California Fish and Game

"Conservation of Wildlife Through Education"

Volume 101 Spring 2015 Number 2

CDFW Photo by Terry Maas

Published Quarterly by the California Department of Fish and Wildlife

CALIFORNIA FISH AND GAME

VOLUME 101 SPRING 2015 NUMBER 2

Published Quarterly by

STATE OF CALIFORNIA
CALIFORNIA NATURAL RESOURCES AGENCY
DEPARTMENT OF FISH AND WILDLIFE
ISSN: 0008-1078 (print)
ISSN: 2331-0405 (online)

--LDA--

California Fish and Game

California Fish and Game is published quarterly by the California Department of Fish and Wildlife. It is a journal devoted to the conservation and understanding of the flora and fauna of California and surrounding areas. If its contents are reproduced elsewhere, the authors and the California Department of Fish and Wildlife would appreciate being acknowledged.

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

Inquiries regarding subscriptions should be directed to the Subscription Manager at 916-322-8911 or by email at scientific.journal@wildlife.ca.gov.

Alternate communication format is available upon request. If reasonable accommodation is needed, call 916-322-8911 or the California Relay (Telephone) Service for the deaf or hearing-impaired from TDD phones at 800-735-2929.

Contents

California Fish and Game 101(2):86; 2015

Notes from the Editor

Wow... it's not yet the end of May, and the spring issue of *California Fish and Game* has hit the streets. This issue, 101(2), of the journal has been in the making for nearly a year, but production of the four special issues celebrating the 100th anniversary of the founding of California's longest-running, continuously published scientific journal took an inordinate amount of time and effort to produce. Fortunately, the Associate Editors, Corresponding Editors, reviewers, and Debra Hamilton, who handles the production and layout of the journal, were able to work both on the special issues as well as papers scheduled to appear in 101(1) and 101(2) simultaneously. Without that ability to multitask, we would be months behind in our publication schedule. Thank you to everyone that has worked so hard over the last 18 months to keep us on track.

I am an avid collector of *California Fish and Game*, and have accumulated individual copies of every issue published to date, with one exception. Although I do have a copy of issue 20(2), it is bound into a former library volume. It has taken >40 years to acquire this collection and, in the process of trying to complete it, I have also accumulated multiple copies of numerous issues. If there are serious collectors among the readership, feel free to let me know of issues that you might need for your collection, and I'll try to help you out. And, if you happen to have a copy of 20(2), published in 1934, please consider me to be a willing recipient should you choose to help me complete my quest.

The current issue of *California Fish and Game*, now in your hands, covers numerous topics. Papers addressing distributional records, physiological responses of fish to crowding during transport, the genetics of wild pigs and their history in California, and predation are included in this issue, illustrating the wide range of topics appropriate to be considered for publication in this journal. Also included is a review of a short book that I suspect many authors will find helpful in their future endeavors. Please note the words of, the late, Kate Wolf that introduce the review. Wordiness is the bane of many editors, and this one is no exception. Additionally, the author of that book, Dr. Bill Dunn, emphasizes the necessity of being "on message" with your audience. Individuals that choose to read this book will very likely become better writers or speakers, or both, as a result of doing so.

Finally, this issue contains a tribute to Regional Manager Kimberly Nicol, who spent her entire career in what is now Region 6. Kim passed away in January of this year following a long and courageous battle with cancer. She began her career as a fisheries biologist, and had a strong interest in reptiles and amphibians, as well as in desert fishes. Those of us that worked with her for many years came to know her as a concerned and effective leader. The critters inhabiting the deserts of southeastern California are better off for Kim's efforts, and she and her work on behalf of those creatures and their habitats will be missed.

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

California Fish and Game 101(2):87-100; 2015

Experimental enhancement of pickleweed, Suisun Bay, California

A. Keith Miles*, Dirk H. Van Vuren, Danika C. Tsao, and Julie L. Yee

U.S. Geological Survey, Western Ecological Research Center, 3020 State University Drive East, Modoc Hall, Room 4003, Sacramento, CA 95819, USA (AKM)

Department of Wildlife, Fish, and Conservation Biology, 1 Shields Avenue, University of California, Davis, CA, 95616, USA (DHVV)

California Department of Water Resources, Division of Environmental Services, 3500 Industrial Boulevard, West Sacramento, CA, 95691, USA (DCT)

U.S. Geological Survey, Western Ecological Research Center, Dixon Field Station, 800 Business Park Drive, Dixon, CA, 95620, USA (JLY)

**Correspondent: keith_miles@usgs.gov*

As mitigation for habitat impacted by the expansion of a pier on Suisun Bay, California, two vehicle parking lots (0.36 ha and 0.13 ha) were restored by being excavated, graded, and contoured using dredged sediments to the topography or elevation of nearby wetlands. We asked if pickleweed (*Sarcocornia pacifica* L, [Amaranthaceae]) colonization could be enhanced by experimental manipulation on these new wetlands. Pickleweed dominates ecologically important communities at adjacent San Francisco Bay, but is not typically dominant at Suisun Bay probably because of widely fluctuating water salinity and is outcompeted by other brackish water plants. Experimental treatments (1.0-m^2 plots) included mulching with pickleweed cuttings in either the fall or the spring, tilling in the fall, or applying organic enrichments in the fall. Control plots received no treatment. Pickleweed colonization was most enhanced at treatment plots that were mulched with pickleweed in the fall. Since exotic vegetation can colonize bare sites within the early phases of restoration and reduce habitat quality, we concluded that mulching was most effective in the fall by reducing invasive plant cover while facilitating native plant colonization.

Key Words: experimental wetlands, pickleweed, restoration, San Francisco Bay, *Sarcocornia pacifica*

The loss of wetlands worldwide from ca. 1900 to present is roughly estimated at about 50% (Fraser and Keddy 2009). In the conterminous United States (U.S.) wetlands are estimated at about 47% of historical area, with the greatest loss reported for the state of California (91%; Goals Project 1999). Events that have affected wetlands at San Francisco Bay, California, exemplify causes and extent of loss that have plagued wetlands globally. The natural shoreline of San Francisco Bay has been developed for mostly urban or agriculture land use over the past 200 years, where tidal marshes alone have declined nearly 80% from about 77,000 to 16,000 ha (Goals Project 1999).

In the past decade, the second largest wetland restoration in the U.S. has been underway at San Francisco Bay. Certain decommissioned agriculture (mainly commercial salt-production ponds) and military lands have been identified for restoration to tidal marshes to offset historical declines. Thus, effective methods for successful wetland restoration are crucial. Methodologies for restoring west coast salt marshes have improved, but instituted guidelines have outpaced the science necessary for success (Zedler 2000).

In 1996, a shipping pier was expanded on Suisun Bay at the Military Ocean Terminal Concord (MOTCO), Concord, California, and in compliance with California state law (California McAteer-Petris Act of 1965 and amended in 1969) tidal marsh restoration was required as mitigation for the pier expansion project. With larger-scaled wetland restoration projects planned for the San Francisco Bay region, we concluded that the mitigation sites identified were ideal for experimentation of wetland recruitment and succession; such studies have been recommended before large-scale restoration projects are instituted (Callaway et al. 1997).

Suisun Bay forms a complex, hydrological interface between the delta of the Sacramento and San Joaquin rivers (hereafter known as "Delta") and San Francisco Bay. Wetlands in Suisun Bay comprise more than 34,000 ha of tidal marsh, managed wetlands, and waterways in the San Francisco Bay Delta region (BCDC 1976). These wetlands are among the largest remaining contiguous wetlands in that region and include more than 12% of California's remaining natural wetlands (California Department of Water Resources 1995). Suisun Bay wetlands support a diversity of plant, fish, and wildlife, including several rare and endangered species, and provide critically important wintering or breeding habitat for migratory waterbirds and fishes.

Brackish water wetlands exemplify the complexity of biotic and abiotic factors that can affect plant community structure (Callaway and Walker 1997). Elevation, salinity, nutrients, and competition are among factors commonly studied that affect species distribution in such habitats (Traut 2005, Morzaria-Luna and Zedler 2014). Suisun Bay represents a region where tidal salt and fresh water mixing is exacerbated by diversion or controlled release of freshwater inflow, and can be challenging in determining those factors most influential in structuring wetland plant assemblages. Drought, climate change, and ensuing sea level rise will only add to the complexity in this region (Callaway et al. 2007, Watson and Byrne 2009).

Pickleweed (*Sarcocornia pacifica* L,[Amaranthaceae]) dominates vegetation expanses of mid-upper tidal elevations in salt marshes along the Pacific coast of North America and the Mediterranean coasts, as well as the main stem of San Francisco Bay (Josselyn 1983). This perennial succulent grows as a low scrub and thrives under saline conditions. In California, pickleweed is essential habitat for species listed as threatened or endangered under state or federal laws, among which are *Reithrodontomys raviventris*

(salt marsh harvest mouse), *Rallus longirostris obsoletus* (Ridgway's rail), and *Chloropyron molle* ssp. *molle* (soft bird's beak), a hemiparasitic plant. Loss of habitat is probably the most serious threat to these species (Foin et al.1997, Bias and Morrison 1999). These species were observed and recorded at MOTCO, and their conservation and enhancement were deemed important natural resource management strategies by the U.S. Navy.

Despite the importance of pickleweed to sensitive species, little study has been conducted on factors affecting pickleweed establishment, probably because it is a hardy species (Zedler 1982) that can tolerate a range of salinities but may be more sensitive to increases in inundation (Woo and Takekawa 2012). However, Pennings and Callaway (1992) suggested that pickleweed can occur in higher densities in the lower marsh zone because of higher tolerance to tidal inundation than some marsh plants. Pickleweed seems to thrive at higher salinities (28‰) but can survive at lower salinities (3‰) (Watsonand Byrne 2009). Although it is widespread in salt marsh habitats in San Francisco Bay, pickleweed is not dominant at Suisun Bay possibly because of increased competition in low salinity sites. At MOTCO (Figure 1), vegetation is dominated by a seeming monoculture of sedges (Cyperaceae; mostly *Schoenoplectus* spp. or *Bolboschoenus* spp. [formerly *Scirpus* spp.]), with very limited open areas for scrub vegetation such as pickleweed.

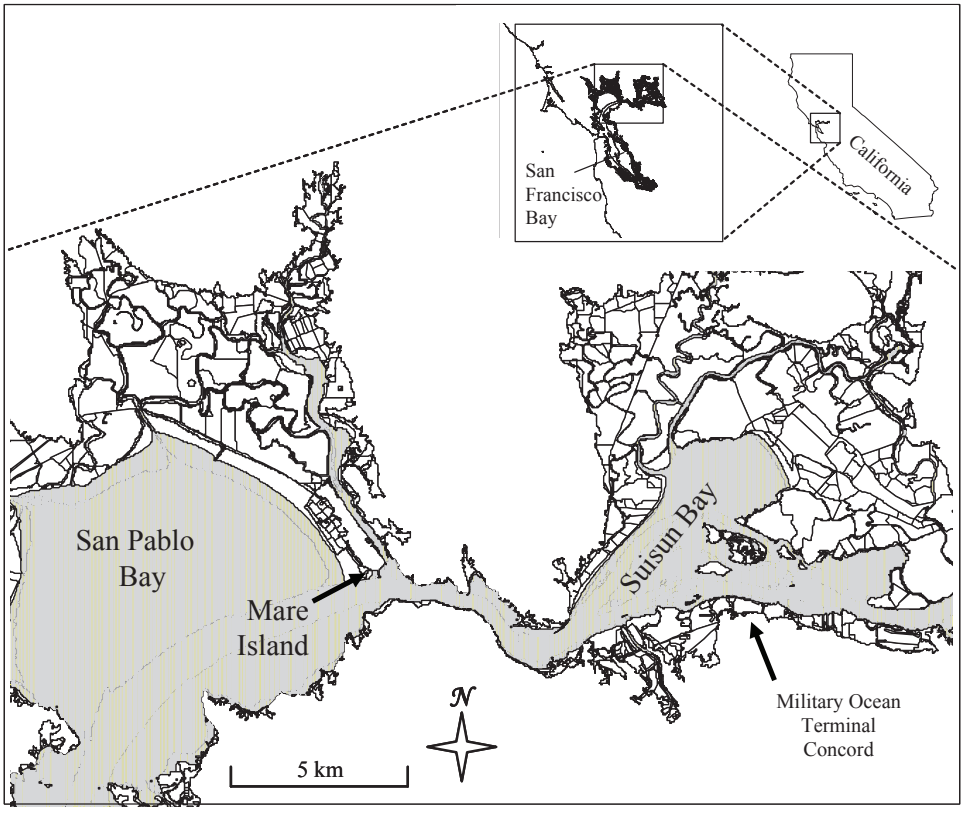


Figure 1.—Location of wetlands in Suisun and San Pablo bays (a.k.a., north San Francisco Bay) used in the study for experimental manipulation, California. (Source: San Francisco Estuary EcoAtlas version 1.50b4, San Francisco Estuary Institute, Oakland, California).

Pickleweed apparently can compete with other structurally dominant marsh plants under certain conditions. For example, gaps created in structurally dominant marsh plant patches can facilitate pickleweed colonization (Boyer and Fong 2005). Restoration methods to facilitate pickleweed establishment have not been studied. While unlikely an issue in habitats dominated by pickleweed, physically marginal habitats or physically disturbed habitats prime for colonization by exotic species like hybrid cordgrass (*Spartina alterniflora* × *Spartina foliosa*) or perennial pepperweed (*Lepidium latifolium*), are such that a diverse native plant community may benefit from assisted recruitment following restoration actions. Pickleweed was not the overall dominant vegetation, but pickleweed patches were not uncommon in the vicinity of the Suisun Bay restoration sites.

Pickleweed colonizes or spreads both by seed and rooting of decumbent or broken stems (Josselyn and Perez 1981). Pickleweed produces a high volume of seeds often considered adequate for recruitment. However, seeding to establish pickleweed may be less effective at the point of restoration because seeds disperse by floating. Some factors that affect pickleweed establishment are soil moisture, soil nitrogen, tidal influences, parasitism, and soil salinity (Covin and Zedler 1988, Osborne 1994, Pennings and Callaway 1996, Callaway et al. 1997). Pickleweed dominates San Francisco Bay marshes in soils that contain 20 to 25% organic material, more than was found in most other plant habitats in the area except those dominated by alkali bulrush (*Bulboschoenus robustus*) or cattails (*Typha* spp.). Soil organic matter might enhance pickleweed growth through increased nitrogen fixation (Covin and Zedler 1988).

We built upon a previous greenhouse study (M. Disney, unpublished data) to apply treatments that might enhance pickleweed colonization in an experimental field study. We included study of seasonality because the timing of restoration efforts may affect the rate and, therefore, success of colonization or recruitment. For example, seeding by pickleweed mostly occurs in fall but germination and vegetative expansion peak in early spring (Josselyn 1983). The objectives of this study were to determine if specific treatments might enhance change of severely disturbed areas to pickleweed habitat, and to document early succession of vegetation at MOTCO; hereafter, the Suisun restoration or Suisun sites. We hypothesized that enriching plots on restored sites with compost, mulching with pickleweed, or seasonal application of pickleweed mulch to increase organic content of the soils or recruitment structure (e.g., seeds, plant cuttings), would enhance rapid colonization or recruitment by pickleweed compared to control plots in areas with low soil salinity or dominance by other marsh plants.

We replicated the two MOTCO sites at Suisun Bay at a third site excavated at the south end of Mare Island (hereafter the San Pablo Bay site), about 10 km west of the Suisun Bay sites. Because these three restoration sites were severely perturbed in their history of use as well as by mechanical excavation, we monitored plots on two reference sites, one each in Suisun and San Pablo bays, to document vegetative structure and temporal change on plots at nearby existing marshes. These reference sites were not compared statistically to the experimental sites; rather, they were used to document biotic and physical patterns on adjacent areas of similar topography in order to understand expected mature succession at the restoration sites. Such information facilitated our understanding of development on the restoration sites relative to existing marsh plant structure.

Materials and Methods

Study area.—The study was conducted at MOTCO, Suisun Bay, and at Mare Island at the far eastern edge of San Pablo Bay (hereafter San Pablo Bay sites; Figure 1). The Suisun restoration area (approximately 38° 03' N, 122° 00' W) was located along the southern shore of Suisun Bay between the cities of Martinez and Pittsburgh. Suisun Bay water salinities range from 0.0 to 11.0‰ during periods of high runoff from the Delta, and from 2.0 to 15.0‰ during the dry season. Tidal amplitude averages 1.63 m, compared to 1.78 m at the mouth of San Francisco Bay.

At Suisun Bay, the two restoration sites were former parking lots. The asphalt layer and base material were excavated, the sites were graded and contoured to the topography or elevation of nearby wetlands, and then both sites were covered with fine-textured dredge material to a depth of about 20 cm by fall 1998. Dredge material was obtained from the Port Sonoma Marina on San Pablo Bay. As required for restoration purposes, this material was tested for inorganic contaminants and levels were determined to be acceptable by the San Francisco Bay Regional Water Quality Control Board prior to use. Resulting elevations (ranging from 0.21 to 0.13 m below mean high-high water [MHHW]) and were similar to that of nearby pickleweed habitat. The sites were approximately 0.36 ha and 0.13 ha. Roads or levees and a slough bordered both sites.

The San Pablo Bay restoration site at the southwest end of Mare Island (38° 04' N, 122° 16' W) was part of a former dredge spoils disposal area that also included unexploded ordnance and some contaminated soil. These materials were excavated in 1997 and the area was contoured into a combination of shallow ponds, levees, and gently sloped areas by summer 1998. Additional dredge material was not applied at the San Pablo Bay site because existing sandy soils were not as extensively excavated as at the Suisun sites, and had similar elevations to nearby upland soils. San Pablo Bay salinities near Mare Island range from 0.0 to 18.0‰ during periods of runoff, and from 12.0 to 25.0‰ during the dry season. Tidal amplitude averages 1.75 m. The San Pablo Bay site was approximately 0.15 ha, and elevations ranged from approximately 0.49 to 0.44 m below MHHW, similar to nearby pickleweed habitat.

Plot treatments.—Thirty, 1.0-m^2 plots were established at each of the three restoration sites, 15 plots at lower and 15 at higher tidal elevations. The lower elevation plots were 0.20 to 0.18 m (below MHHW) at the Suisun and San Pablo restoration sites; the higher elevation plots were 0.15 to 0.13 m at the Suisun sites and 0.44 m (below MHHW) at the San Pablo site. At the San Pablo site and at one of the Suisun sites, some lower-elevation plots were commonly inundated except at extreme low tides. In fall 1998, three replicate plots of each of four treatments and a control were randomly assigned to each of the lower and higher tidal elevations at the three restoration sites. Control plots received no treatment following initial site-wide preparation. The treatments were: (1) mulching with pickleweed in the fall (hereafter fall mulch); (2) mulching with pickleweed in the spring (spring mulch); (3) soil enriched with compost in the fall (enriched); and (4) soil tilled in the fall without adding compost (tilled).

Each 1.0-m^2 mulched plot received about 2.4 kg fresh-weight of pickleweed, which had been obtained from a managed wetland at Mare Island. Entire plants were cut into pieces about 20 cm long, applied by hand onto the selected plots, and then covered with jute mesh that was anchored in place. Enriched plots received enough compost to augment soil organic

material content by an estimated 8%. This was calculated to be an application of about 12.0 kg (dry weight) compost applied per m^2. Compost, produced by a local municipality from green waste, was cultivated into the soil to a depth of about 15 cm using a gasoline-powered rototiller. Fall-mulched, enriched, and tilled-only plots were established in November 1998 and spring mulched plots in May 1999.

We estimated percent cover on each plot using a 1.0-m^2 sampling frame divided into grids by crossing monofilament lines placed at 10, 30, 50, 70, and 90 cm. The sampling frame was aligned on each plot at each sampling interval. Species, duff (dead or decaying material), or bare soil were tallied under the 25 monofilament crosshairs on the frame. Standing water or other materials also were noted. Percent cover per plot was calculated by multiplying the tally for each species or category by four for a total of 100% cover; if, for example, duff and pickleweed occurred under the same crosshair, only pickleweed was recorded but duff was noted. Any live plant species in the plot not recorded by this procedure were noted as trace. Percent cover per plot was collected seasonally beginning in winter (sampling initiated 21 December 1998) 1999 through spring (May) 2000. Overall sampling of both locations could take up to 60 days to complete; therefore, plots were sampled in the same order each season.

In order to compare soil characteristics on the treatment and control plots, two soil cores (2.5 cm diameter × 15.0 cm deep) or samples were extracted from each plot in spring and summer 1999 and winter and spring 2000. Soil samples were homogenized, weighed, air dried, and reweighed, and then sub-sampled for analyses. Organic matter (OM), nitrogen as ammonia (NH_4-N), and nitrate (NO_3-N) were analyzed by the Division of Agriculture and Natural Resources at the University of California, Davis. Approximate salinity and pH of soils was estimated by mixing fixed weight dried soil and volume distilled water and measuring the slurry with a water quality meter.

We monitored two reference sites of about 0.25 ha in size at existing marshes near the restoration sites. Percent cover on fifteen, 1.0-m^2 plots at each reference site was estimated as described above.

Statistical analyses.—Total cover of pickleweed, marsh native, exotic, and total plants on experimental sites were analyzed for differences related to treatment (four treatments vs. control) and elevation (placement in high vs. low tidal range). Total cover of plots observed at the end of the study in spring 2000 were log-transformed and analyzed using mixed factors ANOVA (PROC MIXED; SAS Institute Inc., Cary, NC). We used this model to test the fixed factors (elevation, treatment, and elevation by treatment interactions), while controlling for variation due to the random factor (site). We graphed the means of total cover versus time to interpret patterns of vegetation succession and soil parameters on the treatment and control plots during the study.

Results

Total vegetation cover at both Suisun and San Pablo Bay restoration sites was greater on fall mulched and enriched plots compared to control plots by the end of the study (spring 2000; Table 1); percent cover of exotic plants was greater on enriched or tilled plots compared to the control. Total native cover, largely pickleweed at both Suisun and San Pablo Bay plots, was greater on the fall-mulched treatment plots than all other plots. Native plants (including pickleweed) apparently colonized or thrived better on the higher than lower elevation plots, but exotic plant cover did not appear to be influenced by elevation (Table 1).

Table 1.—Differences in vegetation percent cover by treatments and elevation, at end of study (spring 2000). Includes all Suisun and San Pablo Bay plots. Treatments: fall (FM) and spring mulched (SM), tilled (T), enriched (E), and control (C); High (H) and low (L) tidal elevation.

Cover	Treatment	Elevation	Treatment Means	Elevation Means
Total	$F_{4,77} = 3.94$; $P = 0.006$	$F_{1,77} = 0.11$; $P = 0.745$	FM > E > T > SM > C	-
Exotic	$F_{4,77} = 2.71$; $P = 0.036$	$F_{1,77} = 3.92$; $P = 0.051$	E > T > FM > SM > C	-
Native	$F_{4,77} = 3.87$; $P = 0.007$	$F_{1,77} = 9.89$; $P = 0.002$	FM > C > SM > T > E	H > L[1,2]
Pickleweed	$F_{4,77} = 7.02$; $P = 0.0001$	$F_{1,77} = 25.4$; $P = 0.0001$	FM > SM > C > E > T	H > L[1,3]

[1] Means of treatment that share line did not differ at the 0.05 level of significance.
[2] Interaction of elevation and treatment: $F_{4,77} = 3.18$; $P = 0.01$; i.e., treatment differences for H: FM > C > SM > T > E were different from those for L: FM > E > T > SM > C; similarly for treatments FM & C: H > L; SM & T: H > L; E: L > H
[3] Interaction of elevation and treatment: $F_{4,77} = 2.54$; $P = 0.05$; i.e., treatment differences for H: FM > C > SM > T > E were different from those for L: FM > E > SM > T > C; similarly for treatments FM, SM, & C: H > L; T & E: H > L

Colonization of total or exotic plants on plots initiated in fall 1998 was substantial by spring 1999 at the Suisun sites (Figures 2a, 2b). Fall pickleweed-mulched, enriched, and tilled plots achieved >70% total cover, whereas total cover on the control (untreated) plots was about 50%. Total and exotic cover on plots treated with pickleweed mulch in spring (1999) was lower than that on control plots until spring 2000. Rabbitsfoot grass (*Polypogon monspeliensis*), brass buttons (*Cotula coronopifolia*), and other grasses were the most common exotic species (Table 2). Brass buttons were purposely planted in the Suisun Bay

Table 2.—Percent cover of common (>1% cover) native and exotic plants on treatment and reference plots, averaged across Suisun and San Pablo Bay restoration and reference sites at final (Spring 2000) sampling interval. Totals sum to ~100 % cover due to rounding.

		Treatments					
Scientific name	Common name	Fall Mulch	Spring Mulch	Enrich	Tilled	Control	Ref-erence
Native taxa							
Sarcocornia pacifica	Pickleweed	20.4	8.7	4.2	3.4	8.5	21.6
Distichlis spicata	Saltgrass	--	--	--	--	--	43.6
Triglochin maritima	Arrowgrass	--	--	--	--	--	5.3
Scirpus spp.	Tule	--	0.4	--	0.6	--	7.1
Typha spp.	Bulrush	0.2	--	0.4	1.1	--	0.2
Spergularia macrotheca	Sand spurry	--	--	--	1.5	6.5	--
Phragmites australis	Common reed	0.8	1.3	0.6	0.4	0.5	--
Other native[1]		0.7	0.7	0.1	0.4	0.3	0.2
Total Native		22.1	11.1	5.3	7.2	15.8	78.0
Exotic taxa							
Cotula coronopifolia	Brass buttons	17.5	16.4	33.5	29.5	16.9	--
Polypogon monspeliensis	Rabbitsfoot grass	14.9	13.3	11.4	6.9	1.6	--
Asclepias sp.[2]	Milkweed	--	0.7	1.1	--	--	0.2
Other exotic[3]		1.1	8.9	0.8	--	--	--
Total Exotic		33.5	39.3	46.7	36.4	18.5	0.2
Total Unknown[4]		15.2	5.3	17.7	20.6	17.3	20.0
Total Abiotic[5]		29.1	43.1	29.3	35.4	48.4	0.4

[1] Common (recorded at least two seasons) native species at < 1 average percent cover on some treatment or reference plots: *Atriplex prostrate*, *Epilobium ciliatum*, *Cuscuta salina*, *Juncus balticus*, and *Grindelia stricta*.
[2] Some species of these genera are native in California but were rare in the study sites and not identified to species.
[3] Common exotic species with < 1 average percent cover on some treatment or reference plots: *Lepidium latifolium*, *Lolium multiflorum*, *Bromus madritensis*, *Bassia* sp., and *Lythrum* sp.
[4] Unidentifiable species (e.g., early plant development) or apparent dormant but unidentifiable plant tissue.
[5] Abiotic includes dead plant tissue, water, bare soil, rock, driftwood, jute, and wrack.

region as forage to support waterfowl. Exotic plants were mainly weedy, *r*-selected (quick growing, high recruitment) species that might competitively exclude native vegetation. However, these plants were uncommon at reference site plots and also at most San Pablo Bay plots, and probably were not dominant to pickleweed or later successional vegetation. For the course of this study, exotic cover remained dominant on treatment plots, but was notably lower on the fall-mulched plots than the enriched or tilled plots.

Colonization by native plants was lower than that by exotic plants on the Suisun restoration sites throughout the study, comprising an average of 15–20% cover on the fall-mulched, tilled, and control plots, and <10% cover on the enriched and spring-mulched plots (Figure 2c). Pickleweed dominated early native cover on fall-mulched plots, whereas fat hen (*Atriplex prostrata*) and sand spurrey (*Spergularia macrotheca*) dominated tilled and control plots. Pickleweed cover was notably higher on the fall-mulched than the reference site plots (Figure 2d). Native plants formed nearly 100% cover on the reference plots, and were dominated by saltgrass (*Distichlis spicata*) (Figure 2c). Control and spring-mulched plots had pickleweed cover similar to that on reference plots by spring 2000 (Figure 2d); tilled and enriched only plots had little established pickleweed cover by that time. Notable on the easternmost restoration site at Suisun Bay during the study was colonization by *Chloropyron molle* ssp. *molle*, which is federally listed as endangered and state listed as rare.

