comprehensive assessment of possible determinants of evolved patterns in steelhead trout morphology. Such factors may include elevation gain, hydrology, and water temperature, some or all of which could influence the selection of both physical and physiological traits of steelhead trout relative to the requirements for reproductive migration.

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A synopsis of recent history of California's inland trout management programs: litigation and legislation

DAVID C. LENTZ* AND MARK A. CLIFFORD

California Department of Fish and Wildlife, Fisheries Branch, 830 S Street, Sacramento, CA 95811, USA (DCL)

California Department of Fish and Wildlife, Fisheries Branch, #3 North Old Stage Road, Mt. Shasta, CA 96067, USA (MAC)

*Correspondent: dave.lentz@wildlife.ca.gov

The management of trout fishing, trout fisheries, and the culture and distribution of hatchery-reared trout have been important features of inland fishery management programs for over 140 years. California's fishery managers have striven to respond to the perceived needs and preferences of the state's inland anglers and to include the values of the larger society. Over the decades those needs and values have changed and resulted in changes in the direction of trout management. In this paper we look at a series of events over the past 25 years and examine how those events are influencing the direction of California trout management programs.

Key words: California, conservation, fish hatcheries, legislation, litigation, native trout, *Oncorhynchus* spp., restoration, trout management

A focus on trout fishing in California goes back to the origin of the state's principal agency concerned with fisheries, the Board of Fish Commissioners, established in 1870 by the Governor. Trout were reared at the state's first public fish hatcheries located near the City Hall in San Francisco and the University of California in Berkeley (Shebley 1922). In the late 1800s and early 1900s, many non-native fishes were introduced to California, especially some that were regarded highly as food fishes like American shad (*Alosa sapidissima*), striped bass (*Morone saxatilis*), common carp (*Cyprinus carpio*), catfishes, and black bass. Non-native trout were also early arrivals to California with brown trout (*Salmo trutta*), lake trout, (*Salvelinus namaycush*), and brook trout (*Salvelinus fontinalis*) all arriving prior to 1900 (Dill and Cordone 1997). Brook trout eggs were hatched at the San Francisco hatchery in 1871, the same year shad were introduced in the Sacramento River (Dill and Cordone 1997), and the Fish Commissioners first stocked brook trout in 1872 (Shebley 1917). The early Fish Commissioners were more interested in providing fish for food than for recreation, and sport fishing licenses were not required until 1913 (Dill and Cordone 1997). In the late 1800s, "Sport fishing was actively pursued mainly by wealthy dudes, but most families netted fish

for the table and there were no restrictions on commercial fishing” (Harrell 1970; quoted by Dill and Cordone 1997). In the 1800s, a prevailing sentiment of California society, and the spirit of the day, was that people “did not protect a resource but used it” (Dill and Cordone 1997).

Trout fishing has been among the most important and popular programs of the Department of Fish and Game (CDFG; beginning in 2013, California Department of Fish and Wildlife [CDFW]) through most of its history. In the 1980s trout fishing was the most popular type of fishery, and accounted for about 60% of inland angling effort in California (Fletcher and King 1988). While there may be fewer anglers now per capita, trout continue to be the most sought after target of inland anglers, currently at 59% of the total of all types of fish (USFWS 2011).

The early 1900s saw emphasis on the hatchery production of fingerling trout from eggs collected at dozens of egg-taking stations. Distribution of fingerlings was initiated throughout the state. Additionally, trout were moved from their native streams to many other waters, especially the previously fishless lakes and streams of the high mountains. These introductions were carried out by the “Deputy Fish Commissioners” and transplanting trout was considered a great benefit, even if it was a difficult task (Ellis and Bryant 1920). Even conservation organizations like the Sierra Club were involved in the effort to fix “barren” waters and create sport fisheries through transplants (Rahel 1997).

Beginning in the 1930s, CDFG saw the development of scientific staff with a cadre of fishery biologists to direct freshwater fishery management programs. After World War II, a major expansion of the hatchery program began and extended into the 1960s, a period when most of the current trout hatcheries were built (Leitritz 1970). The catchable trout program was then expanded after heavy angling pressure depleted trout in roadside waters. Catchable trout stocking spread rapidly throughout the state after the war, and the Wildlife Conservation Board provided more than \$4 million for hatcheries (Butler and Borgeson 1965). The post-war era also saw increased stocking of fingerling trout, when CDFG developed its methods for aerial stocking of mountain lakes by dropping fingerlings from tanks in specially outfitted airplanes, a development that made stocking high mountain lakes much more efficient and less expensive.

As a result of the hatchery expansion, production of catchable trout (then 18 to 20 cm rainbow trout [*Oncorhynchus mykiss*]) quadrupled to about seven million fish annually by 1962 (Butler and Borgeson 1965). In 1990, the Department stocked 19 million trout (average weight: 8.1 fish per kg).

The post-war hatchery expansions coincided with a period of dam-building and a huge increase in reservoirs, thus changing the state from one marked by lotic environments with trout and salmon as the featured gamefish to one with significant lentic habitats having both warmwater and coldwater fisheries. Emphases remained, however, on coldwater species for both anglers and fishery managers (Dill and Cordone 1997). Fishery managers also embarked on large chemical treatment projects to eliminate “rough fish”—native and introduced fishes that were deemed to have low “sporting value.” Many miles of streams and large lakes and reservoirs were treated with rotenone to eradicate these fishes considered competitors of trout (Dill and Cordone 1997). One of the largest efforts was the treatment of 256 miles of streams in the Russian River basin, during 1952–1954, which eliminated most of the nongame fish populations (Pintler and Johnson 1956). During this era, an emphasis on natural resource management was practically abandoned and the management of inland sport fisheries was paramount, resembling “that of a big, modern farming program” (Dill

and Cordone 1997). The California Fish and Game Commission's inland fisheries policy stated, "The basic objective should be to supply the best possible fishing for the greatest number of anglers" (CDFG 1953).

By the late-1960s, the social climate was changing rapidly and awareness of environmental issues was becoming increasingly widespread. One result of these societal shifts was the enactment of environmental legislation at both national and state levels. The National Environmental Policy Act (NEPA), its state counterpart the California Environmental Quality Act (CEQA), and the Endangered Species Act of 1973 (ESA) all would have great impact on trout management and natural resource conservation in general. Other examples of this change in the social climate were the establishment of the Clean Air Act and the Clean Water Act.

While hatchery trout programs remained the largest aspect of inland fishery management, the 1970s saw the advent of programs focusing on wild trout management in California as well as on the conservation and restoration of the state's native trout. A social climate that was putting a greater emphasis on native wildlife, natural environments, and increasing ecological knowledge likely contributed to those changes.

Trout management in California has reflected in many ways the prevailing values of the times during its history. Changing values of the angling clientele and of society have altered management priorities, and perhaps for some not as fast as it was needed. Californians have not been shy about using the legislature, the courts, and the ballot to effect changes they want in resource conservation and management. These approaches produced a significant outcome for trout streams and other resources in California when litigation brought by California Trout in the 1980s succeeded at applying the Public Trust Doctrine to Mono Lake, and enforcing long-ignored provisions of the California Fish and Game Code (Code; Sections 5937 and 5946) that required keeping "fish in good condition" downstream of dams. In this paper, we look at examples of these actions and the subsequent outcomes for the state's management of trout.

METHODS

We selected a set of important events of the past 25 years to examine the roles they have played in the recent direction or development of California's inland trout management programs. We reviewed literature from scientific journals, bulletins, proceedings, and other sources often referred to as "gray literature" to inform and build context regarding these events, the times when they occurred, and the affect they had on Department operations. Other sources reviewed included documents such as unpublished reports, manuscripts, departmental letters and correspondence, and planning documents. Moreover, we reviewed legislation that directs current CDFW trout management programs along with decisions and orders resulting from litigation concerning those programs. These sources were used to describe the effects of the events that contributed to the direction of trout management in recent times.

EVENTS IN TROUT MANAGEMENT

Trout Unlimited lawsuit, 1992.—Trout Unlimited (TU) held a long-standing view that the Department's budget resources were spent disproportionately in favor of the hatchery trout program and that management of wild trout, native trout restoration, and coldwater habitat protection were substantially underfunded. Additionally, they believed the stocking of hatchery trout had a suite of negative effects that had never been analyzed through environmental regulatory review (CEQA and NEPA). Trout Unlimited developed and filed a lawsuit against CDFG in 1992 that addressed these concerns and petitioned the court to mandate preparation of an Environmental Impact Report (EIR) concerning the trout hatchery and stocking program. The lawsuit outlined effects on wild trout from stocking hatchery trout that included the effects of disease, competition, predation, and hybridization, along with pollution from hatchery effluents and efficiency issues in both culture and stocking of hatchery trout.

In response, the Department agreed to conduct the environmental review under CEQA in 1993, and consultants were contracted to prepare a draft environmental document in 1994. By 1996, after rounds of review and revisions to the draft, the document was deemed to be inadequate. There were insufficient funds and no mechanism was in place to retain a contractor for additional work on the document. In lieu of re-starting the stalled hatchery and trout stocking environmental document, CDFG initiated development of the Strategic Plan for Trout Management in 1997. This process was established to determine goals and strategies for guidance of all of the Department's inland trout programs into the early 2000s.

Lake Davis chemical treatment, 1997.—The appearance of northern pike (*Esox lucius*) in reservoirs on tributaries to the Middle Fork Feather River, Plumas County, during the 1990s created formidable challenges for CDFG fishery managers. There was great concern this coolwater predator could escape or be illegally moved into the Feather River and gain access to the Sacramento-San Joaquin Delta, which is believed to have very favorable habitat conditions for northern pike. If northern pike had become established in the Delta it would have placed listed salmon (*Oncorhynchus* spp.), steelhead trout (*O. mykiss irideus*), delta smelt (*Hypomesus transpacificus*), and other species of concern at great risk from predation. Additionally, there would be almost no possibility of eliminating that invasive predator from the hundreds of miles of Delta streams and river channels once it became established. It was apparent that containing northern pike in the headwaters and eradicating them from the reservoirs was the best management option to control the expansion of pike into other state waters.

Northern pike were reported by anglers from Frenchman Lake in the 1980s, and in 1988 an angler reported catching a 3.2-kg northern pike (Lee 2001). A chemical treatment of the reservoir and its tributaries using rotenone was successfully implemented in 1991. Northern pike were found downstream of Frenchman Lake in the Middle Fork Feather River during 1992, and a chemical treatment there also appeared successful. Soon after, northern pike were reported in nearby Lake Davis, a popular and productive trout fishery. By the mid-1990s, northern pike had become a dominant species in the reservoir. Planning for a rotenone treatment of Lake Davis had the added difficulties of a larger project area, a fishery with greater contribution to the local economy, and the lake served as a domestic water source for the town of Portola. Departmental fishery managers encountered growing opposition from residents and local governments in Plumas County as the treatment plans developed from 1995 to 1997. The treatment was completed October of 1997 under difficult

and extraordinary circumstances that were the result of actions of local opponents of the project (Lee 2001).