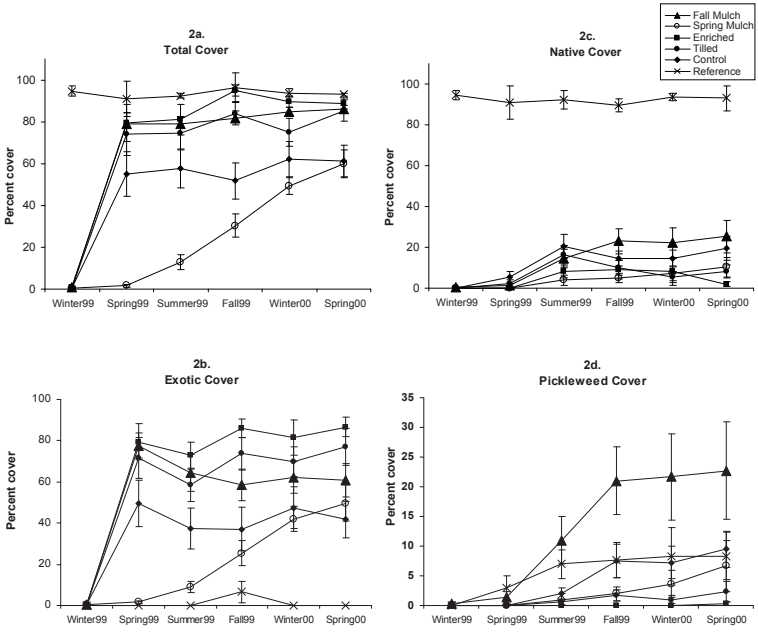
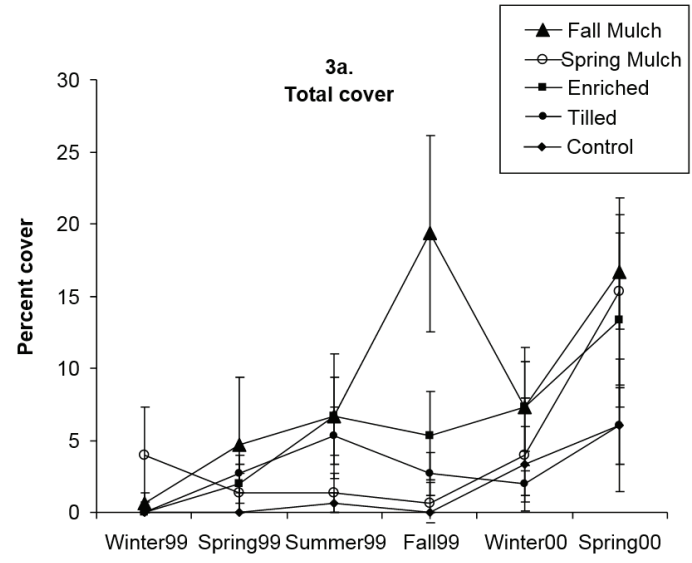


Figure 2.—Mean percent cover of marsh vegetation on treatment (fall mulch, spring mulch, enriched only, tilled only) and control plots, and also nearby reference plots, winter 1999 through spring 2000, at Suisun Bay restoration sites excavated to create new wetlands, Military Ocean Terminal, Concord, California. Bars are standard errors.

At the San Pablo Bay site, total cover was substantially reduced compared to Suisun Bay sites, mainly because of low colonization by exotic species (Figures 3a, 3b). Pickleweed was the dominant cover on all plots, regardless of treatment or control. Pickleweed cover was highest on the fall-mulched plots in fall 1999 and spring 2000, but was comparable to the other plots at other observed seasons.



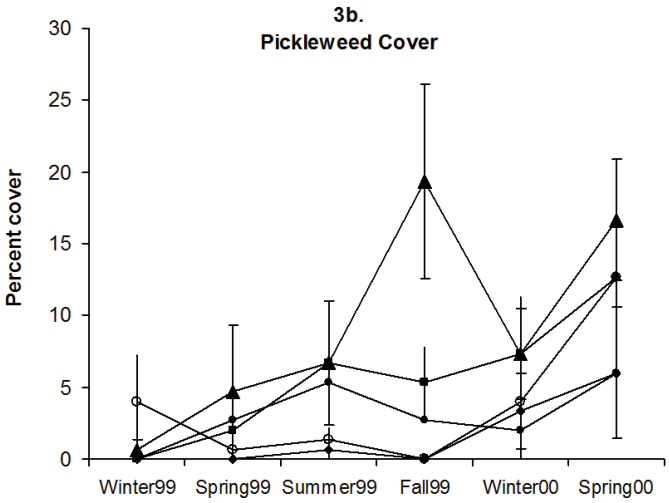


FIGURE 3.—Mean percent cover of marsh vegetation on treatment (fall mulch, spring mulch, enriched only, tilled only) and control plots winter 1999 through spring 2000, at the San Pablo Bay restoration site, Mare Island, California. Bars are standard error. Exotic cover was uncommon on these plots. On reference site plots sampled winter 1999 to spring 2000, mean total cover ranged from 96-100%, of which pickleweed cover ranged from 48-92%.

Levels and changes in soil characteristics generally were similar on all treatment or control plots at both the Suisun and San Pablo Bay restoration sites (Figures 4a-4f). Soil levels for NH_4-N were notably higher at Suisun sites (where dredge-spoil sediments were used) than those at the San Pablo site. Seasonal patterns of change in OM and NH_4-N were similar at both Suisun and San Pablo Bay restoration sites; the exception was that OM on tilled plots was notably greater than that on all other plots at Suisun Bay sites. Nitrogen as NO_3-N was similar in concentration at the Suisun and San Pablo Bay sites, but spiked at different seasons. Approximate soil salinity and pH were similar between treatment and control plots at both Suisun and San Pablo Bay sites; salinity was notably higher at the San Pablo Bay site than the Suisun Bay sites (Figure 4g-4j).

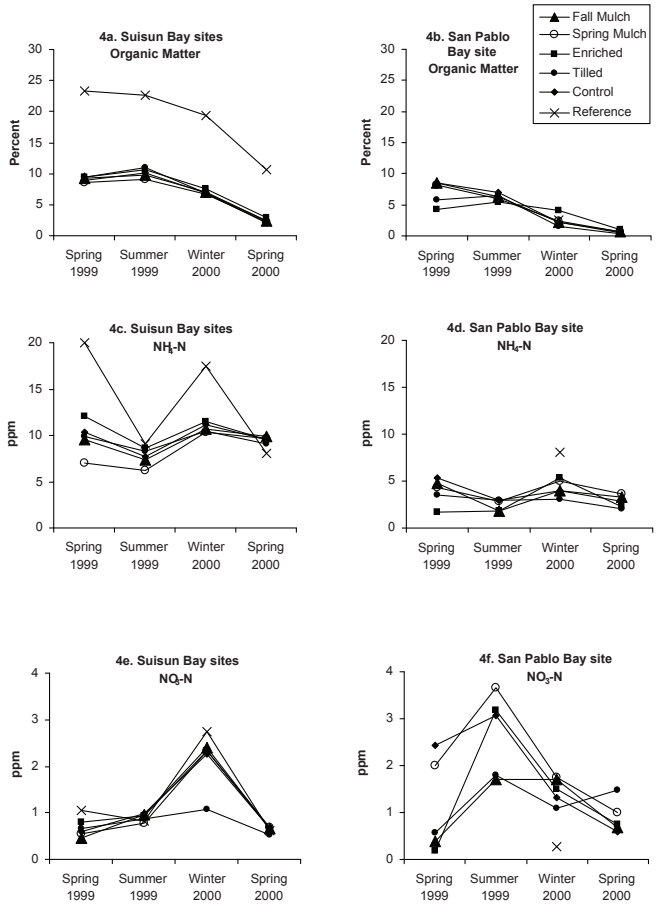


Figure 4.—Percent of organic matter and concentrations of nitrogen (ppm) as ammonia (NH_4-N) and nitrate (NO_3-N), and salinity (ppt) and pH on treatment, control, and reference plots sampled seasonally from spring 1999 to spring 2000.

Discussion

We demonstrated that pickleweed colonization was enhanced through mechanical manipulation, and that application of pickleweed mulch in the fall season was influential in expediting colonization. In a subsequent study, we observed a similarly greater cover of pickleweed on fall-mulched plots (approximately 2 m × 10 m) compared to control plots on a small, restored wetland west of Mare Island at Skaggs Island (Miles 2005). Seeding by pickleweed that was applied in fall probably contributed to the gain witnessed in pickleweed colonization in fall compared with spring treatment. Soil chemistry and structure on most treatment and control plots were similar and probably were not influential in the outcome of vegetative cover on these plots.

Composition of dominant plants on the experimental plots differed from that observed on reference plots, indicating that eventually ground cover on the restoration sites

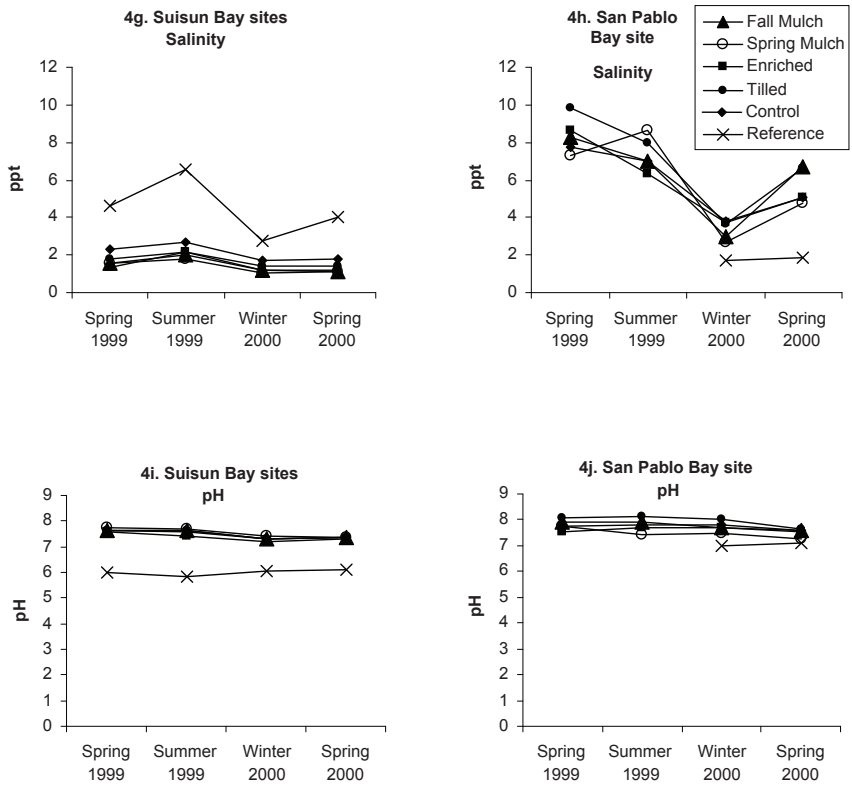


Figure 4 (continued).—Percent of organic matter and concentrations of nitrogen (ppm) as ammonia (NH_4-N) and nitrate (NO_3-N), and salinity (ppt) and pH on treatment, control, and reference plots sampled seasonally from spring 1999 to spring 2000.

could be dominated by saltgrass, and that succession was still in an early state. Subsequent observations (December 2003) at the Suisun Bay sites indicated recruitment of saltgrass on these sites and further colonization by reedy vegetation (e.g., *Typha*, *Phragmites*, *Schoenoplectus*, or *Bolboschoenus*). Surveys of wetlands at the Suisun Bay study area indicated that reed-like vegetation is dominant, with interspersed patches of saltgrass or pickleweed ranging from <1 to about 25 ha based on qualitative surveys we conducted of these wetlands for the U.S. Navy (Miles and Tsao-Melcer 2005). Persistence of patches of plants of lower profile, such as pickleweed and saltgrass, within the higher stature reedy vegetation is probably influenced by the periodic droughts that affect the hydrology of Suisun Bay (Calloway et al. 2007). The resulting higher soil and water salinity at Suisun Bay probably facilitate maintenance of pickleweed and saltgrass, whereas extended occurrence and inundation by freshwater facilitates these reedy species.

Several factors might explain greater colonization or recruitment of exotic vegetation at Suisun Bay restoration sites than at the San Pablo Bay site. The Suisun sites were augmented and graded with dredge-spoil sediments from the Port Sonoma Marina in north San Francisco Bay, whereas the San Pablo Bay site was not augmented. During on-land storage, the dredged sediments may have been inoculated with seeds from plants

in the Port Sonoma area. The dredge-spoil was richer in organic matter than the silt-sand sediments observed at Mare Island, although organic matter was not notably greater on plots at Suisun than San Pablo. Exotic plant colonization was greater at Suisun than San Pablo, but pickleweed cover was comparable at both areas. Colonization by early succession, exotic plants probably did not affect that of native plants via competitive exclusion, or by depletion (e.g., N) or enhancement (e.g., OM) of nutrients.

The Wetland Regional Monitoring Program's EcoAtlas (California Wetlands Monitoring Workgroup [2014]) shows ~400 planned, active, or completed wetland restoration efforts in the San Francisco Bay Delta region. Yet, very few studies in this region have published goals desired, procedures followed, milestones established, or have pre-established success criteria for those restoration efforts (e.g., Marcus 2000). In contrast, there have been a number of published studies of restoration efforts in southern California (e.g., Callaway et al. 1997, Zedler and Callaway 1999, Zedler et al. 2003), where remnant tidal marshes occur in small fragments. While many of the methods suggested from southern California may be applicable to San Francisco Bay, unique habitat qualities such as fluctuating salinities due to estuarine mixing and freshwater flows to Suisun Bay are complex, and require investigation and reporting. Furthermore, as Zedler and Callaway (1999) suggested, restoration efforts rarely follow desired trajectories.

Manipulation of wetland restoration lands in late summer and early fall after the reproductive period probably would be least disruptive to plant and wildlife species in the vicinity of restoration. At sites that may require extensive rehabilitation or excavation, such as former dumps or superfund sites, large-scale tilling of pickleweed mulch would be feasible during contouring to establish proper elevation. For example, in San Pablo Bay the Bell Marin Keys Unit 5 (a former military airfield) and Sonoma Baylands were extensively manipulated (San Francisco Bay Area Wetland Regional Monitoring Program 2014), and excavation and contouring apparently were common practices at southern California coastal restoration or wetland mitigation sites (e.g., Callaway et al. 1997). For this study, pickleweed mulch was obtained from wetlands on Mare Island that were disked in late summer by the U.S. Navy to form a buffer around potentially contaminated sites to discourage colonization by endangered salt marsh harvest mice. Annual execution of this practice indicated that patchwork disking above the root system to obtain mulch probably would not impact the survival of donor pickleweed populations. Mulching with pickleweed may be an economically viable method to enhance establishment of pickleweed in areas where it is not typically dominant.

Morzaria-Luna and Zedler (2014) emphasized that experimental manipulations improve our understanding of ecosystem interactions in novel wetland environments. Ultimately, sea level rise and drought and subsequent increased salinity may accomplish the same results witnessed in experimental manipulation at the Suisun Bay restoration sites (Callaway et al. 2007, Watson and Byrne 2009). Colonization at Suisun Bay could more closely resemble changes observed at the San Pablo Bay restoration site, where pickleweed cover on fall mulch plots and control plots was more similar by the end of the study.

Acknowledgments

The U.S Navy (MIPR number N62474-97-MPC7W05) and U.S. Geological Survey supported this study. We thank M. Disney for assistance with design and field data

collection, and M. Law and S. Spring who assisted in the field. Thanks also to B. Franklin, J. Hemstock, N. Roster, and R. Rugen (former members of the Naval Facilities, Engineering Field Activity West) for their foresight and support. The U.S. Navy provided the land for these restoration efforts. Any use of trade, product, website, or firm names in this publication is for descriptive purposes only and does not imply endorsement by the U.S. Government.

Literature Cited

BCDC (Bay Conservation and Development Commission). 1976. Suisun Marsh protection plan [Internet]; [Cited 2014 December 8]. Available from: http://www.bcdc.ca.gov/publications/library.shtml

Bias, M. A., and M. L. Morrison. 1999. Movement and home range of salt marsh harvest mice. Southwestern Naturalist 44:348-353.

Boyer, K. E., and P. Fong. 2005. Macroalgal-mediated transfers of water column nitrogen to intertidal sediments and salt marsh plants. Journal of Experimental Marine Biology and Ecology 321:59-69.

California Department of Water Resources. 1995. Sacramento-San Joaquin Delta atlas. California Department of Water Resources, Sacramento, USA.

CWMW (California Wetlands Monitoring Workgroup). 2015. EcoAtlas [Internet]; [Accessed 2015 January 12]. Available from: http://www.ecoatlas.org/regions/ecoregion/bay-delta/projects

Callaway, J. C., J. B. Zedler, and D. L. Ross. 1997. Using tidal salt marsh mesocosms to aid wetland restoration. Restoration Ecology 5:135-146.

Callaway, J. C., V. T. Parker, M. C. Vasey, and L. M. Schule. 2007. Emerging issues for the restoration of tidal marsh ecosystems in the context of predicted climate change. Madrono 54:234-248.

Callaway, R. M., and L. R. Walker. 1997. Competition, and facilitation: a synthetic approach to interactions in plant communities. Ecology 78:1958-1965.

Covin, J. D., and J. B. Zedler. 1988. Nitrogen effects on *Spartina foliosa* and *Salicornia virginica* in the salt marsh at Tijuana Estuary, California. Wetlands 8:51-65.

Foin, T. C., E. J. Garcia, R. E. Gill, S. D. Culberson, and J. N. Collins. 1997. Recovery strategies for the California clapper rail (*Rallus longirostris obsoletus*) in the heavily urbanized San Francisco estuarine ecosystem. Landscape and Urban Planning 38:229-243.

Fraser, L. H., and P. A. Keddy (Editors). 2009. The world's largest wetlands: ecology and conservation. Cambridge University Press, New York, USA.

Goals Project. 1999. Baylands ecosystem habitat goals. A report of habitat recommendations prepared by the San Francisco Bay Area Wetlands Ecosystem Goals Project. U.S. Environmental Protection Agency, San Francisco, California, USA and San Francisco Bay Regional Water Quality Control Board, Oakland, California, USA.

Josselyn, M. 1983. The ecology of San Francisco Bay tidal marshes: a community profile. FWS/OBS-83/23. U.S. Fish and Wildlife Service, Division of Biological Services, Washington D.C., USA..

Josselyn, M., and R. Perez. 1981. Sediment characteristic and vegetation colonization. Pages 7-34 *in* T. Niesen and M. N. Josseyln, editors. The Hayward regional

shoreline marsh restoration: biological succession during the first year following dike removal. Technical Report 1. Tiburon Center for Environmental Studies, Tiburon, California, USA.

Marcus, L. 2000. Restoring tidal wetlands at Sonoma Baylands, San Francisco Bay, California. Ecological Engineering 15:373-383.

Miles, A. K. 2005. Tidal marsh restoration, Site 2, Skaggs Island, California. Unpublished Final Report to the U.S. Navy, Daly City, California, USA.

Miles, A. K., and D. C. Tsao-Melcer. 2005. Assessment of wetlands, Military Ocean Terminal, Concord, California. Unpublished Final Report to the U.S. Navy, Daly City, California, USA.

Morzaria-Luna, H. N., and J. B. Zedler. 2014. Competitive interactions between two salt marsh halophytes across stress gradients. Wetlands 34:31-42.

Osborne, M. A. 1994. The effects of tidal and soil-related variables on the growth, morphology, and distribution of the perennial salt marsh pickleweed, *Salicornia virginica* L. in southern California salt marshes. M.S. Thesis, California State University, Long Beach, USA.

Pennings, S. C., and R. M. Callaway. 1992. Salt marsh plant zonation–the relative importance of competition and physical factors. Ecology **73**:681-690.

Pennings, S. C., and R. M. Callaway. 1996. Impact of a parasitic plant on the structure and dynamics of salt marsh vegetation. Ecology 77:1410-1419.

San Francisco Bay Area Wetland Regional Monitoring Program [Internet]; [Cited 2014 December 8]. Available from: http://www.wrmp.org/projectsintro.html

Traut, B. H. 2005. The role of coastal ecotones: a case study of the salt marsh upland transition zone in California. Journal of Ecology 93:279-290.

Watson, E. B., and R. Byrne. 2009. Abundance and diversity of tidal marsh plants along the salinity gradient of the San Francisco Estuary: implications for global change ecology. Plant Ecology 205:113-128.

Woo, I., and J. Y. Takekawa. 2012. Will inundation and salinity levels associated with projected sea level rise reduce the survival, growth, and reproductive capacity of *Sarcoconia pacifica* (pickleweed)? Aquatic Botany 102:8-14.

Zedler, J. B. 1982. The ecology of southern California coastal salt marshes: a community profile. U.S. Fish and Wildlife Service, Division of Biological Services, Washington D.C., USA. FWS/OBS-31/54.

Zedler, J. B. 2000. Progress in wetland restoration ecology. Trends in Ecology and Evolution 15:402-407.

Zedler, J. B., and J. C. Callaway. 1999. Tracking wetland restoration. Do mitigation sites follow desired trajectories? Restoration Ecology 7:69-73.

Zedler, J. B., H. Morzaria-Luna, and K. Ward. 2003. The challenge of restoring vegetation on tidal, hypersaline substrates. Plant and Soil 253:259-273.

Received 15 March 2014
Accepted 16 March 2015
Associate Editor was C. Burton

California Fish and Game 101(2):101-107; 2015

Observations of the non-native Pacific oyster (*Crassostrea gigas*) in San Diego County, California

Jeffrey A. Crooks*, Kai R. Crooks, and Aiden J. Crooks

Tijuana River National Estuarine Research Reserve, 301 Caspian Way, Imperial Beach, CA 91932, USA

**Correspondent: jcrooks@trnerr.org*

Key words: Pacific oyster, *Crassostrea gigas*, biological invasion, exotic species, *Ostrea lurida*, ecosystem engineer

---

California's coastline continues to accumulate species introduced from all over the world. In San Diego County's estuarine and marine waters, the number of reported non-native species now stands at around 100 (Crooks 1998, unpublished data, Preisler et al. 2009). Among the many different taxa of invaders in this region, bivalve molluscs are of particular interest given their potential ecological and economic impacts and the relatively well-documented history of changes in bivalve assemblages over time (Crooks 2001). Bivalves in general are a relatively conspicuous group, their representation in the fossil record and in archeological sites make the deeper history of these organisms accessible, and local molluscan assemblages have also been focal organisms for study by scientists for 150 years. Some of the non-native molluscs reported in the San Diego area include the bay mussel (*Mytilus galloprovincialis*), the Japanese mussel (*Musculista senhousia*), and the Manila clam (*Ruditapes philippinarum*). These are in addition to freshwater invaders of the county, including the Asian clam (*Corbicula fluminea*) and the notorious quagga mussel (*Dreissena rostriformis bugensis*). Despite repeated intentional introductions in San Diego, and California in general, one notable absence in the list of established mollusk invaders has been oysters. Herein we review isolated reports of introduced oysters into San Diego County and nearby areas in the 19th and 20th centuries, and then note what appears to be a successful establishment of the Pacific oyster (*Crassostrea gigas*) that began around the turn of the 21st century.

Movement of several species of non-native oysters into California began in the mid-1800s, driven by a desire to augment the declining fishery of the smaller native oyster, *Ostrea lurida* (Carlton 1979, Kirby 2004). Intentional introduction of Virginia oysters (*Crassostrea virginica*) from the U.S. east coast into California waters (San Francisco Bay) began in the 1860s, and by the 1880s Virginia oysters were planted on trial bases in Alamitos, Newport, and San Diego bays in southern California (Ingersoll 1881, Williamson 1894, Barrett 1963, Carlton 1979). There were some reports of what appears to be short-lived establishment of this species. In Alamitos Bay, Williamson (1894) noted an account that the Virginia oyster ground "embraces the whole of Alamitos and Anaheim Bays," and Townsend (1893)

indicated that "eastern oysters are reported as propagating in San Diego Bay." There was also interest in moving oysters from the lower Sea of Cortez into California, particularly the translocation of *Striostrea prismatica* (= *Crassostrea iridescens*) into the relatively warm waters of San Diego Bay (Gilbert 1891, Rathbun 1894, Moore 1897). As with the Virginia oyster, it appears that there were temporary populations of Mexican oysters to be found in the bay. Ingersoll (1881) highlighted a newspaper article from 1875 that stated that "the raccoon oyster, which is the native Lower California oyster, a bivalve of no mean merit, is found in great abundance in San Diego bay," and Townsend (1893) reported claims of survivors of an "accidental planting" of oysters from Guaymas, Mexico, being occasionally encountered. It is worth noting that the Mexican oyster "closely resembles the Atlantic coast species of the United States" (Rathbun 1894), so it is possible that there was confusion about the identity of the oysters being reported. Nonetheless, it does appear that non-indigenous oysters were temporarily present in San Diego in the late 1800s.

Movement of the Pacific oyster (also known as the giant or Japanese oyster, *Crassostrea gigas*) included introductions to the Pacific Northwest around the turn of the 20th century and to northern California starting in the 1920s (Carlton 1979). Intentional plantings into southern California included imports into Newport Bay in the 1930s, the inland Salton Sea in the 1950s, and San Diego Bay and Catalina in the 1960s (Barrett 1963, Carlton 1979). Small Pacific oysters were also temporarily transplanted as bioindicators in San Diego Bay and other California systems in the 1980s (Smith et al. 1987). Pacific oysters have also been introduced in Baja California, including limited introductions into Estero de Punta Banda near Ensenada (Carlton 1979), and a still-operational Pacific oyster grow-out operation in Bahia San Quintin that began in the 1970s (Islas-Olivares 1975).

Even with this widespread import, persistent populations of Pacific oysters were not reported in California throughout the 19th and 20th centuries, although many oyster associates did successfully invade (Carlton 1979, Grosholz et al. 2012). Occasional instances of wild Pacific oysters were reported, including one specimen found in Mission Bay, San Diego (as *Ostrea laperousi*, noted as "introduced - large 4 to 5 inch specimen" [Wilson 1943]) and a small number in Newport Bay in the 1940s (Carlton 1979). Starting in the year 2000, new incidences of Pacific oysters in southern California began to be reported. Cohen et al. (2005) found Pacific oysters in Los Angeles Harbor in 2000, and LaGrange (2002) reported them from San Diego Bay soon thereafter. This was coupled with other reliable sightings from San Diego Bay and Mission Bay in the early 2000s (C. Gramlich, San Diego State University, personal communication, 2006).

In 2005, we noted the presence of Pacific oysters within Mission Bay, the Tijuana River Estuary, and Oceanside Harbor (Figure 1). Since that time, we have found non-native Pacific oysters in virtually every suitable system in San Diego County, including Oceanside Harbor; Agua Hedionda, Batiquitos, San Elijo, San Dieguito, and Los Peñasquitos lagoons; Mission Bay; the San Diego River flood control channel; San Diego Bay; and the Tijuana River Estuary (Figure 1, Figure 2). Individuals range in size from recruits to specimens reaching 300 mm in length, with instances of multiple year classes present at a given time. Although it can be difficult to distinguish similarly-sized non-native and native Olympia (*O. lurida)* oysters, Pacific oysters can rapidly attain much larger sizes than the natives. Also, limited genetic work on oyster specimens from both Mission Bay and San Pedro (near the Port of Los Angeles) indicates matches with *C. gigas* (Grosholz et al. 2012; J. Asif, California State University Los Angeles, personal communication, 2006). Similar genetic

Figure 1.—Estuaries with observed occurrences of the non-native Pacific oyster (*Crassostrea gigas*) in San Diego County, California, 2005–2014.

Figure 2.—Photographs of the non-native Pacific oyster (*Crassostrea gigas*) in coastal San Diego County, California. (A) approximately100-mm oysters from Mission Bay, 2006; (B) 300-mm oyster from San Diego River Channel, 2011; (C) oysters on Los Penasquitos Lagoon shoreline, 2014; (D) 230-mm oyster from Tijuana Estuary, 2007; (E) abundant oysters on rip-rap in Tuna Harbor, San Diego Bay, 2014.

confirmation was also found for putative Pacific oysters found in San Francisco Bay within the same time frame (Cohen and Weinstein 2008). From these observations and other reports (Tuskes 2012; D. Zacherl, California State University Fullerton, personal communication, 2010; H. Carson, Washington Department of Fish and Wildlife, personal communication, 2014), the Pacific oyster now appears to be established in southern California.

It is probable that the southern California invasion began in the late 1990s, given the conspicuous nature of the Pacific oyster and the fact that work on invasive bivalves in San Diego from the mid-1990s did not detect this species (Crooks 1998). Although ship ballast and fouling are possible invasion vectors, with potential source regions of its native Asia or other areas where established (e.g., the Pacific Northwest), it is likely that aquaculture played a role in this invasion. Aquaculture is an important economic activity in California's coastal waters, and the Pacific oyster is approved for propagation by registered aquaculturists (Grosholz et al. 2012). There is a report of Pacific oyster culture and harvest in San Diego in 1988 (Shaw 1997), but that appears to have been short-lived. Renewed attempts at Pacific oyster culture in Agua Hedionda Lagoon began in the 1990s, and that effort continues today (Conte and Moore 2001, Moore and Moore 2010, Grosholz et al. 2012).

In general, the Pacific oyster is one of the most widely translocated of marine organisms, and it has successfully invaded North America, Europe, South America, Africa, Australasia, and Hawaii (Ruesink et al. 2005, Carlton and Eldredge 2009). Part of what makes the Pacific oyster a successful aquaculture species (and invader) is its relatively broad environmental tolerances (Padilla 2010), including its ability to live within a broad temperature range (Rico-Vila et al. 2009). Within its native Asia, it is found where summer temperatures range from approximately 14° to 29°C and winter temperatures range from -1.9° to 20°C (Carrasco and Baron 2010). Temperatures within San Diego estuaries fall within this range, with summer highs occasionally reaching 28°C and winter lows reaching 6°C (NERRS 2014).

It remains unclear why there should be a successful invasion now, given the failure of previous attempts to deliberately introduce the species both locally and throughout California. Such lags in establishment are not uncommon, however, and invaders are known for their ability to cause "ecological surprises" (Crooks 2011). Also, it is difficult to know the invasion trajectory into the future, as this invasion is still in its early phases. It is possible that the invasion will ultimately fail, but the presence of Pacific oysters across multiple years and multiple systems makes that less likely. If populations in southern California waters do continue to expand and grow, as they have in other areas where they have invaded (e.g., Troost 2010), it will undoubtedly bring changes to the way our estuarine intertidal habitats function as well as in the way we must manage them. Oysters are ecosystem engineers that can alter systems by creating dense biogenic structure and filtering the water column (Ruesink et al. 2005, Crooks 2009, Padilla 2010). The Pacific oyster's potential ability to create "living shorelines" actually exceeds that of the smaller, native *O. lurida*, and this needs to be considered in efforts to restore native oyster habitat in San Diego Bay and elsewhere in southern California. Because Pacific oysters rapidly reach large sizes they could pose problems related to fouling of maritime equipment, infrastructure, and vessels. Finally, Pacific oysters represent an edible and sought-after species, and could be the basis of expanded harvesting activities. However, the lack of recent history of a substantial shellfish harvest in places such as upper San Diego Bay would likely necessitate management approaches that address possible public health risks, such as contamination and disease (e.g., Kaysner et al. 1987, Pauley et al. 1988).