The level of conflict and controversy surrounding the Lake Davis rotenone project was unforeseen by some CDFG fishery managers. Public opinion and reaction also had unforeseen consequences for California and for the use of piscicides within the state and in other western states. After Lake Davis, litigation was filed in Montana to stop a large chemical treatment project of Cherry Creek that was intended to facilitate the restoration of native cutthroat trout (Wilkinson 1999). Plumas County interests were able to influence legislation that provided nearly \$10 million of awards to mitigate economic damages resulting from the Lake Davis treatment project. The ensuing controversy around use of rotenone created enough negative public opinion and opposition that CDFG leaders were reluctant to propose new rotenone projects. Not having rotenone available as a management tool hindered progress in the restoration of native trout in California for more than a decade.

Strategic Plan for Trout Management.—In the 1990s, CDFG fisheries leaders recognized the need for an overarching plan that identified issues and concerns of all of the state's trout management programs. They then began to formulate goals and strategies to address those issues into the future. A previous planning effort that looked at "forward focused management" for trout recommended emphases on stream restoration and protection, protection of quality wild trout fisheries, restoration of native trout, and efficient use of stocked trout (Villa and Deinstadt 1990). Coincidentally, these priorities were expressed as concerns in TU's 1992 litigation.

Development of the Strategic Plan for Trout Management (Trout Plan) became a much more involved and lengthy process than anticipated in 1997 by the Inland Fisheries Chief when, in writing to TU, he expressed the belief that a draft of the plan would be ready within that year. Development of the Trout Plan involved public participation, focus groups, special interest groups, and hundreds of individuals. A framework draft of the Trout Plan was produced and distributed for public review in 1998. The draft document received numerous cycles of internal review by dozens of CDFG personnel, was revised during 1999–2000, and a public review draft was released in June, 2000. The document was finalized in 2003 and signed by the Acting Director in January, 2004.

High mountain lake and stream inventory.—During the 1990s increasing concern was expressed over the declining status of native amphibians and other aquatic species in high elevation lakes and streams. This was especially true for the thousands of lakes in the Sierra Nevada where, historically, there were no native fishes present (Drost and Fellers 1996, Knapp and Matthews 2000). The 1990s was a period that saw efforts such as the Sierra Nevada Ecosystem Project that brought attention to the plight of species like the mountain yellow-legged frog (*Rana sierrae*), which had experienced drastic declines in distribution and abundance (SNEP 1996). Amphibian decline was frequently attributed to the decades of stocking non-native predatory trout by the Department. Fisheries management during the 1990s also incorporated more emphasis on conservation efforts for native non-game species, biodiversity (Winter and Hughes 1997), and ecosystem management approaches (Nielsen 1995).

By 1998, CDFG had initiated a large, multi-year resource assessment program to survey the more than 12,700 high elevation lakes and streams in the Sierra Nevada and Cascade Ranges outside of the national parks. These surveys were intended to gather information on distribution and abundance for native aquatic fauna, introduced trout, and other fishes so that restoration and conservation of native species and management of sport

fisheries could be planned appropriately. Having results of these surveys and assessment data enabled the CDFG to develop a series of Aquatic Biodiversity Management Plans (ABMPs) for watershed basins to guide management that sought to balance the restoration of native amphibian populations while retaining recreational fishing in appropriate waters (Milliron et al. 2004). The ABMPs were first developed for watersheds in the eastern Sierra Nevada and later were prepared for other mountainous areas of the state where conflicts remained between trout management and native amphibian conservation.

In 1999, the decision was made to temporarily discontinue airplane stocking of high-elevation lakes with fingerling trout, a fishery program that had been conducted continuously since the 1950s. This stocking hiatus, in part, helped with determinations of which lakes supported self-sustaining trout populations but also responded to critics concerned with the role of trout stocking in the extirpation of native frogs from much of their historic range. The hiatus also allowed time for survey teams to locate remaining frog populations (M. Lockhart, California Department of Fish and Wildlife, personal communication). The issue of trout stocking impacts to amphibians and other native species remained a contentious issue for CDFG and for organizations that selected it as a focus of litigation against the Department's hatchery and stocking programs.

Assembly Bill 7, 2005.—Concerns brought to the California Legislature during 2005 resulted in passage of Assembly Bill 7 (AB 7), which promulgated several mandates for CDFG trout management. These mandates were codified by revisions to the Code (i.e., Section 13007). First, the law established the Hatchery and Inland Fisheries Fund (HIFF) as part of the Fish and Game Preservation Fund. Then, it required one-third of all sport fishing license fees be deposited into the HIFF for the purposes of supporting the state fish hatcheries, the Heritage and Wild Trout Program, and law enforcement activities related to the trout fishing programs. While having funds dedicated by law for trout programs was helpful, the available funding still resulted in challenges for managers to meet the law's requirements. Progress produced by the expanded funding in the early years was short-lived as production goals increased, along with escalating costs in later years.

A featured element of AB 7 was the explicit linkage of some goals for trout production by the state's trout hatcheries to the number of sport fishing licenses sold annually. Initially, the goal for trout released was set so that the number of pounds of trout produced increased from 2.25 to 2.75 pounds (1 kg to 1.25 kg) of trout released per license sold by 2009. Catchable-sized or larger trout were emphasized and comprised from 1.75 to 2.25 pounds (0.8 kg to 1.0 kg) for those initial requirements. Another requirement was that at least 25% of the total number of trout produced by the state must be native trout species. Those species were described as Heritage Trout in Section 7261 of the Code (i.e., California golden trout (*O. m. aguabonita*), Little Kern golden trout (*O. m. whitei*), Kern River rainbow trout (*O. m. gilberti*), Eagle lake rainbow trout (*O. m. aquilarum*), McCloud redband trout (*O. m. stonei*), Goose Lake redband trout (*O. m. ssp.*), Warner Lakes (Valley) redband trout (*O. m. ssp.*), Lahontan cutthroat trout (*O. clarki henshawi*), Paiute cutthroat trout, (*O. c. seleniris*), coastal cutthroat trout (*O. c. clarki*), and coastal rainbow trout (*O. m. irideus*)). Assembly Bill 7 specified a time table for the hatchery system to meet native trout production percentages and numbers of Heritage Trout species produced. Later, initial AB 7 language was modified to both remove target dates that had expired and clarify native trout production requirements.

Production goals mandated by AB 7 stimulated increases in fish production from 2005 to 2008. However, after 2008 and due in part to the loss of federal grant funding for

the hatcheries, the production mandates have proven difficult for the hatcheries to meet. Conditions such as aging hatchery infrastructure, available space, and adequate water have limited the capacity of the hatchery system to produce sufficient trout. Increasing costs, such as fish feed and truck fuels, have factored into production goal shortfalls, and budgetary restrictions on expenditures for capital improvements to facilities have delayed upgrades at hatcheries intended to enhance production capacity.

For the Heritage and Wild Trout Program (HWTP), the similar benefit of enhanced funding in the first years after passage of AB 7 enabled increases in biologist staffing and enhanced efforts for resource assessment surveys and monitoring. In 2013, HWTP began using HIFF expenditures as the State's financial match for needed federal grants. The combined funding was used to maintain wild trout management efforts. Further legislation (Senate Bill 384, 2007) required HWTP to annually recommend to the California Fish and Game Commission additions of 25 stream miles and one lake for designation as Wild Trout waters. This process revised a previous legislative mandate for special angling regulations (Code Section 1727) to one specifically requiring wild trout management. This mandate, along with required annually increasing workloads, created significant work-planning challenges for HWTP.

Hatchery and stocking program—more litigation.—A special project of environmental law students at Stanford University developed into a lawsuit in 2006 with the environmental groups Pacific Rivers Council (PRC) and Center for Biological Diversity (CBD) as leading plaintiffs in litigation against the Department's hatchery and stocking programs. These plaintiffs had been in discussions with CDFG fisheries leaders, requesting the Department prepare an EIR concerning hatcheries, stocking, and their effects on native species. A budget request for \$1 million to contract for EIR preparation was authorized. As the lawsuit progressed, in May of 2007 the Superior Court ordered CDFG to comply with CEQA and prepare an EIR to address potential environmental effects of its hatchery and stocking programs. In order to receive federal funding (Sport Fish Restoration Act [SFRA] grants) for hatcheries, the document was prepared to also meet federal NEPA requirements by simultaneously developing an Environmental Impact Statement (EIS). The CDFG trout hatcheries and stocking programs are not eligible for SFRA funding until the NEPA process is completed. This resulted in a significant funding shortage from 2008 to the present.

While efforts were made to secure needed funds in addition to the initial \$1 million for the requisite EIR and EIS and CDFG was revising the contract for developing the EIR, the court issued an interim order restricting fish stocking by the Department. The order prevented stocking of non-native fish where native fishes and amphibians from a list of 25 species determined by the plaintiffs were present, or where field surveys had yet to be conducted to ensure those species were absent. Exceptions were negotiated to these restrictions to allow stocking in artificial reservoirs > 400 ha (1,000 acres) in area or in reservoirs less than <400 ha if they were not hydrologically connected to rivers supporting sensitive native species, like the northern red-legged frog (*Rana aurora*). The state court eventually ordered CDFG to complete the EIR and release the associated CEQA determination by January 2010.

Lake Davis, 2007—more pike and more rotenone.—After the 1997 Lake Davis chemical treatment, CDFG faced challenges in rebuilding relationships with the local community. To improve those relationships, surrounding Plumas County waters were stocked with 1.2 million trout, an early opening for the local 1998 trout season was approved, and Lake Davis was re-stocked with more than 2 million trout in 1998 (Lee 2001). Unfortunately, post-project monitoring detected northern pike in the lake during 1999. The Department

initiated efforts to address the situation and established a team located in Portola to develop responses to the expanding numbers of pike. A task force comprised of community members, local government representatives, and Department personnel was formed to develop solutions to the infestation. The director of the Department assured the community that no Proposition 65 chemicals known to be carcinogenic, have reproductive effects, or that persist in the environment would be used by CDFG to treat Lake Davis. The task force then prepared a plan of 12 specific control measures and techniques that did not involve rotenone (CDFG 2000). After several years of implementing the control measures, it became apparent the methods were ineffective. The northern pike population was rapidly expanding and was substantially degrading the trout fishery and its economic benefits to the community. It was then realized rotenone treatment was the only action that might succeed in eradicating northern pike. Prior to the development of control measures by the task force, a CDFG workgroup of about 30 biologists met in September 1999 to review control options, and concluded that draining of the lake and chemical treatment had the highest probability of success (Lee 2001).