Pacific oysters stand out as one of the most transformative invaders of marine ecosystems. Better understanding the role of the Pacific oyster, and how to manage it, will necessitate research on this species in its new setting, including studies of both the oyster population and its interaction with both biotic and abiotic elements of local ecosystems. The Pacific oyster also could advance opportunities for education and public involvement, which are important components of marine management efforts (e.g., West Coast WISE Program 2014). Because Pacific oysters are now becoming one of the most conspicuous biota in our local estuaries, they provide an opportunity to highlight often-hidden changes occurring in the sea.

Acknowledgments

This work was supported by grants to JAC from the Western Regional Panel of the Aquatic Nuisance Species Task Force, the San Diego Foundation's Blasker Environment Grant, as well as the National Oceanic and Atmospheric Administration National Estuarine Research Reserve System. This work also stems in part from Science Fair projects by KRC and AJC. There are many to acknowledge for assisting us and providing information on Pacific oysters in California, including J. Asif, H. Carson, A. Chang, B. Cheng, A. Cohen, M. Genevich, C. Gramlich, J. Lorda, C. Whitcraft, C. Zabin, and D. Zacherl, with a special thank you going to E. Crooks. We also are grateful to the editors and two anonymous reviewers for their thoughtful comments, which improved this manuscript. We dedicate this paper to Jim Carlton, who continues to inspire and advance a deeper understanding of invasion ecology, impacts, and management.

Literature Cited

Barrett, E. M. 1963. The California oyster industry. California Department of Fish and Game, Fish Bulletin 123:1-103.

Carlton, J. T. 1979. History, biogeography, and ecology of the introduced marine and estuarine invertebrates of the Pacific coast of North America. Ph.D. Thesis, University of California, Davis, USA.

Carlton, J. T., and L. G. Eldredge. 2009. Marine bioinvasions of Hawai'i. Bishop Museum Bulletin in Cultural and Environmental Studies 4:1-202.

Carrasco, M. F., and P. J. Baron. 2010. Analysis of the potential geographic range of the Pacific oyster *Crassostrea gigas* (Thunberg, 1793) based on surface seawater temperature satellite data and climate charts: the coast of South America as a study case. Biological Invasions 12:2597-2607.

Cohen, A. N., L. H. Harris, B. L. Bingham, J. T. Carlton, J. W. Chapman, C. C. Lambert, G. Lambert, J. C. Ljubenkov, S. N. Murray, L. C. Rao, K. Reardon, and E. Schwindt. 2005. Rapid assessment survey for exotic organisms in southern California bays and harbors, and abundance in port and non-port areas. Biological Invasions 7:995-1002.

Cohen, A. N., and A. Weinstein. 2008. Exotic oyster survey, removal and research in San Francisco Bay. Annual Progress Report to the California Ocean Science Trust, Oakland, California, USA.

Conte, F., and T. Moore. 2001. Culture of oysters. Pages 500-506 *in* W. S. Leet, C. M. Dewees, R. Klingbeil, and E. J. Larson, editors. California's living marine resources: a status report. California Department of Fish and Game, Sacramento, USA.

Crooks, J. A. 1998. The effects of the introduced mussel *Musculista senhousia* and anthropogenic agents on benthic ecosystems of Mission Bay, San Diego. Ph.D. Thesis, University of California, San Diego, USA.

Crooks, J. A. 2001. Assessing invader roles within changing ecosystems: historical and experimental perspectives on an exotic mussel in an urbanized lagoon. Biological Invasions 3:23-36.

Crooks, J. A. 2009. The role of exotic marine ecosystem engineers. Pages 287-304 *in* G. Rilov and J. A. Crooks, editors. Marine bioinvasions: ecological, conservation, and management perspectives. Springer, Berlin, Germany.

Crooks, J. A. 2011. Lag times. Pages 404-410 *in* D. Simberloff and M. Rejmanek, editors. Encyclopedia of biological invasions. University of California Press, Berkeley, USA.

Gilbert, C. H. 1891. Report on certain investigations regarding the planting of oysters in southern California. Pages 95-97 *in* Bulletin of the United States Fish Commission, Volume 9 for 1889. United States Commssion of Fish and Fisheries, Wasington, D.C., USA.

Grosholz, E. D., R. E. Crafton, R. E. Fontana, J. Pasari, S. L. Williams, and C. Zabin. 2012. Aquatic invasive species vector risk assessments: an analysis of aquaculture as a vector for introduced marine and estuarine species in California. Report to the California Ocean Science Trust, Oakland, California, USA.

Ingersoll, E. 1881. The oyster industry. Pages 201-205 *in* The history and present condition of the fishery industries. Tenth Census of the United States. U.S. Department of Commerce, Washington, D.C., USA.

Islas-Olivares, R. 1975. El ostion japones (*Crassostrea gigas*) en Baja California. Ciencias Marinas 2:58-59.

Kaysner, C. A., C. Abeyta, M. M. Wekell, A. DePaola, R. F. Stott, and J. M. Leitch. 1987. Virulent strains of *Vibrio vulnificus* isolated from estuaries of the United States west coast. Applied and Environmental Microbiology 53:1349-1351.

Kirby, M. X. 2004. Fishing down the coast: historical expansion and collapse of oyster fisheries along continental margins. Proceedings of the National Academy of Sciences USA 101:13096-13099.

LaGrange, J. 2002. *Crassostrea gigas* Thunberg, 1793 in San Diego Bay, California. Festivus 34:91-92.

Moore, H. F. 1897. Oysters and methods of oyster-culture. Pages 263-340 *in* Report of United States Commission of Fish and Fisheries for 1897. United States Commssion of Fish and Fisheries, Wasington, D.C., USA.

Moore, T. O., and J. D. Moore. 2010. Culture of oysters. Chapter 21 *in* Status of the fisheries report. California Department of Fish and Game, Sacramento, USA.

NERRS (National Estuarine Research Reserve System). 2014. System-wide monitoring program, Centralized Data Management Office [Internet]. Accessed 15 October 2014. Available from: http://cdmo.baruch.sc.edu

Padilla, D. K. 2010. Context-dependent impacts of a non-native ecosystem engineer, the Pacific oyster *Crassostrea gigas*. Integrative and Comparative Biology 50:213-225.

Pauley, G. B., B. Van Der Raay, and D. Troutt. 1988. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest)—Pacific oyster. United States Fish and Wildlife Biological Report 82(11.85), United States Army Corps of Engineers, TR EL-82.4:1-28.

Preisler, R., K. Wasson, W. Wolff, and M. Tyrell. 2009. Invasions of estuaries vs the adjacent open coast: a global perspective. Pages 587-617 *in* G. Rilov and J. A. Crooks, editors. Marine bioinvasions: ecological, conservation, and management perspectives. Springer, Berlin, Germany.

Rathbun, R. 1894. Summary of the fishery investigations conducted in the North Pacific Ocean and Bering Sea from July 1, 1888, to July 1, 1892, by the U.S. Fish Commission Steamer Albatross. Pages 357-364 *in* Bulletin of the United States Fish Commission, Volume 7 for 1892. United States Commission of Fish and Fisheries, Washington, D.C., USA.

Rico-Villa, B., S. Pouvreau, and R. Robert. 2009. Influence of food density and temperature on ingestion, growth and settlement of Pacific oyster larvae, *Crassostrea gigas*. Aquaculture 287:395-401.

Ruesink, J. L., H. S. Lenihan, A. C. Trimble, K. W. Heiman, F. Micheli, J. E. Byers, and M. C. Kay. 2005. Introduction of non-native oysters: ecosystem effects and restoration implications. Annual Review of Ecology, Evolution, and Systematics 36:643-689.

Shaw, W. N. 1997. The shellfish industry of California—past, present and future. Pages 57-74 *in* C. L. MacKenzie, Jr., V. G. Burrell, Jr., A. Rosenfield, and W. L. Hobart, editors. The history, present condition and future of molluscan fisheries of North and Central America and Europe, Volume 2. Pacific Coast and Supplemental Topics. United States Department of Commerce, NOAA Technical Report 127.

Smith, D., M. Stephenson, J. Goetzl, G. Ichikawa, and M. Martin. 1987. The use of transplanted juvenile oysters to monitor the toxic effects of tributylin in California waters. Conference Proceedings — Oceans '87:1511-1516.

Townsend, C. H. 1893. Report of observations respecting the oyster resources and oyster fishery of the Pacific Coast of the U.S. Pages 343-372 *in* Report of the Commissioner for 1889-1991. United States Commission of Fish and Fisheries, Washington, D.C., USA.

Troost, K. 2010. Causes and effects of a highly successful marine invasion: case-study of the introduced Pacific oyster *Crassostrea gigas* in continental NW European estuaries. Journal of Sea Research 64:145-165.

Tuskes, P. 2012. Survey of Mission Bay mollusks, San Diego, California. Festivus 44:13-29.

West Coast WISE (Watershed and Invasive Species) Program. 2014. Oregon Sea Grant [Internet; cited 29 April 2014]. Available from: http://seagrant.oregonstate.edu/invasive-species/wise

Williamson, M. B. 1894. Conchological researches in San Pedro Bay and vicinity, including the Alamitos oyster fishery. Annual Publication of the Historical Society of Southern California 3:10-15.

Wilson, E. N. 1943. Shells (alive and dead) I have found in Mission Bay. Conchological Club of Southern California 19:6-8.

Received 29 April 2014
Accepted 30 October 2014
Associate Editor was P. Kalvass

California Fish and Game 101(2):108-130; 2015

Effects of loading density during transport on physiological stress and survival of Sacramento-San Joaquin Delta fishes

Zachary A. Sutphin* and Charles D. Hueth

Fisheries and Wildlife Resources Group, United States Bureau of Reclamation, Denver, CO 80225, USA

**Correspondent: zsutphin@usbr.gov*

Truck transportation from the Bureau of Reclamation's Tracy Fish Collection Facility in California's Sacramento-San Joaquin Delta is the final phase of a multi-component process that results in the capture and release of >50 fish species, thereby preventing entrainment at a downstream water pumping facility. Fish-transport tables (termed Bates Tables) developed in 1955 do not take into consideration the fish loading process, commonly transported sensitive species, or physiological effects of elevated densities. To investigate suitability of the Bates Tables, effects of loading and transport at recommended and twice-recommended transport densities on physiological stress and survival of threadfin shad (*Dorosoma petenense*), Chinook salmon (*Oncorhynchus mykiss*), and striped bass (*Morone saxatilis*) were tested. Density did not have a significant effect on fish survival or physiological stress, as indicated by blood plasma constituent levels. At both densities, mean post-transport (168 hour) survival of all species was high (>98%), and ammonia and carbon dioxide levels increased in transport water as a result of fish metabolism, but levels remained below lethal levels. Among all species tested blood cortisol, glucose, and lactate levels followed a predictive adaptive response, with levels tending to peak immediately following transport and returning to basal levels within 24 hours.

Key words: blood plasma constituents, water quality, adaptive stress response, metabolic rate

---

The truck transportation and release of multiple species and life-stages of fish is the ultimate operation at the Bureau of Reclamation's (BOR) Tracy Fish Collection Facility (TFCF; Byron, CA) necessary to return fish to their natal Sacramento-San Joaquin Delta and prevent entrainment into the Delta-Mendota Canal. Fish transport from the TFCF consists of hauling fish in a cylindrical tank (1.2-m deep, 4.4-m long, mean volume post-transport = 6,455 l) provided continuous pure oxygen via pressurized cylinder and diffusing airstones,

over a maximum distance of 49.9 km (Sutphin and Wu 2008). There is temporal variation in the species of fish transported, water quality during transport, and density of fish salvaged and transported from the TFCF (Sutphin and Wu 2008, CDFG 2013). There are >50 species of fish historically salvaged at the TFCF, and temperatures can approach species-specific lethal levels (Brett 1952, Olson and Foster 1957). Mean transport duration is 59.4 minutes (Craft et al. 2008, Sutphin and Wu 2008). Design, operation, and continued research at the multi-component TFCF is intended to maximize fish salvage while minimizing fish mortality (BOR 2013). However, operations at the TFCF result in the salvage of a constant influx of fish, and there is currently no efficient method to control the amount of fish entering the facility. As a result, elevated densities of fish may sometimes be transported.

To maintain fish health and maximize long-term survival, stressors common during loading and transport operations including handling, confinement, unfavorably high densities, and degraded water quality conditions must be considered (Piper et al. 1982, Berka 1986, Sutphin and Wu 2008). Fish loading and transportation, particularly at high densities, can result in mechanical abrasion, poor water quality conditions, and physiological stress, which can contribute to reduced survivability (Ross and Ross 1999, Urbinati et al. 2004, Carneiro et al. 2009). Exposure to stress elicits a general adaptive physiological and behavioral stress response in most fishes (Pickering 1981), consisting of primary and secondary levels and, if the stressor persists, a tertiary level (Schreck 1981, Bonga 1997, Barton et al. 2002). The primary stress response, as a result of capture, loading, and transport, is generally exhibited as the release of circulating catecholamines and corticosteroids (i.e., cortisol) by activation of the hypothalamus-pituitary-interrenal axis (Barton et al. 2003, Urbinati et al. 2004). As a result, cortisol is commonly measured as an indicator of fish primary stress response (Bonga 1997). The secondary stress response, among other physiological processes, can result in increased blood glucose and lactate, as well as increased heart rate, blood flow, and metabolic rate (Barton and Iwama 1991, Mommsen et al. 1999, Barton et al. 2002), making blood glucose and lactate, as well as measures of metabolism (oxygen consumption (MO_2) and ammonia production (M_{TAN}) common means to measure the secondary stress response in fish (Barton and Schreck 1987, Barton et al. 2002). The secondary stress response elicited in fish during fish-loading and transport, and glucose production in particular, is a response to fish energetic requirements, but can coincide with elevated MO_2, carbon dioxide production (MCO_2), and M_{TAN} rates, accelerating the rate of water quality decline (Bonga 1997, Barton et al. 2002). In truck transport systems, accumulated fish metabolic and excretory byproducts can result in toxic levels of CO_2, total ammonia nitrogen (TAN), and unionized ammonia (NH_3), which can impair performance, health, and survival of fish (Meade 1985, Russo and Thurston 1991). Similarly, elevated MO_2 can result in low O_2 levels, leading to a hypoxic state, which can also contribute to deteriorating health and mortality (Wedemeyer 1996). As a result, maintenance of appropriate water quality is often a limiting factor during fish transport, and is generally considered when developing fish transportation tables (Berka 1986, Emata 2000).

During the initial phases of TFCF development, fish transportation tables, termed Bates Tables, were designed based on simple unreplicated experiments during which the number of juvenile striped bass (*Morone saxatilis*) that can be maintained in stagnant water provided compressed air was estimated (BOR 1955). The activity report provided by BOR (1955) provides minimal experimental detail, but apparently doesn't consider stressors associated with actual transport, only considers the response of one species and,

presumably, doesn't incorporate the additional stressor of fish loading. Because multiple acute disturbances (i.e., capture, loading, transport, and release) tend to result in a cumulative stress response (Barton et al. 1986, Mesa 1994), and the survival of millions of fish annually transported from the TFCF is based on transport tables designed with simple, unreplicated, and vaguely described experiments on a single species, the primary research objective was to quantify effects of loading and transporting fish at recommended Bates Table levels on transport water quality, physiological stress, and post-transport (168 hour) survival of striped bass, Chinook salmon (*Oncorhynchus tshawytscha*), and threadfin shad (*Dorosoma petenense*). To determine if higher fish densities could be transported when deemed necessary by TFCF management, the secondary objective was to evaluate the effects of fish transport at densities twice the level recommended by the Bates Tables on the same parameters.

Materials and Methods

Selection of test species.—The species tested represent a phylogenetically diverse group with distinct life histories, and all have incurred precipitous declines in population abundance in recent decades (Yoshiyama et al. 1998, Moyle 2002, Feyrer et al. 2007). Threadfin shad were selected for testing because they are a Pelagic Organism Decline (POD) species (Sommer et al. 2007), have historically been the most abundant fish in the TFCF salvage, and, because they are a shoaling species, are often salvaged in high densities (CDFG 2013). Striped bass are also defined as a POD and are frequently salvaged at the TFCF, but were also selected because the Bates Tables were developed based on data collected with striped bass (BOR 1955). Of the four distinct populations of Central Valley Chinook salmon, winter-run are classified and protected as endangered and spring-run are classified as threatened under the Endangered Species Act (NMFS 1997). Their current listing status, as well as their use as a key species when considering initial design and development of the TFCF, warranted their use as a species during testing (Bates and Visonhaler 1957). Though all runs of Chinook salmon are salvaged at the TFCF, Fall- and Spring-run are generally the most abundant (Aasen 2011, 2012, 2013). Therefore, fall-run were selected for testing, and were intended as a representative surrogate for all salmon runs.

Fish source and care.—Juvenile Chinook salmon (Central Valley Fall-run) were acquired from the Mokelumne River Fish Hatchery (California Department of Fish and Wildlife, Clements, California), juvenile striped bass were acquired from Keo Fish Farm, Inc. (Keo, Arkansas), and adult threadfin shad were obtained from Hermann's Fish Farm (Robstown, Texas) for testing. All test species were truck-transported in 550-l tanks to BOR's Technical Service Center (TSC; Denver, CO), where they were maintained in two to three continuously aerated 870-l circular tanks. Following arrival at the TSC, fish were maintained at transport water temperatures and provided a daily 2–3 hour prophylactic salinity (NaCl at 6–8 g/l) and paracide green/formalin (22.5 ml) bath for four days to minimize likelihood of pathogenic infection and promote internal osmotic balance that can be compromised as a result of handling and transport procedures (Piper et al. 1982, Wedemeyer 1996). Approximately 7 days following arrival, fish were exposed to gradual changes in temperature, not exceeding 1.0°C/day, until target test temperatures were achieved. Fish were maintained under a natural photoperiod (37° 44' 23" N) with a combination of natural and halogen light sources. Chinook salmon were fed slow sinking pellets (1.5 mm, Bio Oregon®, Longview, Washington), striped bass were fed floating pellets (1.5 mm, Skretting USA, Tooele, Utah),

and threadfin shad were fed a mixture of crumbled dry feed (Skretting USA, Tooele, Utah) and Hikari® dry plankton feed (0.4–0.6 mm, Kyorin Co., Ltd., Japan). Threadfin shad were provided supplemental feedings of live brine shrimp (INVE Aquaculture, Inc., Salt Lake City, Utah). All species were fed at 2–3% body weight per day**.**

Density level and thermal regime.—Species-specific transport densities and thermal regime used during testing were selected to represent the worst-case-scenario for fish transport operations as currently outlined in the Bates Tables. This was ultimately selected as the highest water temperature each species would likely be exposed to during TFCF fish-transport, based on historic TFCF fish salvage data (1992–2009), and the corresponding maximum allowable transport density as defined by the Bates Tables (Craft et al. 2008). Elevated water temperatures were assumed to contribute to the worst-case-scenario during fish transport because elevated temperatures, particularly those approaching lethal levels, result in decreased gas solubility (i.e., lower dissolved oxygen levels), increased metabolic rates (i.e., increased dissolved oxygen intake and ammonia excretion), and immunosuppression (Wedemeyer et al. 1976, Colt 1984). Also, basal conditions, and magnitude and duration of stress response can be affected by thermal regime, often increasing at extremes (Wydoski et al. 1976, Barton and Schreck 1987, Davis and Parker 1990). Therefore, test temperature ranges selected for Chinook salmon, striped bass, and threadfin shad were 20 to 21°C, 26 to 27°C, and 23 to 24°C, respectively.

The Bates Tables isolate species into two categories, Chinook salmon and "other", and do not recommend specific transport densities (i.e., g fish/l), but indicate the percent of a load (up to 100%) as a total number of salvaged fish within a particular size class represents. For example, full loads (100%) of Chinook salmon >7.6 cm transported at 20–21°C, threadfin shad 6.4 to 11.4 cm transported at 26–27°C, and striped bass >11.4 cm transported at 23–24°C, are approximately 9,000 fish, 4,500 fish, and 3,500 fish, respectively, in a 3,785-l truck (original TFCF truck volume). To convert number of fish per unit volume to density (g/l), length-to-weight regression relationships developed from fish captured at the TFCF, were used to estimate weights of Chinook salmon ($y = 0.0074\ x^{3.12}$), threadfin shad ($y = 0.000004\ x^{3.27}$), and striped bass ($y = 0.0092\ x^{3.01}$) at 80, 100, and 150 mm fork length, respectively. Calculated Chinook salmon (11.33 g), threadfin (6.82 g), and striped bass (31.85 g) weights were multiplied by total number of fish, based on a full load (100%) as indicated by the Bates Tables, then divided by the volume (3,785 l) of the TFCF fish-transport truck, to calculate the following recommended maximum densities targeted during testing (Bates) for Chinook salmon, threadfin shad, and striped bass: 26.9, 8.1, and 29.5 g/l. These experimental densities were intended to determine if the maximum densities recommended by the Bates Tables are suitable. Double the recommended maximum densities were also tested to determine if higher transport densities could be used, as deemed necessary or appropriate by facility managers (Bates×2).

Fish marking and pre-treatment holding.—Prior to testing, fish were marked using a fluorescent microsphere solution (New West Technologies, Santa Rosa, California) and isolated as a function of treatment condition (species × density level) into individual 190-l conical holding tanks (76 cm diameter × 61 cm high), intended to simulate the TFCF fish haul-out bucket. Twenty fish of each species were also marked and transferred to 340-l post-transport survival-tanks to serve as a control. Marking fish prior to testing permitted an accurate estimate of transport density, and allowed consolidation of treatment and control fish during post-transport survival assessment. This marking method was deemed minimally

invasive compared to other external marking techniques and, coupled with a 7 days post marking holding period, presumably did not affect test fish stress response (Sharpe et al. 1998, Hayes et al. 2000). Species-specific feeding regime before experimentation and 7 days following marking was the same as outlined during fish holding**.** Test fish were not provided food for ~12 hours prior to experimentation. Water temperature (°C), pH (standard units), total ammonia nitrogen (TAN, mg/l), and dissolved oxygen (DO; mg/l) were measured daily using a YSI Pro-Plus multi-parameter instrument (YSI Inc., Yellow Springs, Ohio), and CO_2 (mg/l) was measured daily using a Oxyguard Carbon Dioxide Analyzer (Water Management Tech, Baton Rouge, Louisiana). The YSI Pro-Plus TAN electrode was calibrated daily during testing. All other probes were calibrated once at the initiation of each experimental period.

Experimental methodology.—To initiate fish loading from a 190-l fish holding tank into a fish transport container, water was drained from an external side 3.8-cm valve until ~30 l water and fish remained in the holding tank. During this process, two 1,000 mL-graduated beakers were partially filled and pre-transport water °C, DO, salinity (NaCl, g/l), pH, TAN, and CO_2 were measured. A fish transport container (30.5 cm long × 25.4 cm diameter, 15.1 l), sealed on the ends with 25.4 cm rubber cap and containing an internal microbubble oxygen diffuser (Point 4 System Inc., Coquitlam, British Columbia, Canada), was oriented below the conical holding tank, filled with tank water (permitting flushed fish to transfer directly to water and not an empty transport tank), and a 7.6-cm valve was opened, permitting remaining water and all fish to pass through a 7.6-cm rubber tube into the fish transport container. Drop height of water and fish from the holding tank to the transport container, was 24.1 cm. One hundred ten grams of non-iodized salt (North American Salt Co., Overland Park, Kansas) was added to each container to achieve salinities near 8 g/l, a value targeted during transport of fish from the TFCF. A 7.6-cm plug was immediately inserted to restrict water and fish spillage, and the transport container was transferred to an enclosed rear-section of a vehicle where the internal microbubbler diffuser was immediately supplied pure oxygen via a pressurized cylinder. Minimal fluctuation in temperatures occur within the transport tanks of trucks at the TFCF (Sutphin and Wu 2008); during testing, atmospheric temperature inside the vehicle was monitored and adjusted in an effort to maintain temperature similar to targeted test temperature. During transport, a 1.9-cm valve was partially opened to permit degassing, a similar practice used during truck transport of fish from the TFCF. Target transport duration was 60 minutes, similar to those recorded during fish transport at the TFCF (Sutphin and Wu 2008).

Following experimental transport, two fish from each replicate treatment were removed from their respective transport container. One fish was immediately immobilized and sampled for blood, and one was transferred to a 9.5-l black bucket, containing ~4.7 l water at the same temperature as pre-transport holding, provided continuous aeration via an air pump, and covered with a black lid. Fish were maintained in quiescent conditions for 1 hour, post transport, to determine if a short-duration holding period would allow fish time to recover from loading and transport stress. Post-transport water quality was also measured. Pre- and post-transport water quality conditions permitted the calculation of species- and density-dependent static M_{TAN} and $M_{CO2,}$ indicators of fish metabolic rate and response to stress (Alsop et al. 1999, Randall and Tsui 2002), as well as Δ°C, ΔpH, maximum TAN (NH_3) and CO_2 levels, and verification of transport NaCl levels. Unionized ammonia levels (NH_3) were calculated based on the NH_3 fraction of TAN (Francis-Floyd et al. 2012).

The remaining fish in each transport container were transferred (water to water) to a 340.7 l survival tank, with each survival tank housing a replicate for Bates, Bates×2, and control. Each survival tank was covered with shade cloth to promote quiescent conditions, and was only partially opened during blood sampling, feeding, and water quality measurements. Fish were removed from survival tanks (two/treatment) using a fine-mesh dip-net, at 24 and 168 hours for blood sampling. Water quality measurements and feeding regime through 168 hours post-transport were the same used during pre-treatment holding. Following 168 hour post-transport survival assessment, all fish were euthanized with a lethal dose of tricaine methanesulfonate (MS-222, Argent Chemical Laboratories, Inc., Redmond, Washington; 200 mg/l) weighed (±0.01 g) and measured for standard and total length (±1 mm) using an electronic balance and measuring board.

Due to the difficulty in capturing a known number of fish during fish loading (e.g., draining from conical tanks into fish transport containers) without causing additional harm or stress to other test fish, effects of the fish flushing process on blood plasma constituents (not metabolic rates or survival) were evaluated independently 7 days following the completion of the previously described treatment conditions for each species tested. To assess effects of flushing, fish were marked and provided the same conditions and care during pre-treatment holding described for subsequent treatment conditions. Following flushing from the holding tank (Flush), two fish were immediately captured, sampled for blood, and processed (weighed and measured) using the same methods described for previous treatments. These fish were sampled to assess if the fish loading or flushing process contributed to elevated stress, allowing the differentiation between effects of fish loading and fish transport on stress. However, it is important to note when interpreting flush data that flushed fish were tested independently and after all other treatment conditions.

To sample blood following loading (Flush), transport (1 hour), 1 hour post transport (2 hour), 24 hours, and 168 hours post transport, captured fish were quickly transferred to a water bath containing a lethal dose of MS-222 (200 mg/l), which resulted in rapid immobilization (<20 sec). Elevated MS-222 dose, coupled with sampling fish within 2 minutes following capture, likely inhibited stress-related increases in plasma cortisol, and ensured measured cortisol levels were a result of treatment conditions and not an artifact of handling and sampling techniques (Barton et al. 1986; Barton 2000, 2002). Immobilized fish were wrapped in Kimwipes® to ensure residual water did not contaminate blood samples, and the caudal peduncle was immediately severed with a scalpel. Heparinized microhematocrit capillary tubes (40-µl) were used to collect blood from the caudal vein and artery, and were immediately centrifuged using a microhematocrit centrifuge (Clay-Adams Autocrit Ultra 3, Franklin Lakes, New Jersey) for five minutes at 12,000 rpm to separate plasma and packed red blood cells. Packed red blood cell volume (Hematocrit, Hct) was measured immediately following centrifuging. The volume of red blood cells was discarded, and the remaining blood plasma was transferred into plastic cryogenic freezing vials and stored in a -80°C freezer. Weights (±0.01 g) and lengths (SL, TL ±1 mm) of each fish were obtained following sampling. Cryogenic vials were later thawed for plasma glucose, lactate, and cortisol measurements. Glucose and plasma were measured using a polarographic analyzer (YSI 2700 Select, YSI Inc., Yellow Springs, Ohio), plasma cortisol levels were measured at Mapes Veterinary Endocrinology Laboratory (University of California, Davis) using a modified enzyme immunoassay. Small volumes of blood recovered from threadfin shad precluded cortisol measurement.

Statistical analyses.—The majority of data did not meet the assumptions of parametric statistics; therefore Two-Way ANOVA on ranks was used to test species and treatment (density, time post transport) differences between morphometrics (weight, length), transport conditions (duration, density, water quality post-transport), metabolism (M_{TAN}, M_{CO2}), blood plasma constituents (Hct, cortisol, glucose, and lactate), and 168 hour post-transport survival. Tukey's Test was employed for all pairwise comparisons. All statistical analyses were conducted using SigmaStat 3.5 software (Systat Software Inc., Richmond, California); the significance level (α) for all analyses was set at 0.05.

Results

Wet weights of test fish were significantly different, for all treatment conditions, across species tested (Table 1; $F_{2,6} = 337.81$, $P < 0.001$). Threadfin shad and striped bass weights were not different across treatment conditions (Tukey's Test, $P > 0.05$). However,

Table 1.—Fish lengths, weights, sample size, and number of fish used per replicate during testing. Lengths and weights are reported as means ± 1 standard deviation.