Unlike the 1997 lake treatment project, CDFG conducted extensive public outreach and collaboration with local stakeholders. The result was a rotenone treatment that was accepted by much of the community and was regarded as a collaborative decision by the community and the Department. The project required an extensive environmental analysis. Few, if any, inland fishery projects undertaken by CDFG match the level of staffing, preparation, special funding (millions of dollars), coordinated implementation, and complexity that resulted. Northern pike have not been found in Lake Davis since this treatment; the project and its local collaboration were notable successes.

Environmental documents.—Following the 2006 lawsuit by PRC and CBD, the Department completed an EIR in January 2010, thereby complying with court order. The resulting document identified several impacts of the hatchery and stocking programs and provided mitigation measures, many related to how hatcheries would be operated in the future. For trout hatcheries, these measures directed the minimizing of contaminants and pathogens in hatchery discharges, best management practices for minimizing risk of disease transmission to native amphibians, and monitoring of influent and effluent for aquatic invasive species. For stocking practices, measures directed the minimizing of unintentional releases, increased stocking of triploid (i.e., sterile) trout to reduce genetic impacts to steelhead and other native trout, and the preparation of hatchery genetic management plans. Other measures addressed informing anglers about control of invasive species like New Zealand mudsnail (*Potamopyrgus antipodarum*) and minimizing angling impacts to sensitive riparian habitats.

An important measure was defining a systematic approach to the CDFG approval process for trout stocking. The Department developed a pre-stocking evaluation protocol, outlined in Appendix K of the environmental document, which required field surveys to be conducted for the presence of certain sensitive, native species (Decision Species) when a stocking location had been determined to have suitable habitat for the species. The Department has implemented the requirements since 2010; however, the NEPA process is unfinished. Two Biological Assessments, one for the USFWS and one for the NMFS, are approaching completion to fulfill compliance with Section 7 of the ESA. Since implementing of the new measures, the Department now stocks about 30% fewer waters in the state. The net result has been a reduction from approximately 1,200 stocking locations in 2005 to about 800 in 2014.

After release of the Final EIR in 2010, CBD and other plaintiffs filed another lawsuit challenging the adequacy of the EIR, contending that analysis of potential project impacts, formulation of mitigation measures, and identification and evaluation of project alternatives did not meet CEQA requirements. In 2012, a Sacramento Superior Court judge ruled that the EIR fully complied with CEQA. Plaintiffs appealed, and the Appellate Court also found the EIR was adequate under CEQA in January 2015 (N. Murray, CDFW, personal communication).

Senate Bill 1148, 2012.—Senate Bill 1148. (SB 1148), introduced by Senator Pavley, had a large focus and influence on CDFG trout management. SB 1148 established direction for both HWTP and hatchery trout programs through revisions and additions to the Trout and Steelhead Management Planning Act of 1979 (Code Section 1725 et seq.) and Code Section 13007 (AB 7). Emphasis in SB 1148 was placed on the importance of managing for native trout, reducing the impacts of stocking hatchery trout on native trout and other native aquatic species, increased emphasis on stocking of native trout, and the importance of integrating stakeholder and public involvement in developing trout management plans and revising the Trout Plan.

For the trout and inland salmon hatchery production program, SB 1148 established new priorities and requirements. Primarily, it required a shift in focus from traditional hatchery trout strains to native trout strains for stocking inland waters. A important aspect to the legislation was the requirement to ensure that all trout stocked for recreational purposes would be sterile through triploidy or other means. Some exceptions were allowed, including for use of surplus brood stock or situations where native trout strains were appropriate for stocking. Department hatcheries had already been using pressure-induced triploidy, but the new law required a rapid expansion of use of this method, development of other techniques, staff training, and acquisition of specialized equipment to meet trout stocking needs. In many cases, new triploid protocols were needed for species for which sterilized eggs had not yet been produced by CDFW, such as inland Chinook salmon (*O. tshawytscha*), brown trout, and brook trout. In other cases, triploidy methods were refined for several strains of rainbow trout, including Eagle Lake rainbow trout, one of the most important hatchery native trout strains stocked widely around the state. The Department commenced on developing new, and revising existing, protocols in 2013.

SB 1148 restated previous legislative direction regarding the importance of wild trout management, cold water habitat protection and restoration, determining appropriate angling regulations for trout waters, and appropriate stocking of hatchery-produced trout. New emphases were placed on prioritizing native trout for stocking and managing wild populations of native trout. Additional requirements for completing and revising wild trout management plans also were included.

The new law also outlined areas of emphasis for the Trout Plan and required updates of the Trout Plan every five years. It required the establishment of an internal department Strategic Trout Management Team to oversee trout management statewide using the framework of the Trout Plan and with responsibility for developing new, watershed-based, trout management plans. The law required management plans and trout stream and lake inventory surveys be made available on the Department's website. A number of education and outreach efforts were also recommended as well as surveys of anglers to determine use, satisfaction, and preferences.