Species	Treatment	Sample Size (*n*)	#fish/ replicate	Standard Length (mm)	Total Length (mm)	Wet Weight (g)
Chinook Salmon	Control	12	20 - 21	123.8 ± 13.7	141.6 ± 15.2	25.5 ± 9.3
	Flush	12	2	114.9 ± 12.4	131.6 ± 13.6	18.11 ± 6.3
	Bates	12	13 - 21	119.3 ± 13.3	136.7 ± 14.8	21.9 ± 7.8
	Bates × 2	12	22 - 40	124.3 ± 13.8	142.2 ± 15.4	25.6 ± 9.2
Striped Bass	Control	12	20	97.3 ± 4.8	115.8 ± 5.3	17.5 ± 2.7
	Flush	12	2	100.1 ± 4.6	118.6 ± 4.7	18.5 ± 2.2
	Bates	12	24 - 37	98.0 ± 5.4	116.5 ± 6.6	18.1 ± 3.6
	Bates × 2	12	50 - 68	97.3 ± 5.8	115.6 ± 6.9	17.8 ± 3.7
Threadfin Shad	Control	8	20 - 21	72.3 ± 14.7	89.7 ± 18.1	7.2 ± 4.9
	Flush	8	2	83.6 ± 12.5	102.6 ± 15.8	10.1 ± 4.6
	Bates	8	14 - 16	77.3 ± 12.5	94.9 ± 15.3	8.4 ± 4.2
	Bates × 2	8	31 - 38	72.8 ± 11.5	89.3 ± 14.1	7.2 ± 3.7

Chinook salmon Bates×2 and control treatments, though not different in weight from each other, were significantly heavier than the other treatment conditions (Tukey's Test, $P < 0.001$). Pre-treatment (168 hour acclimation) and post-treatment (168 hour survival assessment) water quality conditions are reported in Table 2. Transport duration was not different, as a function of treatment, within species tested ($F_{1,2} = 1.7$, $P = 0.20$). However, mean transport durations of threadfin shad (Bates=58.8 minutes, Bates×2=59.8 minutes) were significantly shorter than Chinook salmon (Bates = 60.5, Bates×2 = 60.8 minutes) and striped bass (Bates = 60.2, Bates×2 = 60.2 minutes; $F_{1,2} = 11.2$, $P < 0.001$; Table 3). Transport °C ($F_{1,2} = 1.1$, $P = 0.305$), DO ($F_{1,2} = 0.11$, $P = 0.738$), and TAN ($F_{1,2} = 2.0$, $P = 0.162$) levels were unaffected by fish density level (Table 3). However, final transport CO_2 levels increased with increasing density for all species tested (Table 3; $F_{1,2} = 73.9$, $P < 0.001$). As designed (see Methods) transport densities ($F_{1,2} = 596.9$, $P < 0.001$) and temperatures ($F_{1,2} = 130.9$, $P < 0.001$) were different across species, and typically within the predetermined target range (Table 3). Interestingly, NaCl levels were different across species during testing ($F_{1,2} = 38.8$, $P < 0.001$), but differences in means (Table 3) were small enough that they likely were not biologically relevant.

Table 2.—Pre-treatment water quality conditions, as measured in 190-l conical tanks (treatment) and 870-l tanks (control) 1–7 days before fish transport, and post-transport water quality conditions as measured in 340-l holding tanks during 168 hour survival assessment. Temperature is reported as °C, dissolved oxygen (DO), salinity (NaCl), and total ammonia nitrogen (TAN) are reported as mg/l, and pH is reported in standard units. Values are reported as means ±1 standard deviation.

		Pre-Treatment Conditions					*Post-Transport (168 hours) Conditions*			
Species	Treatment	°C	DO	NaCl	pH	TAN	°C	DO	pH	TAN
Chinook Salmon	Control	20.0 ± 1.3	6.8 ± 0.3	0.2 ± 0.0	7.6 ± 0.3	0.4 ± 0.2	21.4 ± 0.7	6.3 ± 0.2	7.8 ± 0.3	0.3 ± 0.1
	Bates	20.1 ± 1.3	6.8 ± 0.4	0.2 ± 0.0	7.6 ± 0.4	0.4 ± 0.2				
	Bates × 2	20.1 ± 1.3	6.6 ± 0.4	0.2 ± 0.0	7.6 ± 0.3	0.4 ± 0.2				
Striped Bass	Control	22.7 ± 1.3	6.2 ± 0.4	0.4 ± 0.3	7.5 ± 0.2	0.7 ± 0.4	23.3 ± 0.5	6.1 ± 0.3	7.7 ± 0.2	0.4 ± 0.2
	Bates	22.8 ± 1.1	6.3 ± 0.3	0.2 ± 0.1	7.7 ± 0.2	0.4 ± 0.2				
	Bates × 2	22.8 ± 1.1	6.1 ± 0.3	0.2 ± 0.1	7.7 ± 0.2	0.4 ± 0.2				
Threadfin Shad	Control	26.8 ± 0.6	5.6 ± 0.3	0.2 ± 0.0	7.9 ± 0.1	0.7 ± 0.4	26.7 ± 0.7	5.6 ± 0.2	7.9 ± 0.2	0.2 ± 0.0
	Bates	27.1 ± 0.3	5.9 ± 0.1	0.2 ± 0.0	8.0 ± 0.2	0.6 ± 0.3				
	Bates × 2	27.2 ± 0.2	5.8 ± 0.1	0.2 ± 0.0	8.0 ± 0.1	0.1 ± 0.0				

Table 3.—Target and actual densities during fish transport, transport duration, and water quality conditions (temperature = °C, dissolved oxygen = DO (mg/l), salinity = NaCl (mg/l), total ammonia nitrogen = TAN (mg/l), unionized ammonia = NH_3 (mg/l), carbon dioxide = CO_2 (mg/l)) in transport containers at the end of transport during testing.

Species	Treatment	Target Density (g/l)	Actual Density (g/l)	Transport Duration (minutes)	° C	DO	NaCl	pH	Max TAN	Max NH_3	Max CO_2
Chinook Salmon	Bates	26.9	24.5 ± 1.5	60.5 ± 0.9	20.5 ± 1.2	24.0 ± 4.3	8.5 ± 0.2	6.8 ± 0.3	5.8 ± 1.3	0.01	7.0 ± 2.0
	Bates × 2	53.8	48.8 ± 1.6	60.8 ± 0.8	20.5 ± 1.2	24.1 ± 2.5	8.8 ± 0.2	6.9 ± 0.3	6.0 ± 1.1	0.02	12.1 ± 4.3
Striped Bass	Bates	29.5	34.7 ± 3.9	60.2 ± 0.6	23.0 ± 1.0	21.4 ± 6.2	8.4 ± 0.1	6.8 ± 0.1	4.8 ± 0.8	0.01	8.3 ± 2.5
	Bates × 2	59	68.4 ± 5.7	60.2 ± 0.3	23.0 ± 1.0	23.5 ± 1.4	8.6 ± 0.2	6.8 ± 0.0	5.1 ± 0.9	0.01	13.7 ± 4.1
Threadfin Shad	Bates	8.1	8.5 ± 0.2	58.8 ± 0.9	25.8 ± 0.9	19.0 ± 3.4	8.2 ± 0.1	7.1 ± 0.1	4.6 ± 0.2	0.04	4.6 ± 0.9
	Bates × 2	16.2	16.5 ± 0.4	59.8 ± 0.8	26.8 ± 0.2	16.5 ± 2.1	8.3 ± 0.2	7.1 ± 0.0	4.8 ± 0.1	0.03	11.6 ± 0.9

Survival for all species tested, through 168 hours following transport, was >98%, not affected by density level, and not different compared to survival of control fish (Table 4; Two-Way ANOVA on Ranks, $F_{2,4} = 2.5$, $P = 0.09$). Ammonia production rates (M_{TAN}) were

Table 4.—Mean (± 1 standard deviation) percent survival (168 hour post transport), change in temperature (Δ°C), and ammonia (M_{TAN}) and carbon dioxide (M_{CO2}) fish production rates measured during testing.

Species	Density Category	Density (g/l)	Survival (%)	Δ °C	M_{TAN} (mg/g/h)	M_{CO2} (mg/g/h)
Chinook Salmon	Bates	24.5 ± 1.5	98.7 ± 4.4	0.3 ± 0.6	0.23 ± 0.06	0.27 ± 0.08
	Bates × 2	48.8 ± 1.6	99.3 ± 1.6	0.4 ± 0.6	0.12 ± 0.02	0.24 ± 0.10
	Control	NA	100.0 ± 0.0			
Striped Bass	Bates	34.7 ± 3.9	100.0 ± 0.0	0.5 ± 0.8	0.13 ± 0.02	0.22 ± 0.08
	Bates × 2	68.4 ± 5.7	100.0 ± 0.0	0.3 ± 1.0	0.07 ± 0.01	0.19 ± 0.06
	Control	NA	100.0 ± 0.0			
Threadfin Shad	Bates	8.5 ± 0.2	99.0 ± 2.7	-0.9 ± 1.0	0.54 ± 0.05	0.54 ± 0.10
	Bates × 2	16.5 ± 0.4	99.2 ± 1.4	-0.2 ± 0.4	0.28 ± 0.01	0.70 ± 0.06
	Control	NA	100.0 ± 0.0			

significantly different across all species tested ($F_{1,2}$ = 184.4, P < 0.001), and decreased with increasing density level (Table 4; $F_{1,2}$ = 130.6, P < 0.001). Threadfin shad M_{CO2} levels were significantly greater at an elevated density (Bates×2) during transport (Tukey's Test, P < 0.001), but transport density did not affect M_{CO2} levels for Chinook salmon (Tukey's Test, P = 0.25) and striped bass (Table 4; Tukey's Test, P = 0.32). Threadfin shad M_{CO2} levels, at both transport density levels, were significantly greater than those for Chinook salmon and striped bass (Table 4; $F_{1,2}$ = 148.6, P < 0.001).

Chinook salmon, striped bass, and threadfin shad mean Hct levels ranged from 37.4 to 42.1, 28.6 to 37.5, and 38.4 to 49.0% packed cell volume, respectively, and species ($F_{3,6}$ = 92.81, P < 0.001) and treatment ($F_{3,6}$ = 14.74, P < 0.001) specific differences are reported in Figure 1. Chinook salmon, striped bass, and threadfin shad basal Hct levels were

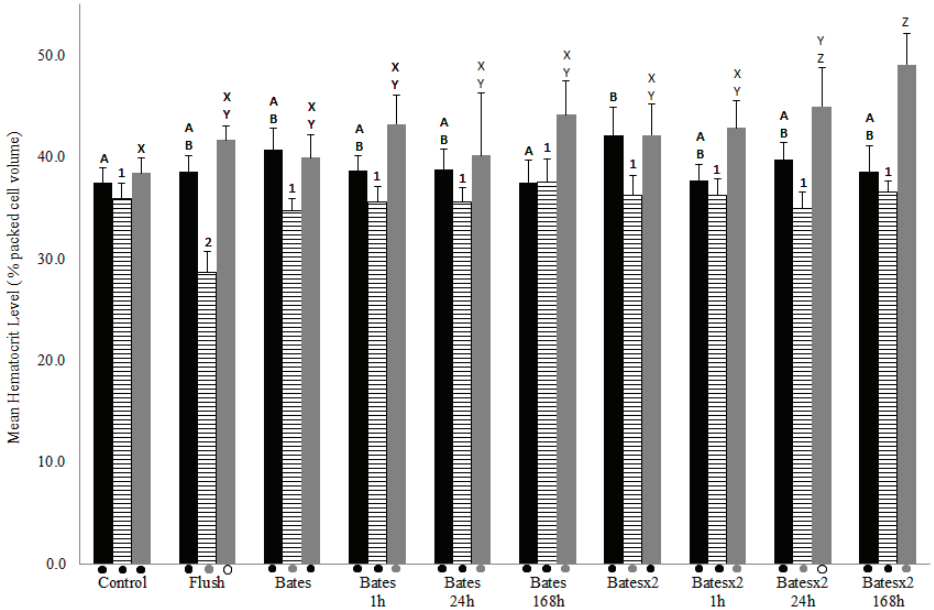


Figure 1.—Mean (±2 *SE*) blood hematocrit levels (% packed cell volume) of Chinook salmon (black), striped bass (horizontal stripes), and threadfin shad (grey) before (control, flush) and after transport at two different density levels (Bates, Bates×2). Different letters (Chinook salmon = A,B; threadfin shad = X,Y,Z) and numbers (striped bass = 1,2) above error bars indicates significant differences across treatment, but within each species only (Two-way ANOVA on Ranks, Tukey's Test). Different colored circles (black, grey, white) below graphs and above treatment titles indicate significant differences across species, but within each treatment condition (Two-way ANOVA on Ranks, Tukey's Test).

indicative of healthy levels as reported for salmon (Mazur and Iwama 1993, Martinelli et al. 1998), as well as other freshwater species (Davis and Parker 1990, Wedemeyer et al. 1990), suggesting fish had not been exposed to chronic stress, and were healthy during testing. Transport density had no effect on Chinook salmon or striped bass blood cortisol level at any sampling period following transport (Figure 2; $F_{9,9}$ = 17.48, P < 0.001). Chinook salmon and striped bass cortisol levels (ng/ml) were significantly higher immediately following

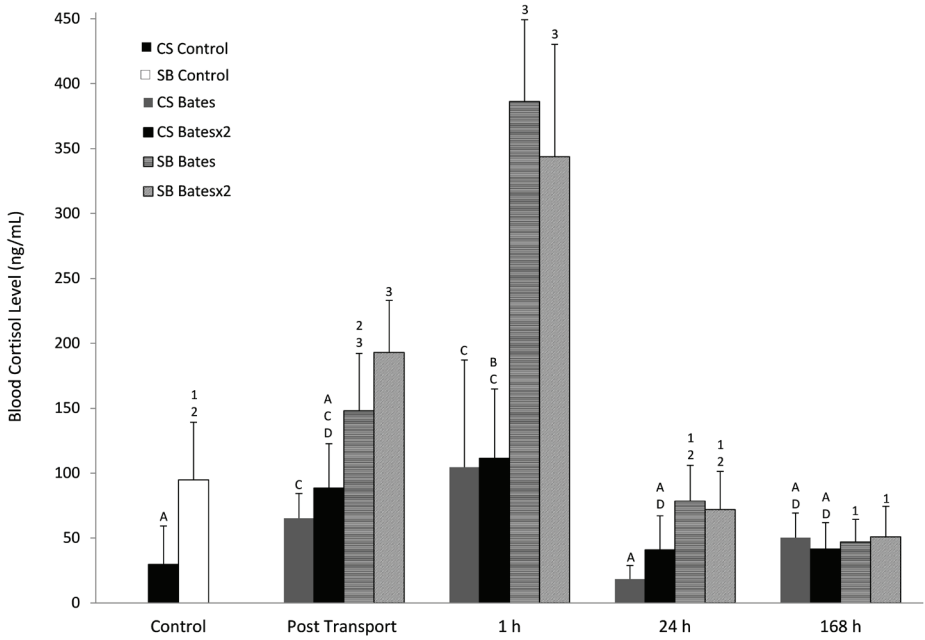


Figure 2.—Mean (±1 *SE*) blood cortisol levels (ng/ml) for Chinook salmon and striped bass before (control) and after transport at two different density levels (Bates, Bates×2). Mean (±1 *SD*) Bates (Chinook salmon = grey, striped bass = horizontal bars) and Bates×2 (Chinook salmon = black, striped bass = perpindicular bars) densities during transport for Chinook salmon and striped bass were 24.5 ± 1.5 and 48.8 ± 1.6, and 34.7 ± 3.9 and 68.4 ± 5.7, respectively. Mean (±1 *SD*) water temperatures during transport for Chinook salmon and striped bass were 20.5 ± 1.2 and 23.0 ± 1.0°C, respectively. Different letters (Chinook salmon) and numbers (striped bass) above error bars indicates significant differences across treatment, but within each species (Two-way ANOVA on Ranks, Tukey's Test).

transport, peaked one hour post-transport, but returned to basal (control) levels within 24 hours post-transport (Figure 2; Tukey's Test, $P < 0.05$). Striped bass cortisol levels were significantly higher than Chinook salmon levels at all sample periods except 168 hours post-transport (Figure 2; Tukey's Test, $P = 0.80$).

In general, transport density had no effect on species-specific blood-glucose level as a function of sampling period before or after transport. The exception: glucose levels of threadfin shad 1 hour post transport, exposed to Bates×2 density levels were significantly greater than threadfin shad at the same time period following transport and exposed to Bates density (Figure 3; $F_{9,18} = 2.87$, $P < 0.001$). At both transport density categories, peak measured blood-glucose levels occurred at 0 (immediately following transport) to 1 hour following transport for all species, and returned to, or below, basal levels within 24 hours post transport (Figure 3). Basal glucose levels were significantly different across all species (Tukey's Test, $P < 0.02$), and striped bass glucose level, transported at Bates density level at 1 hour post transport, were greater than other species tested (Tukey's Test, $P < 0.03$). When transported at Bates×2 density levels and measured at 1 hour post transport, striped bass and threadfin shad glucose levels, though not different from each other, were greater than Chinook salmon levels (Tukey's Test, $P < 0.02$). Striped bass glucose levels at both transport densities and 168 hours post transport were greater than Chinook salmon and threadfin shad levels (Tukey's Test, $P < 0.03$).

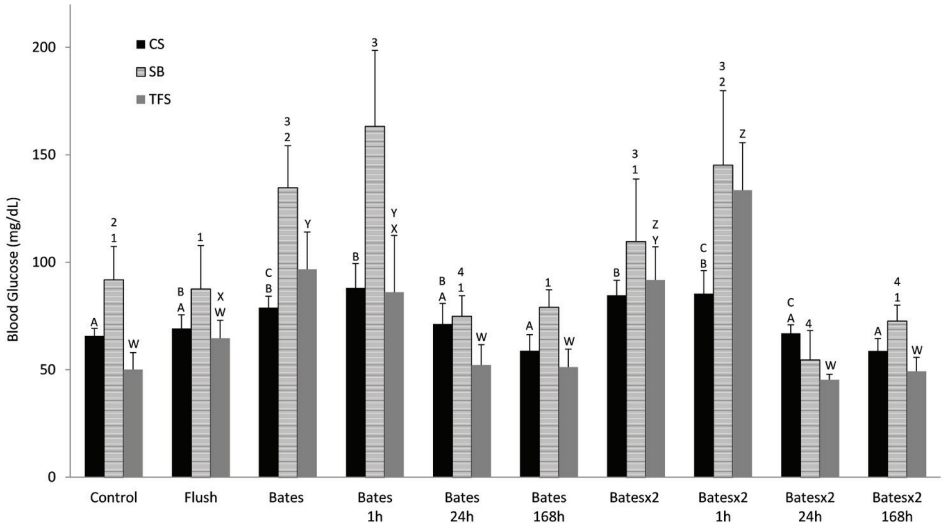


Figure 3.—Mean blood glucose levels (± 2 *SE*) for Chinook salmon (CS, black), striped bass (SB, horizontal bars), and threadfin shad (TFS, grey) before (control, flush) and after transport at two different density levels (Bates, Bates×2). Mean (±1 *SD*) Bates and Bates×2 densities during transport for Chinook salmon, striped bass, and threadfin shad were 24.5 ± 1.5 and 48.8 ± 1.6, 34.7 ± 3.9 and 68.4 ± 5.7, and 8.5 ± 0.2 and 16.5 ± 0.4, respectively. Different letters (CS = A,B,C; TFS = W,X,Y,Z) and numbers (SB = 1,2,3,4) above error bars indicates significant differences across treatment, but within each species only (Two-way ANOVA on Ranks, Tukey's Test).

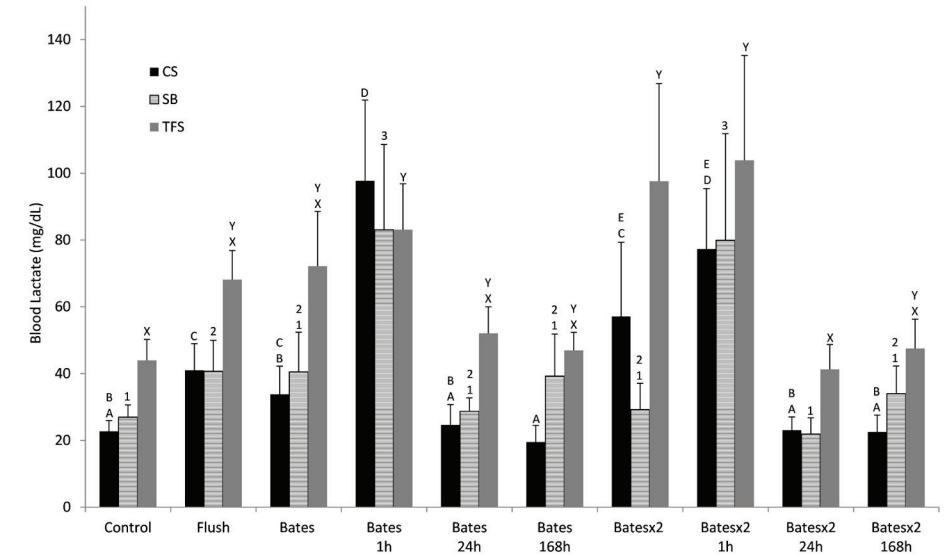


Figure 4.—Mean blood lactate levels (±2 *SE*) for Chinook salmon (CS, black), striped bass (SB, horizontal bars), and threadfin shad (TFS, grey) before (control, flush) and after transport at two different density levels (Bates, Bates×2). Mean (±1 *SD*) Bates and Bates×2 densities during transport for Chinook salmon, striped bass, and threadfin shad were 24.5 ± 1.5 and 48.8 ± 1.6, 34.7 ± 3.9 and 68.4 ± 5.7, and 8.5 ± 0.2 and 16.5 ± 0.4, respectively. Different letters (Chinook salmon = A,B,C,D,E; threadfin shad = X,Y) and numbers (striped bass = 1,2,3) above error bars indicates significant differences across treatment, but within each species only (Two-way ANOVA on Ranks, Tukey's Test).

Similar to what was observed for species-specific cortisol and glucose levels, transport density had no effect on blood lactate levels for any of the species tested, across any sample period, following transport (Figure 4; $F_{9,18} = 29.50$, $P < 0.001$). For all species, and across both density categories evaluated, measured lactate levels were greater than basal levels and peaked at 1 hour post transport, then returned to basal levels by 24 hours post transport (Tukey's Test, $P < 0.05$). Basal lactate levels of threadfin shad were greater than those measured for Chinook salmon and striped bass (Tukey's Test, $P < 0.001$), which were not significantly different from each other (Tukey's Test, $P = 0.98$). At measured peak levels (1 hour post transport), and across both density categories tested, there was no difference in species-specific lactate level. However, at 168 hours following transport and at both density categories, threadfin shad and striped bass lactate levels were not different (Tukey's Test, $P > 0.08$), but were both greater than Chinook salmon levels (Tukey's Test, $P < 0.03$).

Discussion

Basal blood hematrocit levels for all species tested were within reported normal levels for fish, suggesting test fish were not likely anemic or diseased prior to testing (Barton et al. 2002). Similarly, basal cortisol levels for Chinook salmon were similar to those generally reported for salmonids (Barton and Iwama 1991). However, striped bass basal cortisol levels were >3× greater than what has been reported for the species (Davis et al. 1982, Davis and Parker 1986). Though basal glucose levels for striped bass were greater than those for Chinook salmon and threadfin shad, all species basal glucose levels were near the typical range reported for fish (Barton et al. 2002). While it is difficult to ascertain the cause of elevated basal cortisol levels in striped bass, normal basal glucose levels and a nearly identical pre-treatment holding environment across tested species would suggest the elevated levels are perhaps a species specific sensitivity to a stressor not perceived by the other species tested or simply elevated basal cortisol levels common in striped bass.

Immediate post-transport and 168 hours post-transport survival of Chinook salmon, striped bass, and threadfin shad exposed to recommended Bates Table, and twice recommended density levels were high (>98%). In general, reported immediate or en route survival of transported fish is high (>90%; Johnson and Metcalf 1982), even at elevated densities (Staurnes et al. 1994 [560 g/l], Hasan and Bart 2007 [400 g/l]) or for longer durations (Carmichael 1984 [30 hours]). However, long-term survival (≥96 hours) of transported fish varies greatly, and is reportedly dependent on a multitude of parameters including, but not limited to, species, capture or loading technique, transport water quality, and duration. Mazik et al. (1991) reported high (100%) survival of striped bass transported 5 hours in freshwater at a density of 180 g/l, then maintained in 1% NaCl solution for four weeks. Carmichael (1984) also reported elevated survival through 168 hours (>85%) when transporting largemouth bass (*Micropterus salmoides*) at a density of 180 g/l with various combinations of pre-, during, and post-treatments, including NaCl concentration near plasma level. However, in the absence of treatments, and transporting bass at the same density in well water, survival was <20%. When transported in well water, with no additives (e.g., no NaCl), at a density of 200, 300, and 400 g/l for 3 hours, rohu (*Labeo rohita)* experienced 28, 35, and 41% mortality through two weeks post transport, respectively (Hasan and Bart 2007). These results support those reported by others, as well as those of the current study, that suggest use of NaCl, at levels between 0.5 and 8%, placate fish stress response

and contribute to increased survivability (Johnson and Metcalf 1982, Mazik et al. 1991, Swanson et al. 1996) of fish during, or immediately following, handling and transport operations. In response to stress, permeability of fish gills generally increase, resulting in osmoregulatory imbalances (Mazeaud et al. 1977). The addition of NaCl to water during stressful situations (i.e., handling, loading, transport) near the internal plasma concentration of fish minimizes the energetic requirements of osmoregulation (Redding and Schreck 1983). However, the use of NaCl as an additive to improve survival of fish during transport is apparently species specific (Gomes et al. 2003, 2006). For example, juvenile matrinxa (*Brycon cephalus)* can tolerate (100% survival) transport for 4 hours at densities between 83 and 206 g/l without the apparent addition of NaCl or other treatments (Urbinati et al. 2004, Abreu et al. 2008). Therefore, care should be taken to consider species-specific needs, and other practices, particularly pre-transport capture and handling, that may limit survival of fish during transport.

In the current study, fish were transferred from holding tank to transport container by water-to-water. As a result, fish were not exposed to physical damage and additional stress, often associated with netting and physical-handling, which could have compromised survival (Barthel et al. 2003). For example, freshwater drum (*Aplodinorus grunniens*) transported at densities of 60 and 120 g/l for 6 hours experienced 70 and 96% mortality, even with the addition of 5% NaCl, through two weeks following transport (Johnson and Metcalf 1982). Ultimately, Johnson and Metcalf (1982) suggested capture and handling (shore seining and hand counting) contributed most significantly to elevated post-transport mortality. Though post-transport survival of fish in the current study was high, it is important to note transport and post-transport water in the current study is different from SSJD water. Sacramento-San Joaquin Delta water reportedly contains elevated levels of pesticides and heavy metals, as well as pathogens and parasites (Lee and Lee 2004). Stress incurred during fish loading and transport can lead to immunodeficiency, and contribute to increased bacterial (Pickering and Pottinger 1989; Iwama et al. 1997) and parasitic (Woo et al. 1987) infection.

Increasing transport densities, from recommended Bates Table density levels to twice the recommended levels, had no effect on Chinook salmon, striped bass, or threadfin shad survival (immediate or long-term) or measured blood plasma constituents in general (hematocrit, cortisol, lactate, or glucose). There is variability in reported species-specific effects of density during fish transport on long-term survivability and physiological stress response. Abreau et al. (2008) reported survival and cortisol response during 4-hour transport of juvenile matrinxa (*Brycon cephalus*) was not density dependent, and survival of walleye (*Sander vitreus*) fry during 4-hour transport is also reportedly not density dependent (Peterson and Carline 1996). Similarly, only at the highest transport density (350 g/l) was there an increase in mortality, cortisol, and glucose concentrations following 4-hour transport of juvenile jundia (*Rhamdia quelen*), but at lower densities (75, 150, and 250 g/l) there was no effect (Carneiro et al. 2009). However, both Hasan and Bart (2007) and Gomes et al. (2006) indicated long-term survival and cortisol response of rohu and matrinxa, respectively, were density dependent. When studying the same species as Gomes et al. (2006), Urbinati et al. (2004) reported an inverse relationship between transport density and matrinxa cortisol response, but no density-dependent effect on long-term survival. Apparently, the physiological response of fish to density during transport isn't ubiquitous, and other environmental factors, such as life-stage or development (Pottinger et al. 1995), rearing environment (Woodward and Strange 1987, Jentoft et al. 2005), evolutionary history (Barton

et al. 2000), schooling nature (Parker, 1973, Iguchi et al. 2002), and water quality (Pickering and Pottinger 1987), amongst other factors (Barton 1988), may ultimately supersede or confound the response to elevated density during transport. This further exacerbates the need to understand species, and life-stage, specific needs during transport.

In general, the duration of stress response, as represented by peaking cortisol levels shortly (0.5 to 1 hour) post-transport and returning to basal levels within 24 hours, is typical across most freshwater teleosts exposed to handling, loading, or short-duration transport operations, then returned to a placid environment (Barton and Iwama 1991, Barton 2000, Barton 2002). Glucose and lactate levels for tested species followed a similar temporal trend, which is also a common glucose (Robertson et al. 1987, Barton 2000, Abreu et al. 2008, Carneiro et al. 2009) and lactate (Pickering et al. 1982, Pottinger 1998, Davis and Schreck 1997) response across fish exposed to similar stressors. Duration of response to fish-loading and transport stress can have important management-level implications. The inability of fish to fully recover from loading and transport-related stress within a very short duration ($\leq$1 hr) would likely preclude the necessity of a short-duration holding period for fish prior to release following large-scale transport operations.