DISCUSSION

Trout managers have been guided by goals such as the directive to provide and enhance a diversity of angling opportunities found in the Trout Plan. Additionally, managers have sought to respond to the preferences or perceived needs of the trout angling public. In 1990, the daily bag limit for trout was reduced from 10 to five and the size of catchable trout increased from about 6.6 trout/ kg to 4.4 trout/kg. This action was in response to the preferences of anglers that the size of fish caught was more important than the number of fish caught, which was determined in part from a survey of inland anglers (Fletcher and King 1988). Other information from the angler survey and changing demographics of California anglers helped guide the Department in establishing an urban fishing program in 1992. This program provided stocked trout and catfish in more easily accessible areas for anglers seeking fishing places closer to home. However, groups like California Trout and TU were not satisfied with their perception of the Department's emphasis on hatchery trout management solutions. In their view, the emphasis on native trout, wild trout management, and habitat protection was lagging and needing greater emphasis by trout managers (White 1989). Not seeing acceptable progress in these management activities led to the 1992 litigation. While this litigation may not have produced the intended outcome, it prompted the Department to produce the Trout Plan. That plan established protection, restoration, and enhancement of coldwater ecosystems as an equal theme with diverse, recreational angling opportunity. Perhaps overdue, these changes in society's values are influencing the direction of trout management (Panek 1997).

The role of rotenone application as a tool in native trout restoration was substantially reduced in California due to the controversy over Lake Davis treatments. Consequently, the use of rotenone to expand and establish new populations of native trout in California was curtailed from 1996 to 2013. A great deal of progress in the restoration of Lahontan cutthroat trout, Paiute cutthroat trout, and California golden trout was hindered as a result. An ironic aspect of the current native trout management strategy is that "to preserve native trout stocks, much effort today is directed at removing the same trout species introduced so enthusiastically by our fisheries forefathers" (Rahel 1997).

California seems to be a proving ground for environmental litigation. When the Department and federal partners tried to implement rotenone treatments called for under the federal recovery plan for the threatened Paiute cutthroat trout in the early 2000s, opponents of rotenone use were able to stall treatment projects in successive years through lawsuits in both state and federal court. While successful in state court, CDFG was stopped in 2005, literally hours from the beginning of a rotenone treatment of Silver King Creek, Alpine County, by a federal judge of the Eastern District of California. The judge later determined the controversy over rotenone was so great that it warranted an EIS to comply with NEPA. It took until 2013 to complete the NEPA process, address new rounds of litigation, and outlast opposition to again use rotenone for native trout restoration.

The major outcomes from the Lake Davis experience for native trout managers in California are the substantial challenges that now must be faced when considering a chemical treatment of rivers, lakes, or reservoirs. Substantial effort must be put forth in pre-project planning and public outreach, in addition to project implementation. The environmental analyses, pre- and post-project monitoring, and environmental compliance now required

are more involved and more costly than the field projects themselves that, ironically, were instrumental in successfully establishing many new populations of native trout in the 1980s and 1990s. Without infusion of new financial and staff resources, the pace of native trout restoration will be much slower compared to past decades.

Legislation, such as AB 7, was intended to provide the budgetary stability for the trout management programs to consistently progress. Ten years later there are still many challenges to meeting hatchery production goals and to accomplishing wild trout resource assessment and management. Litigation and implementation of the Hatchery EIR have not simplified the hatchery trout program's ability to meet the mandates of AB 7 or the new priorities and requirements of SB 1148. Additionally, a consequence of AB 7 is one that challenges the implementation of one of the goals of the Trout Plan: "Improve the effectiveness and efficiency of how hatchery trout are utilized for providing angling opportunities." With a goal that requires over 1.8 million kg of trout produced annually and fewer locations to stock those fish, fishery and hatchery managers are hard pressed to both produce large amounts of trout and efficiently stock them into appropriate waters. The Trout Plan will need to be revised with these new legislative requirements, mandated priorities for native trout, and legal direction from the EIR in mind.

Societal change of increasing perceived value for native species and natural ecosystems have appeared, perhaps at times disconnectedly, in a variety of legislation, litigation, and events that have directed California trout management. These events clearly are having effects. In the high mountain lakes, trout stocking has been discontinued in more than 90% of the lakes that received hatchery fish prior to 1998. California's hatcheries are experiencing a paradigm shift, including changes in their missions to produce more native trout and triploid "recreational" trout. A new direction has been established to produce the right fish, for the right purpose, for the right location. The restoration of native trout is reinvigorated with planning for new chemical treatments and efforts to remove non-native trout with mechanical means (e.g., electrofishing or nets), but with the primary goal of expanding California's native trout populations. The Trout Plan awaits revisions that will capture these directions and reflect new priorities and goals.

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BOOK REVIEW

The Fish in the Forest – Salmon and the Web of Life

Dale Stokes. 2014. University of California Press, Berkeley, USA. 159 pages. \$29.95 (hard cover). ISBN: 9780520269200

In *The Fish in the Forest - Salmon and the Web of Life*, author Dale Stokes has provided the curious reader a succinct account of the Pacific salmon (genus *Oncorhynchus*). In his easy to read book, Stokes explains in detail how salmon along the Pacific coast of North America have influenced and sculpted the ecology of the temperate ecosystem.

The book is divided into six sections, each addressing a particular aspect of the salmon and its role ecologically and culturally: The Forest and the Fish, Life and Death of a Salmon, The Salmon Signature, Salmon Gestalt, The Salmon Forest, and Full Circle. In each of these sections, Stokes skillfully connects the salmon with the environment in which it survives. As the story unfolds the reader finds him or herself learning ecological principles, biological terminology, and basic concepts of chemistry. Salmon taxonomy is covered as well as the role of indigenous peoples and their cultural connection to salmon. Scattered throughout the book - almost on every page - are outstanding photos by photographer Doc White. The photos supplement the text by providing a glimpse into salmon day to day life, their predators, and the landscape the fish support. A reference section is included at the end of the book.