The magnitude of cortisol increase, from basal to peak levels, for Chinook salmon (3.8×) and striped bass (4.1×) are similar to what is reported for other fish following transportation (Davis and Parker 1986, Maule et al. 1988). Elevated release of cortisol in response to loading and transport stress is a neuroendocrine response; stress functions to stimulate the hypothalamic-pituitary-interrenal axis, leading to the circulatory release of cortisol and other corticosteroid hormones (Randall and Perry 1992, Bonga 1997). Maximum post-stress glucose and lactate levels for all species tested were within the general range reported for other species (Barton et al. 2002). When subjected to a stressor, it is generally accepted that elevated glucose levels, or hyperglycemia, is a physiological response to meet energetic demands to respond to the stressor (Mazeaud and Mazeaud 1981), and blood lactate build-up is a result of increased muscular activity, generally associated with heightened activity or swimming (Driedzic and Hochachka 1978). In general, the severity of a stressor is represented by magnitude of a fish's stress response (Barton et al. 1980, Barton and Iwama 1991). The reported experimental results suggest loading and transporting Chinook salmon, striped bass, and threadfin shad at recommended or twice recommended Bates Table densities results in a stress-response similar to other fish-handling and transport operations.

Species-specific differences in the magnitude of physiological response to loading and transport stress are common (Davis and Parker 1986; Barton and Grosh, as reported in Barton and Iwama 1991). Across most sampling periods during testing, striped bass cortisol levels were greater than levels for Chinook salmon. Similarly, basal glucose level of striped bass, as well as the level 1 hour following transport at the recommended Bates Table density, was greater than levels for other species tested. Threadfin shad basal, immediately post-transport, and 24 hours post-transport lactate levels, as well as en route M_{TAN} and M_{CO2} levels, were greater than those for Chinook salmon and striped bass. As designed, there were distinct differences in test temperatures across species. Water temperature, particularly those outside a preferred range, appear to contribute to elevated cortisol and glucose levels in some fish (Strange 1980, Davis et al. 1984, Barton and Schreck 1987). So, it is possible environmental conditions may have influenced results. However, the two species that exhibited elevated cortisol, glucose, and lactate levels during testing (striped bass and threadfin shad), were likely within or close to their preferred temperature range (Coutant et

al. 1984). Therefore, we ultimately attribute species-specific differences in physiological stress response to genetic differences, as is supported by Davis and Parker (1986), Barton and Iwama (1991), and Barton et al. (2002).

Ammonia and carbon dioxide production rates of fish, though indicators of metabolic rate and response to stress (Alsop et al. 1999, Randall and Tsui 2002), were important measured variables to determine what density levels of fish could be transported over short duration without reaching harmful and acutely lethal levels of TAN, NH_3, and CO_2. Elevated NH_3 and CO_2 can be toxic to fish, and are of particular importance during fish transport operations because stress and increased activity during transport can contribute to increased ammonia and carbon dioxide production rates (Wedemeyer 1996, Alsop et al. 1999, Randal and Tsui 2002). Also, stressors and elevated CO_2, often encountered during transport, can increase fish sensitivity to ammonia (Randall and Tsui 2002). Excessive CO_2 results in a reduction of CO_2 gill excretion, leading to hypercapnia and respiratory acidosis. Elevated NH_3 can result in gill corrosion and nerve damage, impairing osmoregulation and central nervous system functionality (Wedemeyer 1996, Portz et al. 2006).

Ammonia production rates of fish following transport in the current study were greater than rates reported for fasted (Altinok and Grizzle 2004) and recently fed fish (Brett and Zala 1975, Jarboe 1995), as well as those for swimming fish (Sukumaran and Kutty 1977). Results of the current study are supported by Randall and Tsui (2002), who suggested M_{TAN} of fish increased in response to stress and activity. Ammonia production rates of all species tested tended to have an inverse relationship with density, similar to what was observed during the transportation of juvenile matrinxa (Urbinati et al. 2004, Abreu et al. 2008). Results from Fromm and Gillette (1968) and Fromm (1970) suggested ammonia excretion rates decline with exposure to increasing ammonia levels. It is likely higher densities of fish during transport result in rapid increases in ammonia, and elevated levels earlier during the transport process. Once ammonia levels reach a critical level, it is possible reduction in ammonia production in fish is a physiological response to minimize likelihood of exposure to lethal levels (Randall and Tsui 2002).

Recommended maximum CO_2 levels for short-term holding or transport and fish culture operations are reportedly 15–60 mg/l and <5–10 mg/l, respectively (Wedemeyer 1996, Timmons et al. 2002, Portz et al. 2006). Short duration exposure to levels >200 mg/l are often used as a fish anesthetic (Sommerfelt and Smith 1990, Ackerman et al. 2005) and, though dependent on the buffering capacity of transport water (i.e., alkalinity level), exposure to CO_2 levels >85 mg/l may affect survival of fish during transport operations (Amend et al. 1982, Grottum and Sigholt 1996). Recommended maximum NH_3 levels for fish culture operations are reportedly 0.01–0.02 mg/l (Westers 1981, Meade 1985, Wedemeyer 1996), whereas acutely toxic NH_3 levels for freshwater fish, Chinook salmon included, range from 0.32–3.10 mg/l (Ruffier et al. 1981, Thurston and Meyn 1984). Results of the current study indicate transport of fish at recommended, and twice recommended, Bates Table densities should permit transport at or below recommended CO_2 and NH_3 levels, and likely not expose fish to acutely lethal CO_2 or NH_3 levels.

Results of the current study support those reported in the scientific literature indicating, when sufficient means are taken to maintain appropriate water quality conditions (e.g., increase NaCl and dissolved oxygen levels) and transport densities, stress incurred as a result of loading and transport is adaptive and functions to reestablish homeostasis and promote survival. In a review of fish transportation operations conducted by federal, state,

tribal, and private entities, Carmichael and Tomasso (1988) indicated transport densities typically range from 24 to 431 g/l. Therefore, maximum Bates Table densities are on the lower end of the spectrum compared to most other fish transportation operations. Moderate transport densities, paired with elevated NaCl levels during transport, likely result in minimal transport induced mortality of fish from the TFCF. Though evaluating the fish loading process was not a project objective, study results compared to those reported in the published literature suggest water-to-water transfer of fish (i.e., draining water and fish from a bucket to a transport tank) results in high survival, and is therefore a technique that is not only efficient, but one that should be explored during other fish transference operations. Tracy Fish Collection Facility standard operating procedures for fish transportation (TFCF SOP#41) require fish be transported, at a minimum, every 12 hours, and every eight hours when delta smelt (*Hypomesus transpacificus*) are present at the facility. Also, there are multiple fish holding tanks that can be employed at the TFCF in an attempt to regulate the density of fish transported. As a result, transportation of exceedingly high densities of fish (i.e., >Bates Table levels) is infrequent. However, pulses of high densities of fish do occur at the TFCF, and that is why the Bates Tables were developed, and the current study was undertaken. When such conditions arise, or if there are upward shifting trends in populations of fish in the SSJD, results of the current study allow TFCF management to permit transport of fish at up to twice the recommended TFCF densities with the confidence that survival of fish will not be compromised.

All three species evaluated during testing are commonly produced during hatchery or private commercial operations. Chinook salmon and striped bass are produced in hatcheries throughout the coastal western and eastern United States, respectively, for artificial propagation, sport fishing, and research activities (Geiger and Parker 1985, Hilborn 1992). Threadfin shad are often produced and stocked in lentic systems as a supplemental prey item (DeVries et al. 1991). Therefore, results of this study expand beyond operations at SSJD state and federal fish collection facilities, and add to the existing knowledge of species-specific fish handling, loading, and transport requirements. Reported survival data can be used to develop or verify species specific fish transport standard operating procedures. However, the current report extends beyond simply evaluating effects of loading and transport on stress and survival, and details effects on metabolic rate (M_{O2}, M_{TAN}, M_{CO2}). Since reduced oxygen levels, and elevated ammonia and carbon dioxide levels can impair chronic health and survival of fish, data from the current study can be used to ascertain adequate densities levels required to maintain adequate water quality conditions during fish holding necessary to support long-term health and survival of fish.

Acknowledgments

Funding for this project was provided by the U.S. Bureau of Reclamation, Tracy Fish Collection Facilities Research Program. We thank D. Portz, J. Hutcherson, and all anonymous reviewers for suggestions that have helped to improve this document. The study design was improved with input from R. Silva, B. Bridges, and members of the Tracy Technical Advisory Team. J. Hutcherson, and D. Mench provided technical support while caring for fish and conducting laboratory studies. The mention or reference of specific products does not constitute endorsement by the Bureau of Reclamation or Department of the Interior.

Literature Cited

Aasen, G. A. 2011. 2010 Fish salvage at the Tracy Fish Collection Facility. California Department of Fish and Game Bay-Delta Region. Fish Facilities Research and Operations Monitoring Unit, Stockton, California, USA.

Aasen, G. A. 2012. 2011 Fish salvage at the Tracy Fish Collection Facility. California Department of Fish and Game Bay-Delta Region. Fish Facilities Research and Operations Monitoring Unit, Stockton, California, USA.

Aasen, G. A. 2013. 2012 Fish salvage at the Tracy Fish Collection Facility. California Department of Fish and Game Bay-Delta Region. Fish Facilities Research and Operations Monitoring Unit, Stockton, California, USA.

Abreu, J. S. D., A. I. Sanabria-Ochoa, F. D. Goncalves, and E. C. Urbinati. 2008. Stress responses of juvenile matrinxa (*Brycon amazonicus*) after transport in a closed system under different loading densities. Ciencia Rural 38:1413-1417.

Ackerman, P. A., J. D. Morgan, and G. K. Iwama. 2005. Anesthetics. Appendix to CCAC guidelines on: the care and use of fish in research, teaching and testing. Canadian Council on Animal Care, Ottawa, Canada.

Altinok, I., and J. M. Grizzle. 2004. Excretion of ammonia and urea by phylogenetically diverse fish species in low salinities. Aquaculture 238:499-507.

Alsop D. H., J. D. Kieffer, and C. M. Wood. 1999. The effects of temperature and swimming speed on instantaneous fuel use and nitrogenous waste excretion of the Nile tilapia. Physiological and Biochemical Zoology 72:474-483.

Amend D. F., T. R. Croy, B. A. Goven, K. A. Johnson, and D. H. Mccarthy. 1982. Transportation of fish in closed system: methods to control ammonia, carbon dioxide, pH and bacterial growth. Transactions of the American Fisheries Society 111:603-611.

Barthel, B. L., S. J. Cooke, C. D. Suski, and D. P. Philipp. 2003. Effects of landing net mesh type on injury and mortality in a freshwater recreational fishery. Fisheries Research 63:275-282.

Barton, B. A. 1988. Endocrine and metabolic responses of fish to stress. International Association of Aquatic Animal Medicine Proceedings 19:41-55.

Barton, B. A. 2000. Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. North American Journal of Aquaculture 62:12-18.

Barton, B. A. 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology 42:517-525.

Barton, B. A., and C. B. Schreck. 1987. Metabolic cost of acute physical stress in juvenile steelhead. Transactions of the American Fisheries Society 116:257-263.

Barton, B. A., and G. K. Iwama. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Review of Fish Diseases 1:3-26.

Barton, B. A., R. E. Peter, and C. R. Paulencu. 1980. Plasma cortisol levels of fingerling rainbow trout (*Salmo gairdneri*) at rest, and subjected to handling, confinement, transport, and stocking. Canadian Journal of Fisheries and Aquatic Sciences 37:805-811.

Barton, B. A., C. B. Schreck, and L. A. Sigismondi. 1986. Multiple acute disturbances evoke cumulative physiological stress responses in juvenile Chinook salmon. Transactions of the American Fisheries Society 115:245-251.

Barton, B. A., J. D. Morgan, and M. M. Vijayan. 2002. Physiological and condition-related indicators of environmental stress in fish. Pages 111-148 *in* S. M. Adams, editor. Biological indicators of aquatic ecosystem stress. American Fisheries Society, Bethesda, Maryland, USA.

Barton, B. A., H. Bollig, B. L. Hauskins, and C. R. Jansen. 2000. Juvenile pallid (*Scaphirhynchus albus*) and hybrid pallid 3 shovelnose (*S. albus* × *platornychus)* sturgeons exhibit low physiological responses to acute handling and severe confinement. Comparative Biochemistry and Physiology 126A:125-134.

Barton, B. A., A. H. Haukenes, B. G. Parsons, and J. R. Reed. 2003. Plasma cortisol and chloride stress responses in juvenile walleyes during capture, transport, and stocking procedures. North American Journal of Aquaculture 65:210-219.

Bates, D. W., and R.Vinsonhaler. 1957. Use of louvers for guiding fish. Transactions of the American Fisheries Society 112:38-57.

Berka, R. 1986. The transport of live fish: a review. European Inland Fisheries Advisory Commission Technical Paper No. 48. Food and Agriculture Organization of the United Nations, Rome, Italy.

Bonga, S. W. 1997. The stress response in fish. Physiological reviews 77:591-625.

BOR (Bureau of Reclamation). 1955. Activity report for month of August 1955 — Tracy Fish Screen studies. Solano Project Office, Bureau of Reclamation, Sacramento, California, USA.

BOR. 2013. Tracy Fish Collection Facility [Internet]. Peer reviewed technical reports and technical bulletins [cited 2014 July 15]. Available from: http://www.usbr.gov/pmts/tech_services/tracy_research/tracyreports/index.html.

Brett, J. R. 1952. Temperature tolerances in young Pacific salmon, genus *Oncorhynchus*. Journal of the Fisheries Research Board of Canada 9:268-323.

Brett, J. R., and C. A. Zala. 1975. Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon (*Oncorhynchus nerka*) under controlled conditions. Journal of the Fisheries Board of Canada 32:2479-2486.

CDFG (California Department of Fish and Game). 2013. California Department of Fish and Game [Internet]. Fish salvage monitoring [cited 2014 July 15]. Available from: fpt://fpt.delta.dfg.ca.gov/salvage/

Carmichael, G. J. 1984. Long distance truck transport of intensively reared largemouth bass. Progressive Fish Culturist 46:111-115.

Carmichael, G. J., J. R. Tomasso, B. A. Sinnco, and K. B. Davis. 1984. Characterization and alleviation of stress associated with transporting largemouth bass. Transactions of the American Fisheries Society 113:778-785.

Carmichael, G. J., and J. R. Tomasso. 1988. Survey of fish transportation equipment and techniques. Progressive Fish Culturist 50:155-159.

Carneiro, P. C. F., P. H. D. S. Kaiseler, E. D. A. C. Swarofsky, and B. Baldisserotto. 2009. Transport of jundia (*Rhamdia quelen*) juveniles at different loading densities: water quality and blood parameters. Neotropical Ichthyology 7:283-288.

Colt, J. 1984. Computation of dissolved gas concentrations in water as functions of temperature, salinity, and pressure. American Fisheries Society Special Publication 14. American Fisheries Society, Bethesda, Maryland, USA.

Coutant, C. C., K. L. Zachman, D. K. Cox, and B. L. Pearman. 1984. Temperature selection by juvenile striped bass in laboratory and field. Transactions of the American Fisheries Society 113:666-671.

Craft, D., M. Del Hoyo, R. Housewright, and J. Fields. 2008. Semi-continuous water quality measurements at the Tracy Fish Collection Facility: 7-year summary. Tracy Series Report 37. Bureau of Reclamation, Tracy, California, USA.

Davis, K. B., and N.C. Parker. 1986. Plasma corticosteroid stress response of fourteen species of warmwater fish to transportation. Transactions of the American Fisheries Society 115:495-499.

Davis, K. B., and N. C. Parker. 1990. Physiological stress in striped bass: effect of acclimation temperature. Aquaculture 91:349-358.

Davis, L. E., and C. B. Schreck. 1997. The energetic response to handling stress in juvenile Coho salmon. Transactions of the American Fisheries Society126:248-258.

Davis, K. B., N. C. Parker, and M. A. Suttle. 1982. Plasma corticosteroids and chlorides in striped bass exposed to tricaine methanesulfonate, quinaldine, etomidate and salt. Progressive Fish Culturist 44:205-207.

Davis, K. B., M. A. Suttle, and N. C. Parker. 1984. Biotic and abiotic influences on corticosteroid hormone rhythms in channel catfish. Transactions of the American Fisheries Society 113:414-421.

Devries, D. R., R. A. Stein, J. G. Miner, and G. G. Mittelbach. 1991. Stocking threadfin shad: consequences for young-of-year fishes. Transactions of the American Fisheries Society 120:368-381.

Driedzic, W. R., and P. W. Hochachka. 1978. Metabolism in fish during exercise. Pages 503-543 *in* S. Hoar and D. J. Randall, editors. Fish physiology, volume 7. Academic Press, New York, USA.

Emata, C. 2000. Live transport of pond-reared milkfish (*Chanos chunos*) broodstock. Journal of the World Aquaculture Society 31:279-282.

Feyrer, F., M. L. Nobriga, and T. R. Sommer. 2007. Multidecadal trends for three declining fish species: habitat patterns and mechanisms in the San Francisco Estuary, California, USA. Canadian Journal of Fisheries and Aquatic Sciences 64:723-734.

Francis-Floyd, R., R. C. Watson, D. Petty, and D. Pouder. 2012. Ammonia in aquatic systems. Document TA16. Fisheries and Aquatic Sciences Department, University of Florida, Gainesville, USA.

Fromm, P. O. 1970. Section III. Effect of ammonia on trout and goldfish. Pages 9-22 *in* Toxic action of water soluble pollutants on freshwater fish. EPA Water Pollution Control Research Series 18050 DST 12/70.

Fromm, P. O., and J. R. Gillette. 1968. Effect of ambient ammonia on blood ammonia and nitrogen excretion of rainbow trout (*Salmo gairdneri*). Comparative Biochemistry and Physiology 26:887-896.

Geiger, J. G., and N. C. Parker. 1985. Survey of striped bass hatchery management in the southeastern United States. Progressive Fish Culturist 47:1-13.

Gomes, L. D. C., R. Roubach, B. A. S. Cavero, M. Pereira-Filho, and E. C. Urbinati. 2003. Transport of pirarucu (*Arapaima gigas)* juveniles in plastic bag. Acta Amazonica 33:637-642.

Gomes, L. C., C. A. R. M. Araujo-Lima, A. R. Chippari-Gomes, and R. Roubach. 2006. Transportation of juvenile tambaqui (*Colossoma macropomum*) in a closed system. Brazilian Journal of Biology 66:493-502.

Grottum, J. A., and T. Sigholt. 1996. Acute toxicity of carbon dioxide on European seabass (*Dicentrarchus labrax*): mortality and effects on plasma ions. Comparative Biochemistry and Physiology 115:323-327.

Grutter, A. S., and N. W. Pankhurst. 2000. The effects of capture, handling, confinement and ectoparasite load on plasma levels of cortisol, glucose and lactate in the coral reef fish (*Hemigymnus melapterus*). Journal of Fish Biology 57:391-401

Hayes, M. C., S. M. Focher, and C. R. Contor. 2000. High pressure injection of photonic paint to mark adult Chinook salmon. North American Journal of Aquaculture 62:319-322.

Hasan, M., and A. N. Bart. 2007. Effects of capture, loading density and transport stress on the mortality, physiological responses, bacterial density and growth of rohu (*Labeo rohita*) fingerlings. Fish Physiology and Biochemistry 33:241-248.

Hilborn, R. 1992. Hatcheries and the future of salmon in the Northwest. Fisheries 17:5-8.

Iguchi, K. I ., F. Ito, K. Ogawa, N. Matsubara, T. Yodo, and T. Yamasaki. 2002. Reduction of transport stress of ayu by obligated schooling. Fisheries Science 68:849-853.

Iwama, G. K., A. D. Pickering, J. P. Sumpter, and C. B. Schreck. 1997. Fish stress and health in aquaculture. Cambridge University Press, Cambridge, United Kingdom.

Jarboe, H. H. 1995. Diel dissolved oxygen consumption and total ammonia nitrogen production by fingerling channel catfish following feeding at different times. Progressive Fish Culturist 57:156-160.

Jentoft, S., A. H. Aastveit, P. A. Torjesen, and O. Andersen. 2005. Effects of stress on growth, cortisol and glucose levels in non-domesticated Eurasian perch (*Perca fluviatilis*) and domesticated rainbow trout (*Oncorhynchus mykiss*). Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology 141:353-358.

Johnson, D. L., and M. T. Metcalf. 1982. Causes and controls of freshwater drum mortality during transportation. Transactions of the American Fisheries Society 111:58-62.

Lee, G. F., and A. Jones-Lee. 2004. Overview of Sacramento-San Joaquin River Delta water quality issues. G. Fred Lee and Associates, El Macero, California, USA.

Martinelli, T. L., H. C. Hansel, and R. S. Shively. 1998. Growth and physiological responses to surgical and gastric radio transmitter implantation techniques in subyearling Chinook salmon (*Oncorhynchus tshawytscha)*. Hydrobiologia 371/372:79-87.

Maule, A. G., C. B. Schreck, C. S. Bradford, and B. A. Barton. 1988. Physiological effects of collecting and transporting emigrating juvenile Chinook salmon past dams on the Columbia River. Transactions of the American Fisheries Society 117:245-261.

Mazur, C. F., and G. K. Iwama. 1993. Effect of handling and stocking density on hematocrit, plasma cortisol, and survival in wild and hatchery-reared Chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 112:291-299.

Mazeaud, M. M., and F. Mazeaud. 1981. Adrenergic responses to stress in fish. Pages 49-75 *in* A. D. Pickering, editor. Stress and fish. Academic Press, New York, USA.

Mazeaud, M. M., F. Mazeaud, and E. M. Donaldson. 1977. Primary and secondary effects of stress in fish. Transactions of the American Fisheries Society 106:201-212.

Mazik, P. M., B. A. Simco, and N. C. Parker. 1991. Influence of water hardness and salts on survival and physiological characteristics of striped bass during and after transport. Transactions of the American Fisheries Society 120:121-126.

Meade, J. W. 1985. Allowable ammonia for fish culture. Progressive Fish Culturist 47:135-145.

Mesa, M. G. 1994. Effects of multiple acute stressors on the predator avoidance ability and physiology of juvenile Chinook salmon. Transactions of the American Fisheries Society 123:786-793.

Mommsen, T. P., M. M. Vijayan, and T. W. Moon. 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries 9:211-268.

Moyle, P. B. 2002. Inland fishes of California. University of California Press, Berkeley, USA.

NMFS (National Marine Fisheries Service). 1997. NMFS proposed recovery plan for the Sacramento River winter run Chinook. National Marine Fisheries Service, Southwest Regional Office, Long Beach, California, USA.

Olson, P. A. and R. F. Foster. 1957. Temperature tolerance of eggs and young of Columbia River Chinook salmon. Transactions of the American Fisheries Society 85:203-207.

Parker Jr., F. R. 1973. Reduced metabolic rates in fishes as a result of induced schooling. Transactions of the American Fisheries Society 102:125-131.

Peterson, D. L., and R. E. Carline. 1996. Communications: effects of tetracycline marking, transport density, and transport time on short-term survival of walleye fry. Progressive Fish Culturist 58:29-31.

Pickering, A. D. 1981. Stress and fish. Academic Press, New York, USA.

Pickering, A. D., and T. G. Pottinger. 1987. Poor water quality suppresses the cortisol response of salmonid fish to handling and confinement. Journal of Fish Biology 30:363-374.

Pickering, A. D., and T. G. Pottinger. 1989. Stress responses and disease resistance in salmonid fish: effects of chronic elevation of plasma cortisol. Fish Physiology and Biochemistry 7:253-258.

Pickering, A. D., T. G. Pottinger, and P. Christie. 1982. Recovery of the brown trout, *Salmo trutta* L., from acute handling stress: a time-course study. Journal of Fish Biology 20:229-244.

Piper, R. G., I. B. McElwain, L. E. Orme, J. P. McCraren, L. G. Fowler, and J. R. Leonard. 1982. Fish hatchery management. U.S. Department of Interior, Fish and Wildlife Service, Washington, D.C., USA.

Portz, D. E., C. M. Woodley, and J. J. Cech, Jr. 2006. Stress-associated impacts of short-term holding on fishes. Reviews in Fish Biology and Fisheries 16:125-170.

Pottinger, T. G. 1998. Changes in blood cortisol, glucose and lactate in carp retained in anglers' keepnets. Journal of Fish Biology 53:728-742.

Pottinger, T. G., P. H. M. Balm, and A. D. Pickering. 1995. Sexual maturity modifies the responsiveness of the pituitary-interrenal axis to stress in male rainbow trout. General and Comparative Endocrinology 98:311-320.

Randall, D. J., and S. F. Perry. 1992. Catecholamines. Pages 255-300 *in* W. S. Hoar and D. J. Randall, editors. Fish physiology. Academic Press, New York, USA.

Randall, D. J., and T. K. N. Tsui. 2002. Ammonia toxicity in fish. Marine Pollution Bulletin 45:17-23.

Redding, J. M., and C. B.Schreck. 1983. Influence of ambient salinity on osmoregulation and cortisol concentration in yearling Coho salmon during stress. Transactions of the American Fisheries Society 112:800-807.

Ross, L. G., and B. Ross. 1999. Anesthetic and sedative techniques for aquatic animals. Blackwell Science, Oxford, United Kingdom.

Russo, R., and R. Thurston. 1991. Toxicity of ammonia, nitrite, and nitrate to fishes. Pages 58-89 *in* D. E. Brune and J. R. Tomasso, editors. Aquaculture and Water Quality. World Aquaculture Society, Baton Rouge, Louisiana, USA.

Robertson, L., P. Thomas, and C. R. Arnold. 1987. Plasma cortisol and secondary stress responses of cultured red drum (*Sciaenops ocellatus*) to several transportation procedures. Aquaculture 68:115-130.

Ruffier, P. J., W. C. Boyle, and J. K. Kleinschmidt. 1981. Short-term acute bioassays to evaluate ammonia toxicity and effluents standards. Journal of the Water Pollution Control Federation 53:367-377.

Schreck, C. B. 1981. Stress and compensation in teleostean fishes: response to social and physical factors. Pages 295-321 *in* A. D. Pickering, editor. Stress and fish. Academic Press, New York, USA.

Sharpe, C. S., D. A. Thompson, H. L. Blankenship, and C. B. Schreck. 1998. Effects of routine handling and tagging procedures on physiological stress responses in juvenile Chinook salmon. Progressive Fish Culturist 60:81-87.

Sommer, T., C. Armor, R. Baxter, R. Breuer, L. Brown, M. Chotkowski, S. Culberson, F. Feyrer, M. Gingras, B. Herbold, W. Kimmerer, A. Mueller-Solger, M. Nobriga, and K. Souza. 2007. The collapse of pelagic fishes in the upper San Francisco estuary. Fisheries 32:270-277.

Staurnes, M., T. Sigholt, H. P. Pedersen, and T. Rustad. 1994. Physiological effects of simulated high-density transport of Atlantic cod (*Gadus morhua*). Aquaculture 119:381-391.

Strange, R. J. 1980. Acclimation temperature influences cortisol and glucose concentrations in stressed channel catfish. Transactions of the American Fisheries Society 109:298-303.

Sukumaran, N., and M. N. Kutty. 1977. Oxygen consumption and ammonia excretion in the catfish *Mystus armnatus*, with special reference to swimming speed and ambient oxygen. Proceedings of the Indian Academy of Science 86B:195-206.

Summerfelt, R. C., and L. S. Smith. 1990. Anesthesia, surgery, and related techniques. Pages 213-272 *in* C. B. Schreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland, USA.

Sutphin, Z. A., and B. J. Wu. 2008. Changes in water quality during fish-hauling operations at the Tracy Fish Collection Facility. Tracy Fish Collection Facility Studies, Volume 2008-2. U.S. Department of the Interior, Bureau of Reclamation, Technical Service Center, Denver, Colorado, USA.

Swanson, C., R. C. Mager, S. I. Doroshov, and J. J. Cech Jr. 1996. Use of salts, anesthetics, and polymers to minimize handling and transport mortality in delta smelt. Transactions of the American Fisheries Society 125:326-329.

Thurston, R.V., and E. L. Meyn. 1984. Acute toxicity of ammonia to five fish species from the northwest United States. Montana Fisheries Bioassay Laboratory Technical Report Volume 84(4), Montana State University, Bozeman, USA.

Timmons, M. B., J. M. Ebeling, F. W. Wheaton, S. T. Summerfelt, and B. J. Vinci. 2002. Recirculating aquaculture systems. Second edition. Cayuga Aqua Ventures, Ithaca, New York, USA.

Urbinati, E. C., J. S. De Abreau, A. C. Da Silva Camargo, and M. A. Landinez Parra. 2004. Loading and transport stress of juvenile matrinxa (*Brycon cephalus*, Characidae) at various densities. Aquaculture 229:389-400.

Wedemeyer, G. A. 1996. Physiology of fish in intensive culture systems. Chapman Hall, New York, USA.

Wedemeyer, G. A., F. P. Meyer, and L. Smith. 1976. Diseases of fishes: environmental stress and fish diseases. TFH Publications, Neptune, New Jersey, USA.

Wedemeyer, G. A., B. A. Barton, and D. J. Mcleay. 1990. Stress and acclimation. Pages 451-489 *in* C. B. Schreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland, USA.