A common theme is interlaced throughout the book: how salmon influence the environment beyond the streambed. Salmon are a keystone species, meaning that their presence in the ecosystem supports hundreds of other species, either directly or indirectly. The salmon transport nutrients from the ocean to the temperate forest. These nutrients, such as nitrogen and phosphorus, are used by plethora of other species, from bacteria, invertebrates, and parasites, to bears, wolves, and old growth forests. The conveyor belt of nutrients and energy provided by salmon has created the Pacific forests as we see them today. The environmental interconnectedness between the salmon and other species is profound. For example, plant flowering periods and the life cycles of their pollinators are synchronized more so on the spawning cycle of the salmon rather than other typical factors such as photoperiod and temperature.

On page 130 the author summarizes the concept of salmon ecology rather well:

“The net effect of salmon on an ecosystem is formed from the balance between salmon being sources of enrichment and salmon being forces of disturbance, both roles complicated by the geomorphology of the landscape they live in. The same is true of nearly all creatures in the Salmon Forest and across the earth, including bears and eagles and native fishermen. Ecosystem dynamics in our world are rarely formulated with straightforward links that are easily defined and parameterized, or with a convenient separation between forces abiotic and biotic. Instead those dynamics are entwined under the guiding force of natural selection, linking

hundreds of species in complex networks with often subtle feedback loops that provide hidden stability and that are difficult to ascertain. The salmon in the forest provides a unique window through which to observe the mechanisms that shape our planet.”

The Fish in the Forest is a must read for those that appreciate aquatic ecosystems and have an interest in salmon ecology. The co-evolution between salmon and the forests of the Pacific coast represent hundreds of thousands of years of nutrient ebb and flow and the book communicates the interdependence as a result of this relationship. There is a cautionary tale however, that is exposed when reading the book: salmon are in decline and the effects of exploiting rivers by building hydroelectric dams and other structures is having a significant ecological impact on the forests, the fish, and everything else connected. This book is the first step in educating oneself on salmon ecology and making a difference before it is too late.

HOWARD O. CLARK, JR. *Garcia and Associates, 993 Ezie Avenue, Clovis, CA 93611, USA*

BOOK REVIEW

Fishing the River of Time

Tony Taylor. 2012. Greystone Books, Berkeley, California, USA. 216 pages. \$19.95 (soft cover). ISBN: 978-1-77100-057-4

Author Tony Taylor, a geologist by training, has written a whimsically brief glimpse into his long life. He weaves a story that combines several disciplines, including ecology, geology, boating, history, fishing, and the art of observation. He traveled back to Lake Cowichan, in western Canada, after a several decade absence, rented a cabin and awaited the arrival of his grandson. He arrived a few days early to scout out the local rivers in hopes of catching a fish and show his grandson a land he once knew and enjoyed.

The book, however, is much more than meets the eye. It's a journey into the past, present, and future. Taylor enjoys water and especially rivers. Watching the flowing water allows one to reflect and think. Thinking is important; it helps sort out the day's experiences and allows the mind to float hoping to pick up on a different perspective on something so common. While preparing for his grandson's arrival, Taylor was quick to explore the landscape and tells the reader stories about his experiences at Lake Cowichan and its people. Nature is an important theme in the book and Taylor's discussions and storytelling does not disappoint.

Taylor is modest; he does not claim to be an expert on fishing, rivers, or fish. He tries to convey to the reader that there are different approaches to the various questions someone may have about life and learning is a two-way street. Ask questions and listen to others. The wisdom that can be gleaned from simple conversations may surprise you. The pending arrival of the young grandson provided plenty opportunity for sharing and testing the two-way learning paradigm.

Finally the day arrives when Taylor's grandson, Ned, appears at his cabin doorstep. They do not know each other particularly well but the historically and culturally rich backdrop of Lake Cowichan is a setting that facilitates relationships. Not just between people, but between people and the land; a land that encompasses all states of matter: earth, fire, water, and air. Ned is curious about his grandfather and they take an immediate liking to each other. Fishing and the idea of catching a fish is the vehicle used by the characters to become familiar with one another. It does not take long for the conversions to crystalize and Ned and Taylor begin their journey along the river's edge. The older generations have an obligation to pass along stories and knowledge to the next generation and the book exemplifies this age-old tradition very well.

It's not about catching a fish; it's the experience on the river. Watching the water, studying the flows and thinking like a fish. Keen observation is always key. Perhaps when someone is well versed with nature, you become part of the natural cycle. The concept of cycles was always in the back of Taylor's mind; from the spawning fish to the bears that feed on them. The dying and dead fish in turn provide much-needed nutrients to the granitic soils that otherwise could not support the vast Canadian forests. The fish make the difference. Taylor realized this, when he compared the Canadian ecosystem to the soils of Australia,

where Taylor has lived during the past several decades. Ned kept asking questions, and Taylor provided as truthful answers as he could. The two-way learning was clear and true; Taylor learned much from Ned because the grandson had an untainted and innocent look on life.