Westers, H. 1981. Fish culture manual for the state of Michigan. Michigan Department of Natural Resources, Lansing, USA.

Woo, P. T. K., J. F. Leatherland, and M. S. Lee. 1987. *Cryptobia salmositica*: cortisol increases the susceptibility of *Salmo gairdneri* Richardson to experimental cryptobiosis. Journal of Fish Diseases 10:75-83.

Woodward, C. C., and R. J. Strange. 1987. Physiological stress responses in wild and hatchery-reared rainbow trout. Transactions of the American Fisheries Society 116:574-579.

Wydowski, R. S., G. A. Wedemeyer, and N. C. Nelson. 1976. Physiological response to hooking stress in hatchery and wild rainbow trout (*Salmo gairdneri*). Transactions of the American Fisheries Society 105:601-606.

Yoshiyama, R. M., F. W. Fisher, and P. B. Moyle. 1998. Historical abundance and decline of Chinook salmon in the Central Valley region of California. North American Journal of Fisheries Management 18:487-521.

Received 30 October 2014
Accepted 15 April 2015
Associate Editor was K. Shaffer

California Fish and Game 101(2):131-145; 2015

Mitochondrial DNA perspectives on the introduction and spread of wild pigs in California

Rick A. Sweitzer*, Blake E. McCann, Ronald E. Loggins, and Rebecca B. Simmons

The Great Basin Institute, 16750 Mt. Rose Highway, Reno, NV 89511, USA (RAS)

Theodore Roosevelt National Park, Medora, North Dakota 58645, USA (BEM)

Wayne State College, Wayne, NE, USA (REL)

Department of Biology, University of North Dakota, Grand Forks, ND 58202 (RBS)

**Correspondent: sweitzerrick@gmail.com*

Domestic pigs were first introduced to California by Spanish explorers and missionaries in the 1700s and were soon established as feral populations in coastal regions. Feral pigs are currently abundant in mainland California where their presence in 56 of the state's 58 counties is ecologically problematic. We used molecular techniques to inform on an incomplete record of human introductions associated with escape or purposed release of domestic and European-type wild swine in California, and to provide insight on the mechanisms that produced an accelerated expansion dynamic in the state after the 1970s. We developed mtDNA sequence data for 151 tissue or blood samples from wild pigs spanning their distribution in California, and a 550 base pair segment of the control region was used in phylogenetic analysis. Phylogenetic analyses included our data and 904 published sequences for wild and domestic swine from elsewhere in the U.S. and around the world. Gene flow indicative of natural spread in California was assessed from population-level mtDNA sequence relationships for five population groupings, and we assessed mtDNA haplotypes associated with different periods of invasion by partitioning samples originating from "Historic" occupied counties (before 1968), and "Recent" occupied counties. Nine mtDNA haplotypes were identified among wild pigs California, including three that were unique to California, three that were common elsewhere in the United States, two that were known from Hawaii or other Pacific Islands, and one that was known only from Kentucky, USA. Apparent gene flow between Recent and Historic ranges indicated that pigs dispersed at the regional level, and we identified evidence for expansion by anthropogenic and natural processes from

presence of several haplotypes only in Recent range. MtDNA sequence data provided new insight on wild pig expansion in California, including evidence that contemporary translocations promoted hybridization and subsequent spread by natural population growth. Considered together, information on the distribution of common and unique haplotypes and gene flow suggests that range expansion by wild pigs in California is progressing by natural and human-facilitated dispersal, and new introductions from outside of the state. We advocate against additional anthropogenic movement of wild pigs within the state or from other U. S. states because these animals are known to be detrimental to native plants and animals in California ecosystems.

Key words: genetics, mtDNA, haplotypes, sequence, feral pigs, translocation, expansion, invasion, native ecosystems

---

Domesticated swine (*Sus scrofa*) were first introduced to North America during the 1500s, and feral pigs were present in wildlands shortly thereafter. Feral pigs had spread into at least 13 southern tier U.S. states by the late 1800s (Mayer and Brisbin 1991), when interest in hunting in the 1900s led to the importation and anthropogenic dispersal of Eurasian wild boar from Europe, which hybridized with existing feral pigs in many parts of the country (Mayer and Brisbin 1991). The consequence was the expansion of European wild pig hybrids and feral-domestic swine from 17 to 44 U.S. states within the last 35 years (Gipson et al. 1998, McCann et al. 2014). Swine is a generic term for all types of pigs, but terms used by resource managers, hunters and the public to refer to feral pigs, European wild boar, and wild pig hybrids vary locally and regionally in the U.S. For clarity, we provide a narrative overview of relevant terminology for domestic, feral, and European wild swine (Appendix I).

The story of wild pigs in mainland California parallels their continental history. Some domestic pigs that were initially introduced by Spanish explorers and missionaries in coastal regions in the 1500s became feral when they were allowed to forage in the oak woodlands (Barrett and Pine 1980). Subsequent additional releases and escape of domestic pigs from livestock pens led to feral populations in other mainland locations by the early 1900s (Pine and Gerdes 1973). Then, in 1925, Eurasian wild boar hybrids (n =12) from Hooper Bald, North Carolina, were released in Monterey County (Pine and Gerdes 1973). These hybrids dispersed and bred with feral pigs, whose progeny were later translocated to other counties in California (Mayer and Brisbin 1991).

Domestic swine were also brought to the Channel Islands by the Spanish in the 1600s, initially on Santa Cruz Island in association with a Spanish penal colony (Sweitzer 1998). Spanish settlement may have also been the source for feral pigs on nearby Santa Rosa Island (Collins 1981). Reliable records document that a small number of feral pigs were translocated from Santa Rosa Island to Santa Catalina Island in the 1930s (Schuyler et al. 2002). At one time feral pigs occurred on five of California's Channel Islands, but all have since been eradicated (Long 1993, Lombardo and Faulkner 2000, Schuyler et al. 2002, Ramsey et al. 2009).

The anthropogenic dispersal of Eurasian wild pig hybrids likely explains recent range expansion in mainland California based on the hypotheses that hybrids may be more

invasive than feral-domestic pigs, and also because ranch owners with managed hunting preferentially selected pigs with wild boar characteristics for translocation to their properties (Barrett 1977). Wild-living pigs (hereafter wild pigs) were designated game mammals in California in 1957, after which many private landowners introduced wild pigs to their properties for the purpose of fee hunting (Fitzhugh and Loomis 1993). The popularity of wild pigs as a game species increased in the 1970s and 1980s (Barrett 1993), coincident with concern over increasing ecological and agricultural impacts as they became more widespread. In response, in 1992 the California legislature required hunters to purchase wild pig tags to hunt the species, and a portion of the funds that were generated was directed to research on wild pig biology and their ecological impacts (Waithman et al. 1999, Sweitzer et al. 2000, Sweitzer and Van Vuren 2002).

The combination of wild pigs being included in the Annual Hunter Game Take Survey and records from tag returns revealed that wild pigs expanded in range from nine counties in the 1960s to nearly the entire state by 2006 (Loggins 2007). It remains unclear to what extent natural dispersal, anthropogenic transfer, and the types of breeds of domestic or Eurasian wild pig hybrids that were introduced shaped the expansion and current distribution of wild pigs in California. In the absence of complete records, and considering the clandestine nature of many introductions, molecular techniques provide the best opportunity for examining these factors (Spencer and Hampton 2005).

Mitochondrial DNA (mtDNA) has been used successfully for a number of phylogeographic studies of pigs. Previous work has evaluated the adaptive radiation of suids across Eurasia and identified centers of domestication (Giuffra et al. 2000, Luetkemeier et al. 2010). Published mtDNA sequences have become a valuable resource for global analysis of pig ancestry and dispersal patterns (McCann et al 2014), and several prior investigators used mtDNA to identify ancient anthropogenic dispersal of Eurasian wild boar and domestic swine (Fang and Andersson 2006, Vigne et al. 2009, Scandura et al. 2011). There are known intrinsic limitations of mtDNA sequence data regarding stochastic variation and nuclear pseudogenes (Parr et al. 2006), but these factors do not seem to impact overarching phylogenetic relationships of introduced suids (McCann et al. 2014).

In an analysis of mtDNA from 81 wild pigs collected in 30 U.S. states, McCann (2012) clearly identified the translocation of Eurasian wild pigs from Hooper Bald, North Carolina to California. We therefore considered that a regional analysis of mtDNA sequences within California could provide important perspective on the role of Eurasian wild pigs in recent range expansions within California. Here, we (1) evaluate mtDNA variation in California wild pigs; (2) identify mtDNA haplotypes associated with historic and recent (post 1960s) pig expansion; and (3) evaluate likely mechanisms associated with recent invasion.

Materials and Methods

We obtained tissue samples from 151 wild pigs in 23 California counties from 1996 to 2010, spanning recent and historic distribution of the species in the state (Figure 1). We also obtained published mtDNA sequences of wild and domestic swine from 29 other U.S. states, and four other continents (Figure 1). Wild pig tissue samples from throughout California were obtained from USDA Wildlife Services, National Park Service, California Department of Fish and Game, and private organizations involved in sanctioned pig control or eradication programs. For each animal, field personnel collected blood or other somatic

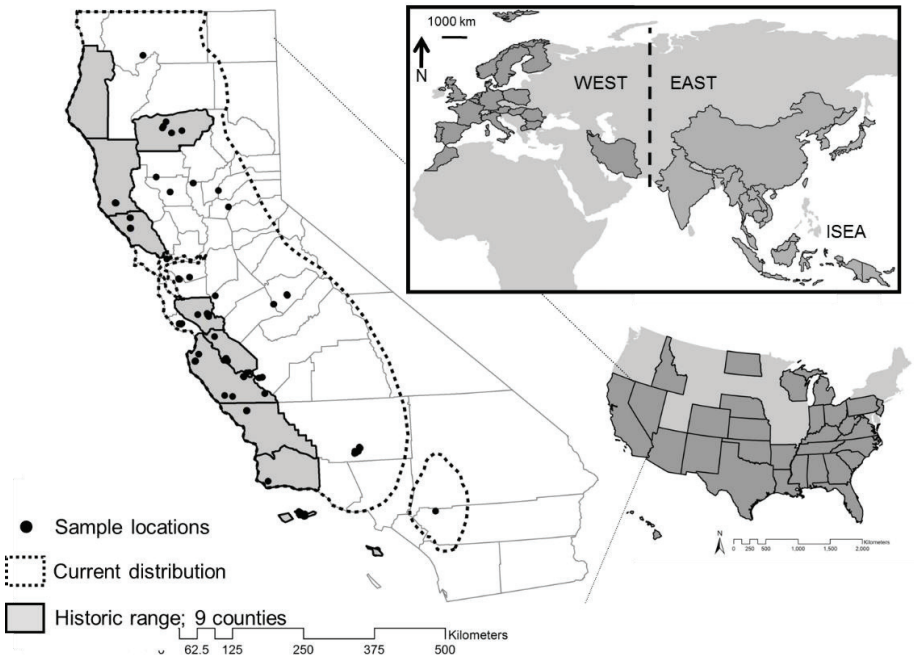


Figure 1.—Distribution of 158 wild pig samples from 23 counties in California, USA, collected from 1996 to 2010 and spanning historic and current invasive range of the species (SCWDS 2010). World geographic location of samples reported among published sequences (n = 904) is represented by country (shaded) in inset, including Western Eurasian (WEST) and Eastern Eurasian (EAST) phylogeographic split, and Island Southeast Asia (ISEA) phylogeography denoted for some wild *S. scrofa* and other *Sus* species (n = 5). Note: Australia, Hawaii, Iceland, and some Pacific Islands are not shown. United States geography for other published wild pig samples is included.

tissue, recorded pelage characteristics, date, and geographic coordinates for each sample. Blood was transferred to FTA cards (Whatman Inc., Florham Park, NJ, USA), air dried, and stored at room temperature, whereas somatic tissue was frozen and stored at -20°C.

We processed sample specimens using standard protocols as previously described by McCann et al. (2014). We used forward primer PigF (5'-ACTCTGGTCTTGTAAACC-3') and reverse primer PigR (5' –TAAGGGGAAAG ACTGGGC-3') to amplify and sequence an ≈550 base pair segment of the mtDNA control region (Okumura et al. 1996, Loggins 2007). We then submitted sequences to online holdings at NCBI Genbank (http://www.ncbi.nlm. nih.gov/). Accession numbers are as follows: AY96871-AY968729, AY968731-AY968742, AY968744-AY968763, AY968765-AY968806, AY973042, JF702003-JF702008, JF702013-JF702016, JF702018- JF702022, JF702038-JF702039, JF702041-JF702048, JF702050-JF702053, JF702055, JF702079-JF702080, JF702082-JF702104, and JQ792040.

We manually aligned and trimmed sequences for wild pigs from California to match a 403 base pair matrix of 81 mtDNA haplotypes identified for *Sus scrofa* sampled from 30 U.S. states and 904 published sequences for wild and domestic swine from around the world, including Eurasia and Island Southeast Asia (Figure 1; McCann et al. 2014). We included sequences for five other species of *Sus* (n =13) in the alignment to serve as outgroups. We collapsed sequences to haplotype using TCS 1.21 with gaps set as fifth

character state (Clement et al. 2000). We used JMODELTEST to determine the most appropriate evolutionary model for phylogenetic analysis (GTR+I+G; Guindon and Gascuel 2003, Posada 2008). We then constructed phylogenies in MRBAYES 3.1.2 (Ronquist and Huelsenbeck 2003) with 10,000,000 Markov Chain Monte Carlo (MCMC) sampling generations and retained consensus tree structure with >75% posterior probabilities. To determine if observed mtDNA variation increases with additional regional sampling in California, we compared the number of observed haplotypes for the 151 newly evaluated individual pigs to those previously identified for seven pigs from California (McCann et al. 2014). We then combined all California samples, resulting in 158 individual specimens for analysis of mtDNA relationships.

We identified mtDNA haplotypes associated with different stages of invasion by comparing their geographic distributions to written histories of introduction (summarized by Mayer and Brisbin 1991) and with other evidence for wild pig mtDNA relationships elsewhere in the U.S. (McCann et al. 2014). To facilitate interpretation of molecular data we grouped the 158 California wild pigs into four discrete clusters of geographic importance: (1) Santa Catalina Island (n = 6); (2) Santa Cruz Island (n = 11); (3) historic mainland (n = 77); and (4) recent mainland (n = 64). We divided mainland samples by "Historic" and "Recent" distributions, and designated Historic as those samples obtained from nine coastal counties occupied by pigs as of 1967 (Waithman et al. 1999). Recent included animals from all other mainland locations in California (Figure 1).

We assessed gene flow among wild pig clusters in California by analyzing population-level mtDNA sequence relationships in ARLEQUIN 3.5 (Excoffier et al. 2005). We compared five population groupings: the four California populations described above, and an arbitrarily assigned population of wild pigs (n = 74) sampled from 29 other U.S. states (29US). We performed a standard analysis of molecular variance (AMOVA) on pair-wise differences with 1,023 permutations to estimate significance. We conducted an exact test of population differentiation with 100,000 MCMC steps based on haplotype frequencies. We then calculated pair-wise F_{ST} and Nm (gene flow) among groups and evaluated results in context of haplotype distributions in California and other states.

Results

We identified 151 haplotypes among all mtDNA sequences, including nine among wild living pigs in California. Phylogenetic analyses revealed three major clades among the 151 mtDNA haplotypes that were associated with geography: Eastern Eurasia (EAST), Western Eurasia (WEST), and Island Southeast Asia (ISEA) (Figure 2). Geography agreed with phylogeny for some groupings, and most haplotypes observed in California were globally distributed and associated with unresolved assemblages in WEST and EAST (M1 and M2, respectively), representing a mixture of domestic swine and Eurasian wild pig hybrids (Figure 2). Three of the nine haplotypes identified for California wild pigs were exclusive to that state (h149, h150, h151), two (h84 and h13) were also found among wild pigs in Hawaii or Pacific Islands, one (h38) matched other continental U.S. wild pigs found only in Kentucky (Table 1), and the other three (h17, h19, h37) were common across the continental U.S. with wide distributions (Table 1). Haplotypes h37, h84, h149, and h151 were confined to single population groupings, and h150 was the only haplotype shared between island and mainland locations (Figure 3, Table 1). Four California haplotypes (h17, h19, h37, h38) were associated with the M1 "mixed" assemblage of wild boar and domestic breeds in WEST, one haplotype (h13) was associated with the M2 mixed assemblage in EAST, and

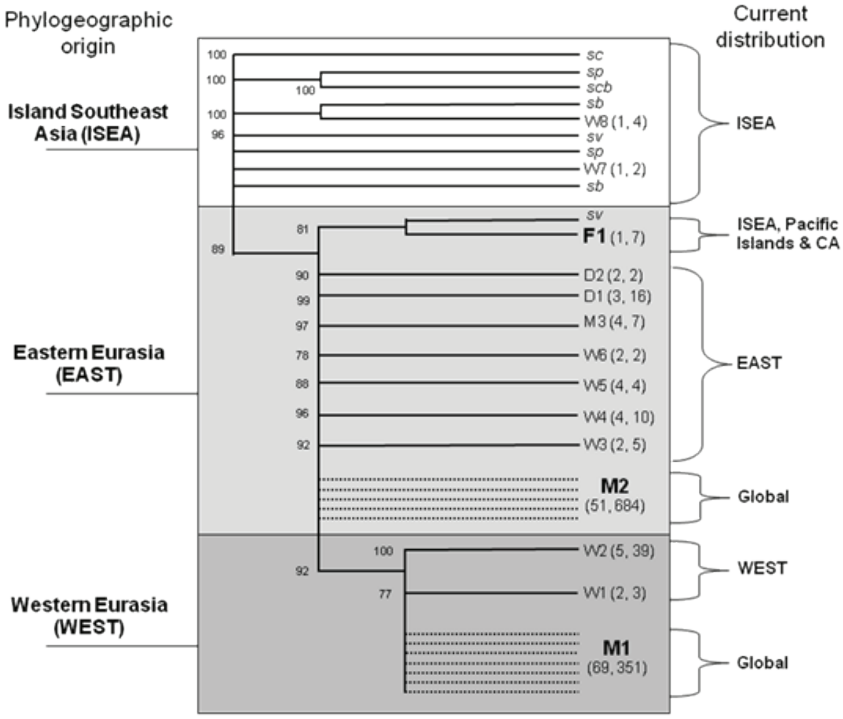


Figure 2.—Cladogram of 151 *Sus scrofa* mtDNA haplotypes constructed for 158 individual pigs from 23 California counties, 74 individuals from 29 other U.S. states, 904 published sequences for *Sus scrofa* from around the world, and 13 sequences from five other *Sus* species for an outgroup. Shaded regions of tree denote overarching phylogeographic associations of mtDNA matching descriptions at left. Current distribution of the haplotypes is denoted by bracketed descriptions at right. Phylogenetic assemblages in tree are described by pig type within EAST and WEST in tree; W (Eurasian Wild Boar), D (domestic), M (mixed, shared between domestic pigs and Eurasian Wild Boar), and F (feral). The five other *Sus* species are abbreviated; *sb* (*S. barbatus*), *sc* (*S. celibensis*), *scb* (*S. cebifrons*), *sp* (*S. philippensis*), and *sv* (*Sus verrucosus*). Groups containing haplotypes found in California are in bold. Dotted lines in the tree indicate polytomies from unresolved phylogenetic assemblages; number of lines is proportional by an approximate factor of ten to the count of haplotypes in each branch. Numbers at nodes indicate mean posterior probabilities (≥75% shown) and numerals in parentheses indicate number of haplotypes and number of individual sequences, from left to right.

one (h84) was linked to the monophyletic F1 group that includes feral pigs observed only in ISEA and on Pacific Islands (Table 1, Figure 2).

In California, WEST mtDNA was more common than EAST (Table 1). Two WEST haplotypes in particular, h17 (n = 59 individuals) and h19 (n = 68 individuals), had wide distributions spanning both Historic and Recent pig range (Figure 3). WEST h150 was found on both Santa Cruz Island and Santa Catalina Island (representing Historic range on the Channel Islands), as well as in Recent range in mainland California (Figure 3). WEST h37 was found in Historic range only, whereas EAST h13 was in Recent range only (Figure 3). Haplotypes h149, h150, and h151 grouped with WEST, but were found only in California.

Table 1.—California geography and breed details for nine mitochondrial DNA haplotypes identified among 158 wild pigs sampled in California from 1996 to 2010. Phylogeographic "Origin" descriptors EAST, WEST and ISEA correspond to a division of mtDNA lineages in Eurasia and elsewhere identified through analysis of a ~400 base pair region of the mtDNA control region. Descriptors M1 and M2 represent unresolved assemblages of mixed domestic and Eurasian wild boar with WEST or EAST phylogenies, and F1 represents feral type pigs from a single ISEA haplotype found only on Santa Catalina Island, Hawaii, and South Pacific Islands. More details are provided by McCann et al. (2014).

Haplotype (Origin)[1]	California region	Phylogenetic group	No. pigs in California	In other US states?[2]	Breed information for haplotype
h13 (EAST)	Recent	M2	8	Yes, HI only	~16 Asian and European domestic breeds
h17 (WEST)	Historic, Recent	M1	59	Yes, six	Linked to Hooper Bald Eurasian wild pig hybrids, and 12 European domestic breeds
h19 (WEST)	Historic, Recent	M1	68	Yes, sixteen	>20 European domestic breeds
h37 (WEST)	Historic	M1	1	Yes, ten	12 European domestic breeds, 1 Asian wild
h38 (WEST)	Historic, Recent	M1	2	Yes, KY only	9 European domestic breeds
h84 (ISEA)	S. Catalina Island	F1	3	Yes, HI only	Feral pigs only
h149 (WEST)	Historic	M1	1	No	Unique to California
h150 (WEST)	S. Catalina and S. Cruz Islands, Recent	M1	15	No	Unique to California
h151 (WEST)	Historic	M1	1	No	Unique to California

[1] h13 was also in China, Korea, Germany, Spain, Japan, Hawaii, Australia, Thailand, Italy and the United Kingdom; h17 was also in Belgium, Germany, Iberian Peninsula, Iceland, Italy, Macedonia, Norway, Spain, and the United Kingdom; h19 was also in Corsica, Finland, France, Germany, Hungary, Holland, Iberian Peninsula, Italy, Portugal, Sweden, and the United Kingdom; h37 was also in Austria, Bulgaria, Germany, Hungary, Iberian Peninsula, Indonesia, Italy, Korea, Morocco, Poland, Portugal, Spain, and the United Kingdom; h38 was also in Austria, Corsica, Denmark, France, Germany, Hungary, Iberian Peninsula, Italy, Portugal, Sardinia, and the United Kingdom; h84 was also in Papua-New Guinea and Vanuatu.
[2] h17 was also among pigs in AR, MS, NC, ND, NV, and TN; h19 was also among pigs in AL, AR, CO, FL, GA, HI, KY, LA, MI, ND, OH, OK, TN, TX, WI, and WV; h37 was also among pigs in AZ, KS, LA, ND, NJ, NE, NM, OK, PA, TX, and VA.

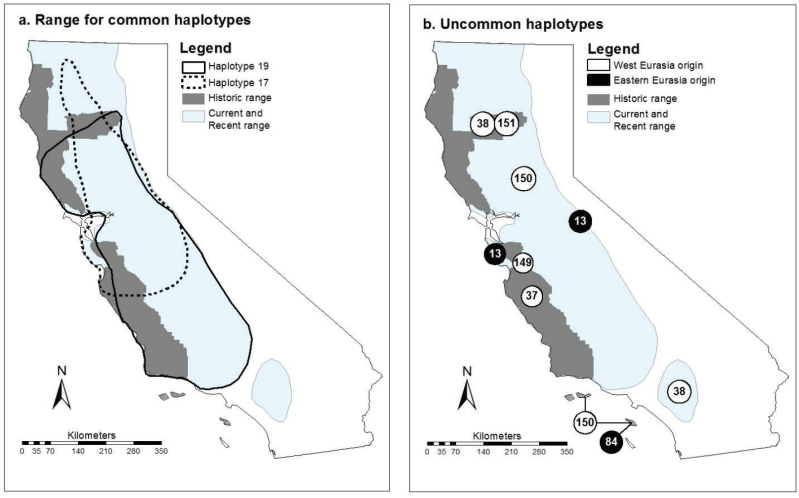


Figure 3.—Distributions of nine mtDNA haplotypes identified among 158 wild pigs sampled in mainland and island locations from 23 counties in California from 1996 to 2010. Haplotypes h17 and h19 were common and widespread with estimated ranges outlined in panel a. Haplotypes that were uncommon with limited distributions (panel b) are the numbers in open (WEST origin) or black circles (EAST origin, except h84 with an ISEA origin). Historic range (nine counties) is shaded gray, and Recent range is shaded light blue.

Population-level mtDNA variation between the five population groupings was significant ($AMOVA_{4, 228}$; $P < 0.001$). Exact tests of population differentiation were highly significant ($\alpha<0.001$) for all but Historic-Recent ($P = 0.002$, $SE \pm 0$ .001) and Santa Catalina Island-Santa Cruz Island ($P = 0.030$, $SE \pm 0.001$). All population F_{ST} measures were significant except Recent and 29US, for which numbers of migrants were estimated as exceptionally high when compared to other populations within the state (Table 2).

Table 2.—Population genetic measures of F_{ST} (bottom half matrix) and *Nm* (top half matrix) for five populations assigned by geography and history of invasion: wild pigs from 29 U.S. states (29US; $n = 74$), historic mainland (Historic; pigs from mainland sites within nine counties of historical occurrence in California; $n = 77$), SCI (Santa Cruz Island California; $n = 11$), SCAT (Santa Catalina Island; $n = 6$), and recent mainland (Recent; pigs from mainland California sites other than the nine historical counties of occurrence; $n = 64$). Significance of *F* statistics between populations is denoted in the lower half of the matrix as follows: **P*<0.05, ***P*<0.01, ****P*<0.0000.

	29US	Historic	SCI	SCAT	Recent
29US	0	4.72864	2.03882	1.71671	98.47133
Historic	0.09563***	0	1.01018	0.33321	8.44260
SCI	0.19695*	0.33109***	0	0.44595	2.65806
SCAT	0.22556*	0.60009***	0.52857*	0	1.07818
Recent	0.00505	0.05591***	0.15833*	0.31682***	0

Discussion

Our phylogeographic result of EAST, WEST, and ISEA groupings agrees with results of other investigators evaluating the mtDNA control region in pigs (Alves et al. 2003, Gongora et al. 2004, Larson et al. 2005), which increases confidence in the accuracy of our dendrogram of sequence relationships associated with wild pigs in California (Figure 2). The unresolved nature of M1 and M2 haplotypes within respective WEST and EAST branches of the phylogeny likely represents an increased rate of nonsynonymous changes in the mtDNA genome resulting from domestication, as described for dogs (Bjornerfeldt et al. 2006). Humans have impacted both the genetic composition and geographic distribution of pigs globally, resulting in a lack of phylogenetic resolution for some mtDNA lineages (McCann et al. 2014). Representatives of M1 and M2 groups with domestic and Eurasian wild pig associations have achieved extensive geographic distributions in North America through anthropogenic dispersal (McCann et al. 2014), and are also prevalent among wild pigs in California (Table 1, Figure 3).

The WEST phylogeographic association for most of the haplotypes (seven of nine) identified in California was not surprising given the major European influence on early settlement of the state. Also, 93% of the 158 wild pigs sampled were linked to a WEST phylogeographic group, including 17 of the 20 feral pig samples from the two islands (Table 1). Geographic distribution of EAST haplotypes was restricted to one island and two spatially separated mainland locations (Figure 3). Both WEST and EAST haplotypes in California were associated with multiple domestic swine breeds, feral pigs, or wild boar from Europe or Asia (Table 1; McCann 2012), but only four of the nine haplotypes were found elsewhere in the continental United States (Table 1). The presence of haplotypes in California from

the EAST+ISEA region (Figure 1) indicates anthropogenic transfer of pigs independent of Spanish settlement of California. This result was consistent with an historical account indicating that domestic pigs from the EAST+ISEA region were commonly onboard trade ships that visited coastal regions of California in the mid-1800s (Dana 1840), and sailors likely traded some of those pigs for local supplies prior to return voyages.

Evidence for historic introductions and range expansion.—Although it is not possible to develop a definitive timeline of Historic invasion based upon mtDNA lineages, insights can be gained from assessment of molecular relationships in light of phylogeny and introduction histories. Haplotypes h19, h37, and h38 are associated with a variety of modern domestic breeds (Table 1), which suggests a recent domestic source for these wild pigs in California (Table 1). The lack of current breed references for unique haplotypes h149, h150, and h151 suggests they may represent ancestors of livestock from the Spanish settlement period, and that those mitochondrial lineages are now absent in modern domestic swine. This hypothesis is supported by the distribution of h149, h150, and h151 primarily within the Historic range of pigs in mainland and island locations (Figure 3). Also, the sharing of h150 between Santa Catalina Island and Santa Cruz Island corroborates a common origin for populations on the Channel Islands resulting from introductions by the Spanish (Mayer and Brisbin 1991, McCann 2012).

Spanish settlement likely also explains the occurrence of h150 on the California mainland because there is no record of anthropogenic transfer between island and mainland sources. Notably, h150 was found among wild pigs sampled from the Sutter Buttes in Sutter County, an area identified as Recent expansion range based on the lack of Annual Hunter Game Take survey records before 1967 (Waithman et al. 1999). Of relevance is that the Sutter Buttes region had been privately owned with no public access and no hunting until after 2003 (Sutter Buttes Regional Land Trust 2015), and it is likely that feral pigs were present in Sutter County but not harvested by hunters prior to 1967.

Haplotypes h17 and h19 were the two most common in both Historic and Recent range in California, and their current distributions likely represent a history of human transfer followed by local population growth and expansion (Pine and Gerdes 1973, Waithman et al. 1999). Haplotype h17 was previously linked to Eurasian wild pig hybrid stock translocated to California from Hooper Bald, North Carolina in 1925 (McCann 2012). The geographic distribution of h17 in California is consistent with the known and documented anthropogenic dispersal of Eurasian wild pig hybrid animals within the state to the north and east of the original introduction site in Monterey County (Figure 3; Mayer and Brisbin 1991). Nevertheless, h17 was also found further to the north, east, and south of areas of known anthropogenic transfer, and it is apparent that once Eurasian wild pig hybrids were translocated outside of Monterey and Tehama counties, they then expanded their distribution into new areas of the state as populations increased (Figure 3).

Haplotype h19 was the most common among the 158 pigs sampled (Table 1), with an expansive coastal and inland distribution from central California to northern California (Figure 3). Haplotype h19 is also widely distributed in 16 other U.S. states with a genetic linkage to >20 different breeds of domestic swine (Table 1; McCann et al. 2014). We therefore conclude that the distribution of feral-domestic pigs with the h19 haplotype in California is indicative of anthropogenic transfer, followed by local expansion resulting from population growth and dispersal. Further we have photographic evidence (pelage characteristics; McCann et al. 2003) that feral-domestic pigs (h19) are interbreeding with Eurasian hybrids

(h17) within their broad zone of overlap (Figure 3). This is important because, based on our research here, we now know that wild pigs of all types in California are of mixed heritage (M1 hybrids; Figure 2), and fully capable of invasion and range expansion resulting from vigorous population growth.

Haplotypes h13 and h38 were uncommon in the state and their role in range expansion is less clear. Feral-domestic h13 was the only EAST mtDNA lineage in California, suggesting that it does not link to livestock associated with Spanish settlement (Table 1). Further, h13 was found exclusively within Recent range (Figure 3). Feral-domestic h38 was identified in both Historic and Recent range (Figure 3), and h38 was the only haplotype among wild pigs in a population that was recently established in southern California (Figure 3; detailed by Loggins 2007). These observations indicate that feral-domestic pigs with h13 and h38 Eastern Eurasian heritage were recently introduced to California, either by escape from livestock pens, or purposed anthropogenic transfer from out of state (Table 1). The spatial separation between wild pig sample locations for both h13 and h38 suggests that anthropogenic transfer was the more likely source (Figure 3).

Assessment of population relatedness revealed lack of differentiation for wild pigs in Historic vs. Recent range, coupled with high levels of gene flow between the ranges (≈8 migrants/generation; Table 2). In contrast, there was genetic differentiation and very low gene flow between Santa Cruz Island and Santa Catalina Island (Table 2), consistent with no records of anthropogenic transfer of wild pigs after the 1930s. It is not always possible to separate natural dispersal from anthropogenic dispersal based solely on molecular evidence, but human facilitated gene flow (= anthropogenic transfer) was obvious for haplotypes h13 and h38 based on distance and presence of geographic barriers to animal movement between the sampling locations (Figure 3). Wild pigs also naturally disperse on their own (Waithman et al. 1999), accounting for animals with haplotypes h17 and h19 occurring in proximity within regions (Figure 3).

An important observation for future consideration is that Loggins (2007) surveyed fewer samples for mtDNA analyses, and additional sampling by our work identified presence of several additional mtDNA haplotypes in California. It is therefore possible that expanded sampling in Humboldt County (historically invaded; Waithman et al. 1999) and along the eastern margin of the San Joaquin Valley (Sierra Nevada foothills) would identify presence of previously unknown haplotypes.

Haplotype patterns among feral pigs on the Channel Islands.—We interpret the low mtDNA diversity observed on Santa Catalina Island (two haplotypes) and Santa Cruz Island (one haplotype) as due to genetic drift associated with isolation or bottlenecks from culling and mast failures (Baber and Coblentz 1986). Both populations endured periodic reduction from the 1940s onward, prior to eventual eradication in the early 2000s (Schuyler et al. 2002, Ramsey et al. 2009). The low mtDNA variability on the islands coupled with presence of abundant feral pigs prior to eradication suggests that wild pigs are resilient even at low genetic diversity.

The discovery of h84 on Santa Catalina Island is interesting, as this haplotype has been observed nowhere else except islands elsewhere in the Pacific Ocean (Figure 2; Larson et al. 2005, Loggins 2007). A possible pathway for introduction of h84 to California was the third voyage of Captain Cook from 1776 to 1779 (Cook 1968, Loggins 2007). Cook transferred livestock between many islands in the southern Pacific Ocean and also visited the Oregon coast, although we could find no records of visits to mainland California or the

Channel Islands. Due to the apparent absence of this haplotype on Santa Cruz Island and the lack of samples for Santa Rosa Island (the source of pigs for Santa Catalina Island), the origin of h84 in California remains unclear.

Importance for management and conservation.—Sequence variation in mtDNA among wild pigs provided valuable insights on the introduction history and on the subsequent, but undocumented, translocation and liberation of wild and domestic pigs that occurred after the 1950s (Waithman et al. 1999). Considered together, information on the distribution of haplotypes and gene flow suggests that range expansion by wild pigs in California is progressing on multiple fronts through population growth coupled with natural dispersal, anthropogenic dispersal, and new introductions from outside of the state (McCann 2012). Natural expansion was not limited to Eurasian wild pig hybrids, however. We found strong indications that feral-domestic wild pigs have moved into new areas independent of anthropogenic transfer.

Identification of new mtDNA lineages among wild pigs in California also suggests that public interest in hunting wild pigs continues to shape the molecular profile of California's wild pigs (McCann et al 2014). California state officials suspect recent importation and release of wild pigs from elsewhere in North America (B. Gonzales, California Department of Fish and Wildlife, personal communication), and our results indicate that this very likely has occurred. Considering the pervasive spread of wild pigs already in progress, additional introductions will be detrimental and should be vigorously discouraged to limit the spread of livestock and zoonotic diseases (Benfield et al. 1999, Jay et al. 2007), and to prevent further damage to native plants and animals, which has been focused in California oak woodland ecosystems (Sweitzer and Van Vuren 2002, Cushman et al. 2004, Grinde 2006, Loggins 2007, Wilcox and Van Vuren 2009).

Acknowledgments

We thank the North Dakota Experimental Program to Stimulate Competitive Research (ND EPSCoR), University of North Dakota Department of Biology, and University of North Dakota Graduate School for funding of this research. Thanks to Wildlife Disease Biologist Brandon Schmit, and multiple field agents with the United States Department of Agriculture, Wildlife Disease Program for collection of pig tissue samples from throughout the United States. Thanks also are extended to the National Park Service, Institute for Wildlife Studies, North Dakota Game and Fish Department, and many other state and private organizations for collection of samples that made this work possible. We thank M. Malek, K. Drees, T. Jahraus, T. Mitchell, A. Bergstrom, and S. Johnson for laboratory and computational contributions, and V. Tkach, and R. Newman critical comments that improved the manuscript. J. Wilcox and an anonymous reviewer also provided comments that improved the manuscript.

Literature Cited

Alves, E., C. Ovilo, C. M. Rodriguez, and L. Silio. 2003. Mitochondrial DNA sequence variation and phylogenetic relationships among Iberian pigs and other domestic and wild pig populations. Animal Genetics 34:319-324.

Baber, D. W., and B. E. Coblentz. 1986. Density, home range, habitat use, and reproduction in feral pigs on Santa Catalina Island. Journal of Mammalogy 67:512-525.

Barrett, R. H. 1977. Wild pigs in California. Pages 111-113 *in* G. W. Wood, editor. Research and management of wild hog populations. Belle Baruch Forest Science Institute, Clemson University, Georgetown, South Carolina, USA.

Barrett, R. H., and D. S. Pine. 1980. History and status of wild pigs, *Sus scrofa*, in San Benito County, California. California Fish Game 67:105-117.

Benfield, D. A., J. E. Collins, S. A. Dee, P. G. Halbur, H. S. Joo, K. M. Lager, W. L. Mengeling, M. P. Murtaugh, K. D. Rossow, G. W. Stevenson, and J. J. Zimmerman. 1999. Porcine reproductive and respiratory syndrome. Pages 201-232 *in* B. E. Straw, editor. Diseases of swine. Eighth edition. Iowa State University Press, Ames, USA.

Bjornerfeldt, S., M. T. Webster, and C. Vila. 2006. Relaxation of selective constraint on dog mitochondrial DNA following domestication. Genome Research 16:990-994.

Clement, M., D. Posada, and K. S. Crandall. 2000. TCS: A computer program to estimate gene genealogies. Molecular Ecology 9:1657-1660.

Collins, P. 1981. The origin and present status of feral pigs on the California Channel Islands. Santa Barbara Museum of Natural History, Santa Barbara, California, USA.

Cook, J. 1968. The journals of Captain James Cook on his voyages of discovery. Boydell and Brewer, Rochester, New York, USA.

Cushman, J. H., T. A. Tierney, and J. M. Hinds. 2004. Variable effects of feral pig disturbances on native and exotic plants in a California grassland. Ecological Applications 14:1746–1756.

Dana, R. H. 1840. Two years before the mast: a personal narrative of life at sea. Harper, New York, USA.

Excoffier, L., G. Laval, and S. Schneider. 2005. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50

Fang, M., and L. Andersson. 2006. Mitochondrial diversity in European and Chinese pigs is consistent with population expansions that occurred prior to domestication. Proceedings of the Royal Society B: Biological Sciences 273:1803-1810.

Fitzhugh, L. E., and J. B. Loomis. 1993. An economic analysis of fee hunting for wild pigs in California. Pages 21-22 *in* W. Tietje and R. H. Barrett, technical coordinators. Conference summaries of the wild pig in California oak woodland: ecology and economics. San Luis Obispo, California, May 25-26. Integrated Hardwood Range Management Program, University of California, Berkeley, USA.

Gipson, P. S., B. Hlavachick, and T. Berger. 1998. Range expansion by wild hogs across the central United States. Wildlife Society Bulletin 26:274-278.

Giuffra, E. J. M. H., J. M. H. Kijas, V. Amarger, O. Carlborg, J. T. Jeon, and L. Andersson. 2000. The origin of the domestic pig: independent domestication and subsequent introgression. Genetics 154:1785-1791.

Gongora, J., P. Fleming, P. B. S. Spencer, R. Mason, O. Garkavenko, J-N Meyer, C. Droegemueller, J. H. Lee, and C. Moran. 2004. Phylogenetic relationships of Australian and New Zealand feral pigs assessed by mitochondrial control region sequence and nuclear GPIP genotype. Molecular Phylogenetics and Evolution 33:339-348.

Grinde, A. 2006. Ecological effects of wild pigs (*Sus scrofa*) in California oak ecosystems. M.S. Thesis, University of North Dakota, Grand Forks, USA.

Guindon, S., and O. Gascuel. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Systematic Biology 52:696-704.

Jay, M. T., M. Cooley, D. Carychao, G. W. Wiscomb, R. A. Sweitzer, L. Crawford-Miksza, J. A. Farrar, D. K. Lau, J. O'Connell, A. Millington, R. V. Asmundson, E. R. Atwill, and R. E. Mandrell. 2007. *Escherichia coli* O157:H7 in feral swine near spinach fields and cattle, central California coast. Emerging Infectious Diseases 13:1908-1911.

Larson, G., K. Dobney, U. Albarella, M. Fang, E. Matsoo-Smith, J. Robins, S. Lowden, H. Finlayson, T. Brand, E. Willerslev, P. Rowley-Conwy, L. Andersson, and A. Cooper. 2005. Worldwide phylogeography of wild boar reveals multiple centers of pig domestication. Science 307:1618-1621.

Loggins, R. E. 2007. Historic origin, range expansion, and habitat suitability of introduced wild pigs (*Sus scrofa*) in California. Ph.D. Dissertation, University of North Dakota, Grand Forks, USA.

Lombardo, C. A. and K. R. Faulkner. 2000. Eradication of feral pigs, (*Sus scrofa*) from Santa Rosa Island, Channel Islands National Park, California. Pages 300-306 *in* D. R. Browne, K. L. Mitchell, and H. W. Chaney. Proceedings of the Fifth California Islands Symposium. Santa Barbara Museum of Natural History, Santa Barbara, California, USA.

Long, W. E. 1993. The Santa Rosa Island wild pig eradication project. Page 40 *in* W. Tietje and R. H. Barrett, technical coordinators. Conference summaries of the wild pig in California oak woodland: ecology and economics. San Luis Obispo, California, May 25-26. Integrated Hardwood Range Management Program, University of California, Berkeley, USA.

Luetkemeier, E. S., M. Sodhi, L. B. Schook, and R. S. Malhi. 2010. Multiple Asian pig origins revealed through genomic analyses. Molecular Phylogenetics and Evolution 54:680-686.

Mayer, J. J., and I. L. Brisbin. 1991. Wild pigs in the United States: their history, comparative morphology, and current status. University of Georgia Press, Athens, USA.

McCann, B. E. 2012. Genetic relationships of wild pigs (*Sus scrofa*) in North America: geographic origins and genotypic distribution of the species with implications for management. Ph.D. Dissertation, University of North Dakota, Grand Forks, USA.

McCann, B. E., D. K. Davie, and G. A. Feldhamer. 2003. Distribution, habitat use, and morphotypes of feral hogs (*Sus scrofa*) in Illinois. Transactions of the Illinois State Academy of Science 96:301-311.

McCann, B. E., M. Matthew, R. Newman, B. Schmit, S. Swaffort, R. Sweitzer, and R. Simmons. 2014. Mitochondrial diversity supports multiple origins for invasive pigs. Journal of Wildlife Management 78:201-213.

Okumura, N., N. Ishiguro, M. Nakano, K. Hirai, A. Matsui, and M. Sahara. 1996. Geographic population structure and sequence divergence in the mitochondrial control region of the Japanese wild boar (*Sus scrofa leucomystax*), with reference to those of domestic pigs. Biochemical Genetics 34:179-189.

Parr, R. L., J. Maki, B. Reguly, G. D. Dakubo, A. Aguirre, R. Wittock, K. Robinson, J. P. Jakupciak, and R. E. Thayer. 2006. The pseudo-mitochondrial genome influences mistakes in heteroplasmy interpretation. BMC Genomics 7:185-198.

Pine, D. S, and G. L. Gerdes. 1973. Wild pigs in Monterey County. California Fish and Game 59:126-137.

Posada, D. 2008. jModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25:1253-1256.

R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramsey, D. S. L., J. Parkes, and S. A. Morrison. 2009. Quantifying eradication success: the removal of feral pigs from Santa Cruz Island, California. Conservation Biology 23:449-459.

Ronquist, F., and J. P. Huelsenbeck. 2003. MRBAYES 3: bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572-1574.

Scandura, M., L. Lacolina, and M. Apollonio. 2011. Genetic diversity in the European wild boar *Sus scrofa*: phylogeography, population structure and wild × domestic hybridization. Mammal Review 41:125-137.

Schuyler, P. T., D. K. Garcelon, and S. Escover. 2002. Eradication of feral pigs (*Sus scrofa*) on Santa Catalina Island, California, USA. Pages 274-286 *in* C. R. Veitch and M. N. Clout, editors. Turning the tide: the eradication of invasive species. World Conservation Union, Species Survival Commission, Invasive Species Specialist Group, Gland, Switzerland and Cambridge, United Kingdom.

SCWDS (Southeastern Cooperative Wildlife Disease Study). 2010. National feral swine mapping system; 2015 January 3. Southeastern Cooperative Wildlife Disease Study, University of Georgia. Available from: http//:www.feralswinemap.org

Spencer, P. B. S, and J. O. Hampton. 2005. Illegal translocation and genetic structure of feral pigs in Western Australia. Journal of Wildlife Management 69:377-384.

Sutter Buttes Regional Land Trust. 2015. Preserving our lands and heritage through conservation [Internet]. Sutter Buttes Regional Land Trust website; accessed 6 January 2015. Available from: http://www.sutterbutteslandtrust.org/about-the-land-trust/

Sweitzer, R. A. 1998. Conservation implications of feral pigs in island and mainland ecosystems, and a case study of feral pig expansion in California. Pages 24-34 *in* R. O. Baker and A. C. Crabb, editors. Proceedings of the 18th Vertebrate Pest Conference, Costa Mesa, California, USA.

Sweitzer, R. A., I. A. Gardner, D. H. Van Vuren, W. M. Boyce, and J. D. Waithman. 2000. Estimating sizes of wild pig populations in the north and central coast regions of California. Journal of Wildlife Management 64:531-543.

Sweitzer, R. A., and D. H. Van Vuren. 2002. Rooting and foraging effects of wild pigs on tree regeneration and acorn survival in California's oak woodland ecosystems. Pages 218-231 *in* R. B. Standiford, D. McCreary, and K. L. Purcell, technical coordinators. Proceedings of the 5th Symposium on Oak Woodlands: oaks in California's changing landscape. USDA Forest Service, General Technical Report PSW-GTR-184. Pacific Southwest Research Station, Albany, California, USA.

Vigne, J-D., A. Zazzo, J.-F. Saliege, F. Poplin, J. Guilaine, and A. Simmons. 2009. Pre-Neolithic wild boar management and introduction to Cyprus more than 11,400 years ago. Proceedings of the National Academy of Science 106:16135-16138.

Waithman, J. D., R. A. Sweitzer, D. Van Vuren, J. D. Drew, A. J. Brinkhaus, I. A. Gardner, and W. M. Boyce. 1999. Range expansion, population sizes, and management of wild pigs in California. Journal of Wildlife Management 63:298-308.

Wikipedia Contributors. 2015. Feral pig [Internet]. Wikipedia, The Free Encyclopedia. Accessed 6 May 2015. Available from: http://en.wikipedia.org/wiki/Feral_pig

Wilcox, J. T., and D. H. Van Vuren. 2009. Wild pigs as predators in oak woodlands of California. Journal of Mammalogy 90:114-118.

Received 9 February 2015
Accepted 12 May 2015
Associate Editor was J. Villepique

Appendix I: Terminology commonly used for wild and domestic swine

Swine is a generic term commonly used by livestock producers and many state and federal resource managers for all types of domestic, feral and wild pigs. **Eurasian** or **European wild boar** refers to all wild, non-domesticated pigs, which are the ancestors of most domestic breeds of pigs. **Domestic pigs** are swine that are penned and associated with active animal husbandry. **Feral pigs** are domestic pigs that have escaped captivity in the recent or distant past, are no longer associated with active husbandry, and are descended from domesticated individuals. Feral pigs are also commonly referred to as **feral hogs** or **feral swine**, particularly in the southeastern U.S. and Texas. The terms **razorback** and **wild hog** are American colloquialisms, loosely applied to any type of feral pig, wild boar or wild pig hybrid (Wikipedia 2015).

European wild boar are sometimes called **Russian wild boar**, but the species occurs throughout Europe, and European wild boar is more appropriate. European wild boar freely interbreed with feral pigs wherever populations came into contact, leading to **European hybrid wild pigs** with a range of intermediate phenotypic characteristics (Mayer and Brisbin 1991). Phenotypic characteristics do not reliably identify ancestry, however (Mayer and Brisbin 1991), and genetic analysis is the most reliable method for verifying presence of European wild boar hybrids within feral pig populations (McCann et al. 2014). In areas where there is a known history of introduction and interbreeding of Eurasian wild boar with feral pigs, hybrid wild pigs are often referred to as wild boar, which is technically incorrect from a genetic perspective.

California is one of the regions in the U.S. where wild pigs from North Carolina that were thought to be pure or near pure European wild boar were translocated in the 1920s (McCann et al. 2014), pen reared, and then released into the wild for hunting. Once in the wild, the North Carolina-origin wild pigs interbred with feral pigs where feral pigs were already present (Waithman et al. 1999). Feral and European wild pig hybrids have been managed as a big game mammals in California since 1957 (Sweitzer et al. 1999), and are officially referred to as **wild pigs** by the California Department of Fish and Wildlife. Most hunters, landowners, and the general public also use wild pigs when referring to feral pigs and European hybrid wild pigs in the state. Because free ranging swine in California include a diversity of feral and wild type ancestors, wild pigs is an appropriate term for them.

California Fish and Game 101(2):146-148; 2015

A swimming deep-sea peneaoid shrimp photographed off California

Mary K. Wicksten* and Linda A. Kuhnz

Department of Biology, Texas A&M University, College Station, TX 77843, USA (MKW, LAK)

Monterey Bay Aquarium Research Institute, 7700 Sandholdt Road, Moss Landing, CA 95039, USA (MKW, LAK)

**Correspondent: Wicksten@bio.tamu.edu*

Key words: Penaeoidea, *Plesiopenaeus*, abyssal shrimp

During a study of long-term cycles in deep benthic communities, the remotely operated vehicle (ROV) *Doc Ricketts* photographed a large deep-sea shrimp (Figure 1) west of Morro Bay (35° 10' N, 122° 59' W, 3,949 m, Monterey Bay Aquarium Research Institute (MBARI) station M, 24 May 2011). Further details of the study location, methods, and

Figure 1.—Penaeoid shrimp swimming across the sea floor at 3,949 m, 35° 10' N, 122° 59' W, on 24 May 2011 at Monterey Bay Aquarium Research Institute (MBARI) station M. Scale (distance between laser dots) = 29 cm, from which we estimated the total length of the shrimp to be 15 cm. Photograph by ROV *Doc Ricketts*, courtesy of Ken Smith, MBARI.

results are available in Kuhnz et al. (2014). Laser dots on the photograph are 29 cm apart, from which the total length of the shrimp can be estimated at 15 cm. During 2007–2012, this shrimp was seen in eight quantitative video transects but in low abundance—no more than one shrimp per 100 m^2, and was not collected.

The shrimp is shown swimming above the surface of the sediment by means of laterally extended pleopods, characteristic of a penaeoid shrimp (order Decapoda, superfamily Penaeoidea; see Pequegnat 1983, plates XXXIVA and LD for photographs of similarly swimming penaeoid shrimps). The few other shrimp-shaped crustaceans that live at comparable depths in the northeastern Pacific (Superfamily Sergestoidea, four superfamilies within infraorder Caridea and order Lophogastrida) swim either by moving their pleopods up and down along the ventral surface of the abdomen, or hop for short distances by jerking the abdomen. Members of these taxa differ from the shrimp in the photograph by size, ridges on the carapace, color marks, or spinules. Midwater sergestoids or carideans are unlikely to swim close to the sea floor.

Although the photograph is blurred, the individual photographed is most likely *Plesiopenaeus armatus* (Bate, 1881; family Aristeidae). This deep-sea shrimp can be recognized by its large size, red color, reflective corneae of the eyes, dorsal carinae on the abdominal somites, and the characteristic laterally spread pleopods. In the Gulf of Mexico, *P. armatus*, matched to specimens, was photographed by ROV as it paddled with its pleopods in similar fashion to the shrimp in the photograph (M. Wicksten, unpublished data). Bracken-Grissom et al. (2012) noted that the larval stage known as *Cerataspis monstrosa* has taxonomic priority over the adult *P. armatus*, but applied to the International Commission on Zoological Nomenclature to use its plenary action to suppress *Cerataspis* in favor of *Plesiopenaeus*.

Plesiopenaeus armatus is among the largest deep-sea shrimps, reaching a total length of at least 22.5 cm (Texas A&M University Biodiversity and Research Collection catalog number 2-6444). The only other penaeoids known to live at a similar depth in the northeastern Pacific are benthic species of *Benthesicymus* (family Benthesicymidae), considerably smaller than the shrimp in the photograph and having extremely slender legs and a short rostrum. Definitive identification of the shrimp in the photograph would require a specimen and examination of the characteristic copulatory organs (Perez Farfante and Kensley 1997:figure 20).

Perez Farfante and Kensley (1997) provided records of *P. armatus* at 2,562–4,300 m from the North and South Atlantic, Gulf of Mexico, Indian Ocean, and Pacific Ocean from off the Philippines and Japan to the central North Pacific and Hawaii, as well as "off the north-west coast of U.S.A. [sic]" but did not provide a citation for the latter record. Pequegnat (1983) reported the shrimp at 1,800-3,740 m and included it in lists of shrimps from the upper to lower abyssal zones of the Gulf of Mexico. There are insufficient records to determine if the species occurs deeper in certain geographic locations than others. This species was not reported from the northeastern Pacific by McLaughlin and Camp (2005), and Wicksten (2012) did not include it in her recent monograph. Investigators working on the biota of the lower continental slope should look for this shrimp.

Acknowledgments

K. Smith (MBARI) served as chief scientist for the ROV project that detected the apparent *P. armatus* reported herein. This work was sponsored by a grant from the David and Lucile Packard Foundation.

Literature Cited

Bate, C. 1881. On the Penaeidae. Annals and Magazine of Natural History 8(5):169-195.

Bracken-Grissom, H., D. Felder, N. Vollmer, J. Martin, and K. Crandall. 2012. Phylogenetics links monster larva to deep-sea shrimp. Ecology and Evolution 2:2367-2373.

Kuhnz, L., G, Ruhl, C. Huffard, and K. Smith Jr. 2014. Rapid changes and long-term cycles in the benthic megafaunal community observed over 24 years in the abyssal Pacific. Progress in Oceanography 124:1-11.

McLaughlin, P., and D. Camp (co-chairs). 2005. Common and scientific names of aquatic invertebrates from the United States and Canada: crustaceans. American Fisheries Society Special Publication 31:1-533.

Pequegnat, W. 1983. The ecological communities of the continental slope and adjacent regimes of the northern Gulf of Mexico. United States Department of the Interior Minerals Management Services Contract no. AA851-CT1-12.

Perez Farfante, I., and B. Kensley. 1997. Penaeoid and sergestid shrimps and prawns of the world. Memoires du Museum National d'Histoire Naturelle 175:1-233.

Wicksten, M. 2012. Decapod crustacea of the Californian and Oregonian zoogeographic provinces. Zootaxa 3371:1-307.

Received 18 November 2014
Accepted 19 November 2014
Associate Editor was N. Kogut

California Fish and Game 101(2):149-157; 2015

Observations of predation and loss among leopard sharks and brown smoothhounds in San Francisco Bay, California

Ronald A. Russo

East Bay Regional Park District, Oakland, CA 94605, USA (Retired)

Present address: 4960 E 12th Drive, Bellingham, WA 98226, USA

**Correspondent: ronsheri@comcast.net*

Key words: great blue heron, Caspian tern, cause-specific mortality, sloughs, mudflats, eelgrass beds, elasmobranch, predation

---

The San Francisco Bay is the largest along the Pacific coast of the United States (Conomos et al 1985). With deepwater channels, shallow mudflats, and marsh sloughs and channels, it supports a variety of elasmobranchs including leopard shark (*Triakis semifasciata*), brown smoothhound (*Mustelus henlei*), spiny dogfish (*Squalus suckleyi*), soupfin shark (*Galeorhinus galeus*), sevengill shark (*Notorynchus cepedianus*), bat ray (*Myliobatis californicus*) and big skate (*Raja binoculata*).

Little detailed information has been published on the predation or mortality of leopard sharks, especially newborn and young-of-the-year (YOY) in San Francisco Bay (Russo and Herald 1968, Ebert 1986, 1991). Preliminary surveys indicate that marsh sloughs and channels are primary nursery grounds for leopard sharks, while brown smoothhounds generally use near-shore eelgrass beds (R. Russo, East Bay Regional Park District, unpublished data). As observations made incidentally during these surveys, predation on leopard sharks and brown smoothhounds by other sharks and piscivorous birds in the area is first reported here. Bycatch of leopard sharks in the commercial shrimp fishery and other observations of leopard shark kills are also reported as potential impacts to the leopard shark population.

Between 1970 and 2001, fishing trips were conducted monthly at various locations, primarily between coordinates 37° 48' N, 122° 22' W, the San Francisco Bay Bridge, and 37° 27' N, 122° 01' W at the entrance of Alviso Slough at the south end of San Francisco Bay (Figure 1). All specimens captured were identified, sexed, and measured in centimeters total length (cm TL) in a natural position. Newborn leopard sharks were defined as specimens showing unhealed and visible natal scars between the pectoral fins and measuring from 17 to <25 cm TL; YOY up to 50 cm TL with healed or absent natal scars; juveniles as 51–<100 cm TL; and sexually mature adults as >105 cm TL (Ebert 2005).

Sampling methods included rod-and-reel (3–5 h), long-lining (45–90 min), and otter trawl (7–15 min). Long-lines were two 6-mm thick, 152-m long nylon lines rigged with 5/0 hooks baited with squid every meter and capturing juvenile and adult sizes. Smaller size specimens were captured with a 1.3 cm mesh, 4.8-m otter trawl.