The book is a delightful read. It brims with wisdom and is a refreshing pause in our ever-busy lives. Taylor writes on page 193, “Anglers don’t fish to catch fish; if we did, we would net them. We hunt fish, which are very smart, in order to outwit them, and we are less successful than we like to think. Many great anglers have said fishing is a ridiculous passion because fish cost far more to catch than they would to buy in a market, but we continue the pursuit because it enables us to think. The mystery of water fascinates us.” Certainly this statement can be considered a metaphor of life. In the end, they finally catch a fish, but it’s not about the fish, it’s about the journey.

HOWARD O. CLARK, JR. *Garcia and Associates, 993 Ezie Avenue, Clovis, CA 93611, USA*

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L. Lovell	Los Angeles County Sanitation District

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T. Nosal	California Department of Fish and Wildlife
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E. Parnell	Scripps Institution of Oceanography
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T. Wilcoxon	Millikin University
D. Wilken	Santa Barbara Botanic Garden
J. Willoughby	Willoughby Ecological Consulting
C. Witham	Independent Consultant
H. Wittmer	Victoria University of Wellington, New Zealand
D. Wright	California Department of Fish and Wildlife
L. Yamanaka	Pacific Biological Station, Canada



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BOOKS RECEIVED AND AVAILABLE FOR REVIEW

Copies of the following books have been received, and are available for review by interested parties. Individuals interested in preparing a formal review that will be published in *California Fish and Game* should contact the editor (Vern.Bleich@wildlife.ca.gov) with their request to do so.

ANTYPOWICH, L. 2012. A hunting we did go. True mountain adventures. Xlibris LLC, Bloomington, Indiana, USA. 213 pages. \$19.95 (soft cover).

DUNN, W. C. 2014. Becoming a compelling communicator for conservation. The essential reference for everyone who desires to make a difference. CreateSpace, Scotts Valley, California, USA. 69 pages. \$9.95 (soft cover).

GOTSHALL, D. W. 2012. Pacific Coast inshore fishes. Fifth edition. Sea Challengers, Monterey, California, USA. 363 pages. \$9.99 (E-Book).

JORGENSEN, M. C. 2015. Desert bighorn sheep: wilderness icon. Sunbelt Publications, San Diego, California, USA. 143 pages. \$29.95 (soft cover).

KIRKWOOD, S., AND E. MEYERS. 2012. America's national parks: an insider's guide to unforgettable places and experiences. Time Home Entertainment, Inc., New York, New York, USA. 208 pages. \$24.95 (hard cover).

LOVE, M. S. 2011. Certainly more than you want to know about the fishes of the Pacific coast: a postmodern experience. Really Big Press, Santa Barbara, California, USA. 650 pages. \$29.95 (soft cover).

INFORMATION FOR CONTRIBUTORS

California Fish and Game is a peer-reviewed, scientific journal focused on the biology, ecology, and conservation of the flora and fauna of California or the surrounding area, and the northeastern Pacific Ocean. Authors may submit papers for consideration as an article, note, review, or comment. The most recent instructions for authors are published in Volume 97(1) of this journal (Bleich et al. 2011), and are accessible through the California Department of Fish and Wildlife web site (www.dfg.ca.gov/publications).

Planning is in progress to provide an avenue for authors to submit manuscripts directly through the web site, and to enable restricted and confidential access for reviewers. In the meantime, manuscripts should be submitted by e-mail following directions provided by Bleich et al. (2011). The journal standard for style is consistent with the Council of Science Editors (CSE) Style Manual (CSE 2006). Instructions in Bleich et al. (2011) supersede the CSE Style Manual where differences exist between formats.

Authors of manuscripts that are accepted for publication will be invoiced for charges at the rate of \$50 per printed page at the time page proofs are distributed. Authors should state acceptance of page charges in their submittal letters. The corresponding author will receive a PDF file of his or her publication without additional fees, and may distribute those copies without restriction. Plans are underway to make the complete series of *California Fish and Game* available as PDF documents on the California Department of Fish and Wildlife web site.

LITERATURE CITED

- BLEICH, V. C., N. J. KOGUT, AND D. HAMILTON. 2011. Information for contributors to *California Fish and Game*. *California Fish and Game* 97:47-57.
- CSE (COUNCIL OF SCIENCE EDITORS). 2006. Scientific style and format: the CSE manual for authors, editors, and publishers. 7th edition. The Rockefeller University Press, New York, USA.



The Scientific Journal *California Fish and Game* celebrates its 100th Anniversary with four special collector editions.

The California Department of Fish and Wildlife (CDFW) has published the highly respected scientific journal *California Fish and Game* continuously for an entire century. To commemorate the Centennial Anniversary of the journal, CDFW is publishing four special issues in Volume 100.

Promoting “Conservation of Wildlife Through Education”, *California Fish and Game* is an internationally recognized, peer-reviewed research publication of interest primarily to scientists active in the fields of conservation, ecology, and natural resource management. It focuses on the fish and wildlife resources of western North America and the eastern North Pacific Ocean, but occasionally includes material from elsewhere.

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“I’m proud to have been the editor of this important scientific journal for the past several years and to guide it through publication of its centennial volume” said Dr. Vern Bleich, Editor-in-Chief. “Material published in the journal represents the important work that scientists, both within CDFW and elsewhere, are doing on behalf of conservation.”

The first issue of volume 100 focused on research and conservation of the vegetation resources in California; the second focused on marine ecology and management, and the third on wildlife ecology. This special issue features an introduction by Fran Pavley, Chair of the Senate Natural Resources and Water Committee, and additional remarks co-authored by CDFW Director Charlton H. Bonham and E. Philip Pister, retired CDFW fisheries biologist.