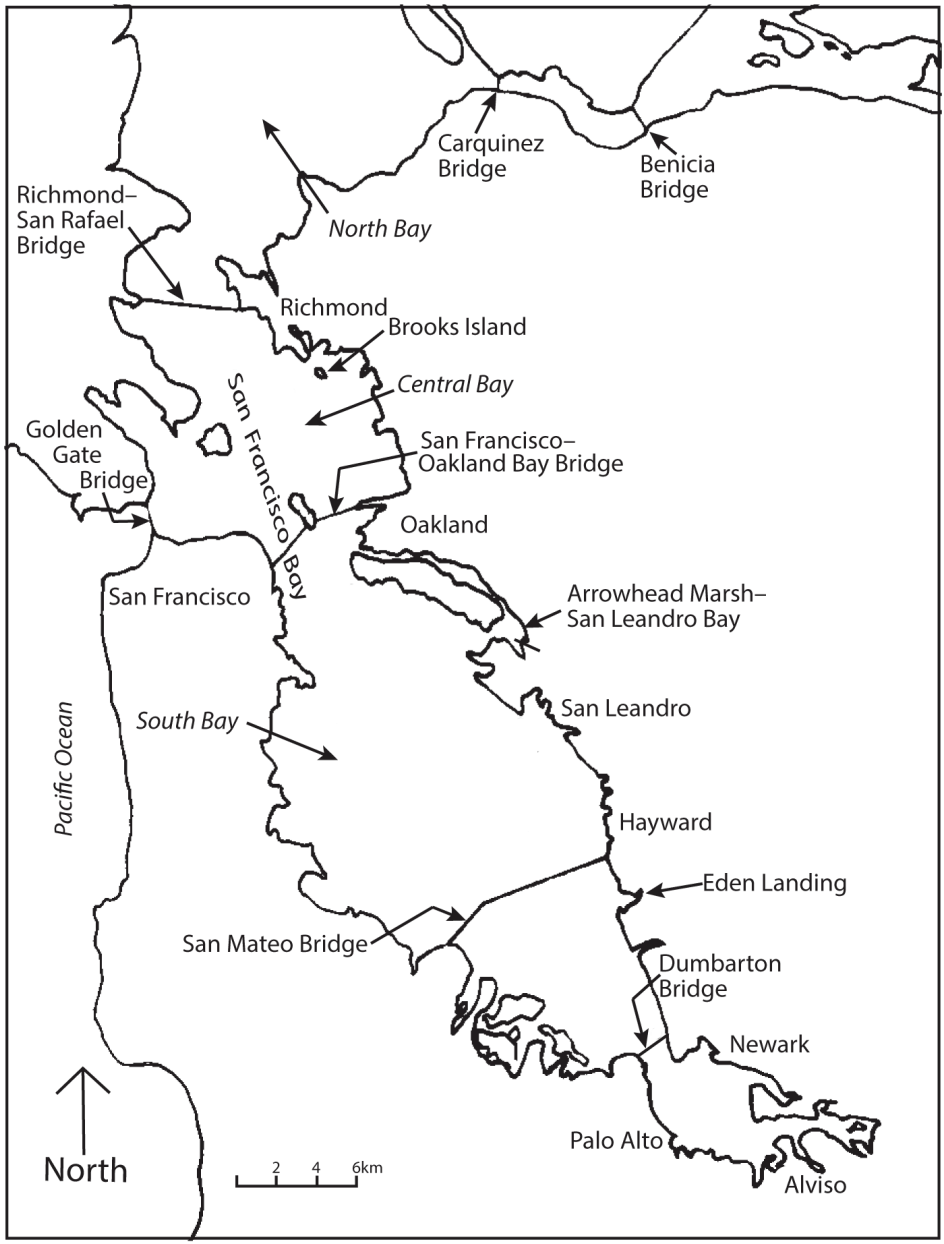


Figure 1.—Map of San Francisco Bay, California, indicating key locations mentioned in the text. Map courtesy of East Bay Regional Park District, Oakland, California.

Between 1970 and 1996 all of the methods described above were used to capture 3,790 elasmobranchs (Russo 2013). From 1997 to 2001 rod-and-reel was used to catch an additional 331 elasmobranchs for a combined total of 4,121 specimens. Of this total 2,478 (60.1%) were leopard sharks and 842 (20.4%) were brown smoothhounds ranging from

newborns to sexually mature adults. Long-lines captured 1,782 YOY, juvenile, and adult leopard sharks, but no newborns. Otter trawls captured 245 newborn and YOY leopard sharks, but often resulted in the fatality of the newborns. Rod-and-reel captured 451 newborn and YOY leopard sharks. Narrow, shallow Mowry and Newark sloughs and similar habitats were avoided by choice, and use of otter trawl and rod-and-reel methods was restricted to the larger Guadalupe and Alviso sloughs, as well as open water.

Leopard sharks and other elasmobranchs were attacked while caught on long-lines during 12.8% of the sets. Since no sixgill shark (*Hexanchus griseus*) were caught south of the San Francisco Bay Bridge and none on long-lines, the observed sevengill sharks were assumed to be the predators. Predation on long-line sets was limited to a few sharks per line, and occasionally the attacking sharks became hooked. On 16 June 1975, for example, a male sevengill shark 190.5 cm TL was caught on a long-line <1.8 km southeast of Hunter's Point with the caudal fin and posterior 1/3 of the body of a male leopard shark hanging out of its mouth.

On other occasions, however, retrieved long-lines contained mostly bodiless heads. On 12 October 1975, a long-line was retrieved following a 1.5-h set approximately 4 km west of the San Leandro Marina in South San Francisco Bay in 6.7 m of water with leopard sharks on 34 consecutive hooks and only four whole bodies remaining, similar to Ebert (1991). The rest of the hooks had the heads of leopard sharks severed over the gill slits or just caudal of the first dorsal fin (Figure 2). Two juvenile sevengill sharks measuring 113

Figure 2.—Severed heads of three sharks, as removed from a long-line in South San Francisco Bay, California. The head at the left is that of a spiny dogfish shark, while the two heads at right are those of leopard sharks. Photo by author.

and 128 cm TL were caught on this same line with large portions of leopard sharks in their stomachs. On 28 June 1976, another long-line set 2 km off Hunter's Point was retrieved with 28 leopard sharks, 26 of which had been severed behind the head. A sevengill shark

measuring 185.4 cm TL became entangled in the line and hooks as it attempted to swallow and an entire leopard shark measuring 71.1 cm TL subsequently was removed from the sevengill shark's mouth and stomach.

On several occasions newborn and juvenile sevengill sharks were either captured in inshore nursery areas or observed preying on newborn or YOY leopard sharks. On 15 May 1980, for example, a female sevengill shark measuring 101.6 cm TL was caught inshore by rod-and-reel in 2.2 m of water among eelgrass, where we had been catching newborn and YOY leopard sharks, just west of the San Leandro Marina. This sevengill shark contained three newborn brown smoothhounds and a newborn leopard shark its stomach. On 18 August 1996, five young sevengill sharks (49–61 cm TL) were caught by rod-and-reel in 1.8 m of water approximately 0.5 km west of the entrance to the San Leandro Marina within a span of 35 minutes, suggesting that a loose group of those predators was patrolling the mudflats and eelgrass beds. On 30 July 2000, a YOY leopard shark (40 cm TL) was caught by rod-and-reel in less than 2 m of water 0.75 km west of the San Leandro Marina. As the leopard shark was brought to the surface, I noticed a churning action in the water within 10 cm of its tail, which continued all the way to the side of the boat. Once the leopard shark was within reach, I observed multiple bite attempts by a young sevengill shark that I had watched pursue the leopard shark for at least 5 m at the surface. Once alongside the boat, the sevengill shark was netted and brought aboard for examination, along with the small leopard shark. The sevengill shark measured 46 cm TL and still showed the natal scar between its pectoral fins which, combined with its length, indicated it was a newborn (Ebert 1989).

On 4 July 2001, a 112 cm TL sevengill shark was caught by rod-and-reel in 1.6 m of water at the entrance to San Leandro Bay (Arrowhead Marsh and Airport Channel, just east of the Oakland Airport) that had swallowed a juvenile male leopard shark (51 cm TL) whole. Similar observations were made involving both leopard sharks and brown smoothhounds (newborns, YOY, and juveniles) in inshore eelgrass beds or near the entrances to salt marshes along the eastern shoreline of South San Francisco Bay.

On 20 April 2000, I visited a Caspian tern (*Hydroprogne caspia*) nesting colony on Brooks Island Shoreline Preserve near the Richmond Harbor and observed a tern returning to the colony with a newborn leopard shark estimated to be 19 cm TL in its beak (Figure 3). Keith Larsen (Department of Fisheries and Wildlife, Oregon State University) and other researchers were conducting a study of the nesting and food habits of Caspian terns at Brooks Island and Eden Landing, just south of the east end of the San Mateo Bridge. Based on time away from the colony, researchers thought that terns were utilizing local mudflat and marsh environments for food (Roby et al. 2009). Larsen reported that on several occasions he witnessed Caspian terns returning to the breeding grounds of the island with what he thought were young leopard sharks. Based on 35 mm slides he showed me of terns with prey items in their beaks, I confirmed the identifications of both newborn leopard sharks and brown smoothhounds.

On 3 February 2009, Gail West (G. West, photographer, 6 April 2009, personal communication) observed a great blue heron (*Ardea herodias*) attacking a young leopard shark (Figure 4) at the shoreline near Damon Slough, San Leandro Bay. The attack occurred over a period of 18 minutes, with the heron jabbing the shark with its beak multiple times about the head and gill areas before swallowing it. The leopard shark appeared to be approximately 53 cm TL, as estimated by using the beak-length formula for herons (Bayer 1985) and several of West's photographs.

Figure 3.—A Caspian tern with a deceased newborn leopard shark in its beak. Photo courtesy of Bird Research Northwest.

Figure 4.—Frontal view of a great blue heron with a young-of-the-year leopard shark in its beak. Photo courtesy of G. West.

On 23 May 1979 while aboard a commercial shrimp trawler operated by Captain Tom Laine in the lower reaches of Alviso Slough, as well as the open waters just north of the entrance to the slough and south of Calaveras Point in the southernmost end of San Francisco Bay, I observed six consecutive trawls. Each trawl set caught 136–181 kg of bay shrimp (*Crangon* spp.) along with 40–60 newborn leopard sharks (17–21 cm TL), resulting in 328 newborns for the day's total bycatch; neither YOY leopard sharks nor newborns of other elasmobranch species were caught. The mass and weight of shrimps captured in each trawl likely contributed to the near 100% mortality of these newborns upon dumping the net. The few newborns still showing signs of life were relocated to laboratory aquaria the same day in aerated buckets of seawater, but all died within 24–36 h after capture.

Following the initial report of dead and dying sharks along the East Bay shoreline (Russo and Herald 1968) and the preliminary chemical findings of leopard sharks (Russo 1975), I continued to collect data on the annual kill of elasmobranchs until the mid-1980s. While the numbers of dead or distressed sharks on East Bay beaches never approached those observed in 1967 (1,000 specimens in one month at Alameda), a few to several dozen dead sharks and rays were often discovered on intertidal mudflats at low tide. For example, between 28 May and 5 September 1982, 80 sharks and rays were collected from a 1-km stretch of shoreline at Alameda's Robert Crown Memorial State Beach. Affected elasmobranchs included 33 (41.2%) brown smoothhounds, 29 (36.2%) leopard sharks, 11 (13.8%) bat rays, five (6.3%) spiny dogfish, and two (2.5%) sevengill sharks. Most of these sharks and rays were already dead when found on the beach, but five (6.3%) were still alive. These individuals were taken out into 1 m of water and released, but all returned to the beach within 15 minutes after release. Additionally, on 2 August 1982, I found 29 leopard sharks ranging in size from 50 to 119.4 cm TL ($\bar{x}$=104.3 cm) that had been shot or stabbed multiple times. All were found close together at low tide on the shoreline of Hayward.

While these results confirm earlier work identifying sevengill sharks as apex predators (Ebert 1986), new information is provided that shows sevengill sharks preying on leopard sharks and brown smoothhounds in their nursery areas. Although the predation of long-line caught sharks was generally limited to just a few individuals on some lines, the loss of 88–93% (30/34, 26/28) of long-line caught specimens during two sets suggests that aggregations of sevengill sharks, or "packs" as described by Ebert (1991), were involved in such predation events. Further research is needed to determine the extent to which newborn and YOY sevengill sharks enter smaller sloughs and channels to hunt for leopard sharks.

Quantitative results of research at Eden Landing near Hayward revealed that leopard shark newborns comprised 11.9% (*n*=72) of the total number of fish (*n*=604) brought in to the Caspian tern nesting colony there, but comprised 92.3% of the 78 sharks identified at this site; brown smoothhound newborns comprised 7.7% (Roby et al. 2003). In 2009, the number of leopard shark newborns taken by Caspian terns at this breeding colony comprised only 4.6% (*n*=81) of the totals number of fish taken (*n*=1,770), but accounted for 56.3% (*n*=81) of the total number of sharks taken (*n*=144) with brown smoothhound newborns comprising 34.0% (*n*=49) and unidentified shark newborns comprising 9.7% (*n*=14); I suspect these unidentified individuals were brown smoothhounds because, aside from leopard sharks, no other newborn elasmobranchs were ever captured during our study (Roby et al. 2009, Collis et al. 2012). It is possible that leopard shark newborns, being in shallow marsh channels and sloughs near nesting colonies, especially at low tide, are more vulnerable to Caspian tern predation than brown smoothhound newborns that tend to occupy eelgrass beds where

smoothhounds possibly benefit from their coloration and the protective cover of eelgrass blades and may be more difficult for terns to see.

The observation of a great blue heron capturing and eating a leopard shark YOY presents two new biological elements in the life history of leopard sharks: (1) great blue herons are predators of leopard shark newborn and YOY in their marsh shallows, the extent of which is unknown at this time; and (2) given the estimated size of the leopard shark (53 cm TL) captured at this event, newborn and YOY leopard sharks can remain in, or at least re-visit, the shallow sloughs and channels of salt marshes for several months following parturition, feeding on small invertebrates including worms, small shrimps and crabs, and marine pill bugs (Russo 1975). This observation raises questions concerning the extent of newborn and YOY loss to this predator, and whether or not other avian marsh predators such as great egrets (*Ardea alba*) or snowy egrets (*Egretta thula*) engage in similar predation.

Given a single day event involving 328 newborn leopard sharks caught in a commercial trawl, the overall impact of this kind of bycatch loss to recruitment for this species in the South Bay is largely unknown, as no attempts were made to analyze the annual catch loss due to the unknown number of commercial operators and the length of time this fishery has existed. While the South Bay commercial shrimp fishery appears to have ceased in recent years, a North Bay shrimp fishery near marshlands still exists (R. Bartling, California Department of Fish and Wildlife, personal communication, 1 February 2012); I suggest this fishery should be monitored carefully for impacts to leopard sharks.

The cause of dead, disabled, or disoriented sharks along the beaches and shorelines areas of San Francisco Bay remains a mystery and has resulted in considerable public concern with numerous articles continuing to appear in local newspapers. While some research on this problem involving domoic acid has been done (Schaffer et al. 2006), the cause remains unknown. Future investigations should consider the possible role of heavy metals, chlorinated hydrocarbons (Russo 1975), and rodenticides, as well as meningoencephalitis associated with *Carnobacterium* sp., which is known to cause similar symptoms (Schaffer et al. 2013; M. Okihiro, California Department of Fish and Wildlife, personal communication, 19 January 2015) in the stranding deaths of San Francisco Bay elasmobranchs and the disorientation and beaching of live leopard sharks and brown smoothhounds (Russo and Herald 1968).

Previous suggestions that nursery areas in shallow coastal waters provide critically important habitat, food, shelter, and opportunities to evade predators for several species of elasmobranchs (Springer 1967, Medved and Marshall 1981, Snelson et al. 1984, Talent 1985, Branstetter 1990) may be applicable to certain regions of the United States, but not to the West Coast. Newborns <55 cm TL may indeed escape predation in Gulf of Mexico and East Coast shark nurseries (McCandless et al. 2007), but leopard sharks and brown smoothhounds <55 cm TL in San Francisco Bay nurseries clearly are vulnerable to attack as evidenced by events described here.

The abundance of small prey items for newborn leopard sharks inhabiting marsh sloughs and channels, as well as near-shore eelgrass beds, may simply outweigh the risk of predation for this species. In view of the documented predation on newborn and YOY leopard sharks by avian predators in salt marshes, and by sevengill sharks in near-shore eelgrass beds, neither environment is predation-free as once presumed. The use of leopard shark and brown smoothhound nursery areas by sevengill sharks, Caspian terns, and great blue herons in capturing newborns, combined with take from the commercial shrimp fishery, as well as annual shark kills add several elements to the overall complex biology of all species mentioned and the ecology of marsh sloughs and channels. As reported here

for the first time, newborn and YOY leopard sharks are key elements in the diets of several predators, as well as being vulnerable to exploitation, and these factors must be considered in any management plan developed for this species.

Acknowledgments

I wish to express my appreciation to K. Collis and K. Larsen who provided me with photographs and summaries of their findings on Caspian terns, as well as all those who assisted me in field collections over the years, but especially K. Burger, P. Alexander, and my sons R. Russo Jr., K. Russo, and B. Russo, who were instrumental in all rod-and-reel captures between 1986 and 2001. I also thank S. Smith and D. Ebert for their review of the initial data included in this manuscript. And finally, I thank M. Okihiro for sharing his interests in the stranding of elasmobranchs and G. West for her photographs and detailed description of the heron event.

Literature Cited

Bayer, R. D. 1985. Bill length of herons and egrets as an estimator of prey size. Colonial Waterbirds 8:104-109.

Branstetter, S. 1990. Early life-history implications of selected carcharhinoid and lamnoid sharks of the northwest Atlantic. Pages 17-28 *in* H. L. Pratt, S. H. Gruber, and T. Taniuchi, editors. Elasmobranchs as living resources: advances in the biology, ecology, systematics and the status of the fisheries. U.S. Department of Commerce, National Oceanic and Atmospheric Administration Technical Report NMFS 90.

Collis, K., D. D. Roby, K. W. Larson, L. J. Adrean, S. K. Nelson, A. F. Evans, N. Hostetter, D. Battaglia, D. E. Lyons, T. Marcella, and A. Patterson. 2012. Trends in Caspian tern nesting and diet in San Francisco Bay: conservation implications for terns and salmonids. Waterbirds 35:25-34.

Conomos, T. J., R. E. Smith, and J. W. Gartner. 1985. Environmental setting of San Francisco Bay. Hydrobiologia 129:1-12.

Ebert, D. A. 1986. Aspects on the biology of hexanchid sharks along the California coast. Pages 437-449 *in* T. Uyeno, R. Arai, T. Taniuchi, and K. Matsuura, editors. Indo-Pacific fish biology: proceedings of the second international conference on Indo-Pacific fishes, Tokyo, July–August 1985. Ichthyological Society of Japan, Tokyo, Japan.

Ebert, D. A. 1989. Life history of the sevengill shark, *Notorynchus cepedianus* Peron 1807, in two northern California bays. California Fish and Game 75:102-112.

Ebert, D. A. 1991. Observations on the predatory behaviour of the sevengill shark, *Notorynchus cepedianus*. South African Journal of Marine Science 11:455-465.

Ebert, D. A., and T. B. Ebert. 2005. Reproduction, diet, and habitat use of leopard sharks, *Triakis semifasciata* (Girard), in Humboldt Bay, California, USA. Marine and Freshwater Research 56:1089-1098.

McCandless, C. T., N. E. Kohler, and H. L. Pratt Jr. (editors). 2007. Shark nursery grounds of the Gulf of Mexico and the East Coast waters of the United States. American Fisheries Society, Bethesda, Maryland, USA.

Medved, R. J., and J. A. Marshall. 1981. Feeding behavior and biology of young sandbar sharks, *Carcharhinus plumbeus* (Pices: Carcharhinidae), in Chincoteague Bay, Virginia. Fishery Bulletin 79:441-447.

Roby, D. D., K. Collis, S. K. Nelson, K. Larson, C. Couch, and P. J. Klavon. 2003. Caspian tern nesting ecology and diet in San Francisco Bay and interior Oregon. Final 2003 Annual Report to the U.S. Fish and Wildlife Service. Oregon State University, Corvallis, USA.

Roby, D. D., K. Collis, L. J. Adrean, D. S. Battaglia, D. E. Lyons, S. K. Nelson, A. Patterson, C. Spiegel, Y. Suzuki, and C. Wolf. 2009. Caspian tern nesting ecology and diet in San Francisco Bay and interior Oregon. Final 2009 Annual Report to the U.S. Fish and Wildlife Service. Oregon State University, Corvallis, USA.

Russo, R. A. 1975. Observations on the food habits of leopard sharks, *Triakis semifasciata*, and brown smoothhounds, *Mustelus henlei*. California Fish and Game 61:68-81.

Russo, R. A. 2013. Observations on the ectoparasites of elasmobranchs in San Francisco Bay, California. California Fish and Game 99:233-236.

Russo, R. A., and E. S. Herald. 1968. The 1967 shark kill in San Francisco Bay. California Fish and Game 54:215-216.

Schaffer, P., C. Reeves, D. R. Casper, and C. R. Davis. 2006. Absence of neurotoxic effects in leopard sharks, *Triakis semifasciata*, following domoic acid exposure. Toxicon 47:747-752.

Schaffer, P. A., B. Lifland, S. Van Sommeran, D. R. Casper, and C. R. Davis. 2013. Meningoencephalitis associated with *Carnobacterium maltaromaticum*-like bacteria in stranded juvenile salmon sharks, *Lamna ditropis*. Veterinary Pathology 50:412-7.

Snelson, F. F. Jr., T. J. Mulligan, and S. E. Williams. 1984. Food habits, occurrence,and population structure of the bull shark, *Carcharhinus leucas,* in Florida coastal lagoons. Bulletin of Marine Science 34:71-80.

Springer, S. 1967. Social organization of shark populations. Pages 149-174 *in* P. Gilbert, R. F. Mathewson, and D. P. Rall, editors. Sharks, skates, and rays. Johns Hopkins University Press, Baltimore, Maryland, USA.

Talent, L. G. 1985. The occurrence, seasonal distribution, and reproductive condition of elasmobranch fishes in Elkhorn Slough, California. California Fish and Game 71:210-219.

Received 8 August 2014
Accepted 11 February 2015
Associate Editor was N. Kogut

Kimberly A. Nicol, 1956–2015

Long time Department family member and Regional Manager, Kim Nicol passed away on 20 January 2015 following an extended and courageous battle with cancer. Kim worked in Region 6 for more than 25 years, and became the manager of the Inland Deserts Region in July 2010. In that capacity, Kim employed a firm leadership style that quickly earned her the respect and admiration of many Regional Staff and Department Administrators.

Kim was born and raised in Richmond, California and graduated from Richmond High School. Following graduation from California State University, Sacramento, she began her life-long career with the California Department of Fish and Game (later to become the California Department of Fish and Wildlife) as a fishery biologist. She also had special interests and expertise in the biology and conservation of amphibians and reptiles; she put that interest to good use, and became a respected authority and advocate for reptiles, placing a strong emphasis on the conservation of desert tortoise. As a biologist, she also contributed in many meaningful ways to the conservation of desert fishes, especially the desert pupfish and Mojave chub.

As her career progressed, Kim worked upward through the biologist and environmental scientist series, gaining insight and experience that provided her the background to effectively handle the many aspects of regional management. Kim also served as Acting Branch Chief for the Habitat Conservation Planning Branch for an interim period, further demonstrating her commitment to the Department and its critically important mission. As Regional Manager, Kim's demonstrated leadership and her commitment to, and understanding of, the biological resources in the mountains and deserts of southeastern California were valuable contributions to the conservation and management of California's fish and wildlife, albeit in a politically challenging and ecologically diverse part of the state. Kim was also fun to be around, and evenings filled with good food and wine will be remembered by those of us fortunate enough to have worked closely with her for many years.

Kim actively participated in an effort to re-structure the Department's employee recognition program to better reflect the core values of the Department. She worked closely with numerous others to create the current Employee Excellence Awards Program, which embodies the commitments and values exemplified by Kim and many other Department employees. In recognition of her dedication, actions, and commitment, the California Department of Fish and Wildlife Employee Excellence in Leadership Award will be re-named in her honor.—*Friends and Colleagues of Kimberly A. Nicol*

California Fish and Game 101(2):159-160; 2015

Book Review

Becoming a compelling communicator for conservation: the essential reference for everyone who desires to make a difference.

William C. Dunn. 2014. On-Demand Publishing, Scotts Valley, California, USA. 69 pages (Soft cover). $9.95. ISBN 14948858832

> *"...Grandpa was a man of few words. He had a way of not wanting to say any more than he thought would be heard..."*
>
> Kate Wolf, 1981
> *Eyes of a Painter*

Bill Dunn, a friend and colleague for more than three decades, claims that the inspiration for this book originated during the first national meeting of The Wildlife Society in Albuquerque, New Mexico, back in 1994. Indeed, we sat through 45 minutes of a mostly droll and boring presentation one evening, following which we questioned the speaker's ability to convey a meaningful message, and wondered why we had wasted our time listening to him.

Although this book came to fruition many years later, it is the result of continuing frustrations with speakers and writers that "waste so much time" trying to get their messages across. As Kate Wolf implied long before that meeting in Albuquerque, saying more than will be heard wastes time for many people, including the speaker (or author) and audience (or readership). It is my hope that the guidance and admonitions included in this short book will result in vastly improved oral presentations in the future. As an editor, I also hope that authors will take advantage of Bill's suggestions, and present only necessary information and not include every possible detail in their contributions.

This little book consists of six chapters, each of which is devoted to enhancing one or more aspects of communications skills and making the speaker (or writer) more effective at delivering his or her message. As noted in the introduction, the book is not an exhaustive treatise but, instead, is a concise compilation of the essential lessons of communication, and written with the intent that the end result would be an enhanced ability to accomplish conservation. Chapter 2, Laying the Foundation, emphasizes the need to ensure that the message is clear, concise, and complete. In this chapter, Bill emphasizes that it is imperative that individuals be well prepared to deliver a message; if nothing else, he notes that audiences will appreciate the fact that you, as a speaker, value their time!

Chapter 3, The Written Word, contains sage advice that will help an author organize, analyze, and present scientific data with clarity and directness. Bill believes that students of natural science should prepare all papers assigned in their core courses using the framework of peer-reviewed articles, with the intended result that scientific writing would be second nature by the time those students become professionals. During graduate school, I received similar advice from the professor under whom I studied ornithology, advanced ornithology, and field research methods: write every paper you produce, whether a class assignment or

an original piece reporting results of your research, as if it is going to be submitted for peer review. That advice has served me well, and the advice conveyed by Bill in Chapter 3 will serve the readership well.

In Chapter 4, The Spoken Word, Dr. Dunn emphasizes that oral presentations must be informative, creative, compelling, and thought provoking. In this chapter, he addresses ways to structure an effective presentation, create clear and informative visual graphics, and how to speak so the message will be well received.

In Chapter 5, Special Situations, recommendations for addressing decision makers, and others for addressing public audiences are provided. The message delivered is that being succinct with the written word is the most productive strategy: it is better that an administrator asks for additional information than for less. With respect to public meetings, strong emphasis is placed on time management, completeness, and follow-up. The advice included in this chapter will make the communicator more effective, even if the subject at hand is not appreciated by the audience.

In the final chapter, Dr. Dunn concludes that the speaker or author has control over how the message is presented, and that doing it well at every opportunity—whether spoken or written—will benefit conservation. He ends with the hope that the lessons included will help increase support for nature and move conservation forward. I suspect that he is correct; I encourage those that have not given much thought to what they present and how they go about doing so to take advantage of Bill's more than thirty years of experience, and the advice presented. I wish this book had been written years ago!

Vernon C. Bleich, *California Department of Fish and Wildlife, Office of Communications, Education and Outreach, 1416 9th Street, Sacramento, CA 95814, USA*

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 a 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).

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).

Sjaastad, E., and K. E. Svensson. 2015. Small ambassadeurs: the legendary light-line fishing reels. Schiffer Publishing, Atlen, Pennsylvania, USA. 256 pages $45.00 (hard cover).

Releasing salmon into the San Joaquin River. DWR photo by John Chacon

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.

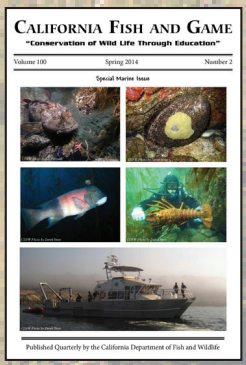
Authors of manuscripts that are accepted for publication will be invoiced for charges at the rate of $50 per printed page at the time page proofs are distributed. Authors should state acceptance of page charges in their submittal letters. The corresponding author will receive a PDF file of his or her 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.

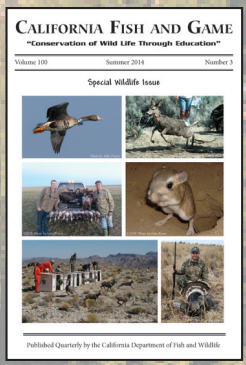
Literature Cited

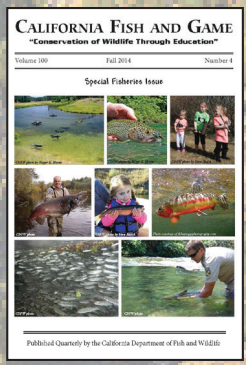
Bleich, V. C., N. J. Kogut, and D. Hamilton. 2011. Information for contributors to *California Fish and Game*. California Fish and Game 97:47-57.

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.

Celebrating 100 Years!







CALIFORNIA FISH AND GAME, CALIFORNIA'S LONGEST-RUNNING, CONTINUOUSLY PUBLISHED PROFESSIONAL JOURNAL, BEGAN PUBLICATION IN 1914 AND VOLUME 100 HAS JUST BEEN COMPLETED.

Each of the special issues comprising Volume 100 is dedicated to a specific topic: Native Plants, Marine Biology, Wildlife and Freshwater Fisheries. Hard copies of each of these collectable special issues can be ordered at the journal's website, *dfg.ca.gov/publications/journal/contents.html* for a small fee per issue until Aug. 15, 2015. After that the hard copies may no longer be available. Electronic versions of each of the special issues can also be downloaded from the same web address at no charge.

We invite interested parties to help celebrate 100 years of history by purchasing hard copies of these special issues.