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Notes From the Editor

I am happy that our second issue of 2020 is continuing the trend of increased number of articles that we began with issue one of this year. We have 6 great articles in this issue encompassing a variety of topics: endangered California condors, mountain lions, mule deer, and freshwater, marine, and anadromous fish.

[Special Note: as this issue goes to print, California, our country, and the world have been overwhelmed by something unprecedented in most of our lifetimes—the outbreak of the novel coronavirus COVID-19. I want to thank all of my editors and the authors for this issue for working hard and efficiently from home to get the issue completed. I would also like to especially thank my layout editor, Lorna Bernard, for her hard work to get this issue out on time—it would not have happened without all of the effort she put into it.]

We had two great guest editors for this issue to take on the marine article. Armand Barilotti started with the Department in September 2019. He obtained his bachelor's degree in marine biology from UC Santa Cruz in 2008, and his master's degree in biology from California State University, Long Beach (CSULB) in 2016. For his master's, he studied the movements of white croaker and California halibut in the Ports of Los Angeles and Long Beach and examined their use of pier habitat. While in school, he assisted the juvenile white shark tagging project at the CSULB Shark Lab and was fortunate enough to swim in the water with and tag over a half dozen sharks. Armand has had a diverse career prior to joining the Department, including working as a scientist helping restore kelp forests and abalone populations, a subtidal technician conducting SCUBA surveys of the kelp forest marine life, manager of an abalone farm, collector of marine life for public aquariums and research institutions, and a deckhand/cook aboard a sportfishing vessel out of San Diego. And Heather Gliniak, an Environmental Scientist in our Los Alamitos regional office, attended CSULB as a presidential scholar, and graduated with a bachelor's degree in marine biology and a minor in chemistry. Heather briefly worked as a chemist at CRG Marine Laboratories before being hired by the Department in late 2006 as a marine biologist on the Fisheries Independent/SCUBA Assessment Project. In early 2007, Heather became a Department diver and loves to get in the water any chance she gets. Presently, she is involved with monitoring and conducting research on populations of nearshore finfish species in southern California, such as Barred Sand Bass, Kelp Bass, and California Halibut. Heather has also served for several years on the southern California district board of AIFRB and is currently acting as Vice Director.

A reminder that many of the Journal's Special Issues will be coming out this year! We have explored the impacts of fire, cannabis, and human recreation on fish and wildlife resources. Look for those in spring and summer of this year; the recreation issue should be out within weeks of this one. Additionally, we are now accepting submissions for two new special issues—one on the California Endangered Species Act (CESA) and one on Human-Wildlife Interactions. If you would like to find out more about our Special Issues, please see our webpage: https://www.wildlife.ca.gov/Publications/Journal/Special-Issues

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Editor-in-Chief
California Fish and Wildlife Journal

California Fish and Wildlife 106(2):99-138; 2020

Comparative Analyses of Upstream Migration in a Multispecies Assemblage of Fish in Response to Highly Managed Flow Regimes

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Few, if any, studies have distinguished among anthropogenic factors (extrinsic drivers) acting on populations of sympatric species of fish within a single watershed or riverine system. Yet adaptive management requires knowledge of whether co-existing taxa with different life-histories are influenced by similar extrinsic as well as intrinsic factors to determine whether they vary in their population dynamics, hence conservation needs. Using data from weir and hatchery hard-counts, we evaluated the effects of anthropogenic-induced altered flow regimes in relation to annual and seasonal patterns of local migration and run-timing in a multispecies assemblage of adult fish inhabiting a large highly managed riverine system. Here, we test the hypothesis that annually managed flows (hydrographs) have altered the migration patterns and run-time schedules in a sympatric assemblage of six taxa composed of both anadromous and non-anadromous species inhabiting the upper Trinity River, California. Results of our analyses provide evidence to support our hypothesis that highly managed flow regimes implemented since 2003 have altered local migration and run-time schedules in a significant and concordant way among all species examined relative to species-specific baseline post-dam flow-type patterns. Whereas counts of all species fluctuated considerably on an annual basis, counts of all taxa decreased strongly and significantly from 2003 to 2018. This decrease coincided with establishment of the Trinity River Restoration Program in 2002 and subsequent highly managed hydrographs in combination with periodic pulse flow augmentations beginning in 2003, irrespective of two periods of three consecutive years of regional drought. We hypothesize that altered annual and seasonal patterns of local migration and run-timing in a suite of taxonomically and ecologically differentiated species with highly divergent life history strategies owing to local adaptation are likely a function of altered extrinsically-driven flow regimes on fluvial ecosystem processes and the fisheries resources they support. Our study suggests that further investigations into the effects of flow management on migration and life history requirements in sympatric multispecies assemblages of non-anadromous and anadromous species of fish inhabiting the upper Trinity River are warranted and necessary for both hatchery- and natural-origin spawning fish, particularly if highly managed flow regimes continue indefinitely.

Key words: anadromous, baseline, fish, flow-type, managed flows, migration patterns, non-anadromous, pulse flows, river

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Riverine flow regimes are key drivers of lotic ecosystem structure and function (Bunn and Arthington 2002; King et al 2003; Kennard et al. 2007). In multispecies assemblages of non-anadromous and anadromous species of fish, temporal variation in hydrological events are also a significant driver of fish abundance, population dynamics, and patterns of migration (Biggs et al. 2005; McManamay et al. 2013; Walton et al. 2016). Such variability may severely constrain estimates of population size and interpretations of the effects of altered flows on seasonal patterns and timing of migration, habitat use, and rates of survival in resident taxa (Crisp 1993; Cunjak et al. 1998; Haxton et al. 2010). Additionally, the biotic integrity of freshwater fish assemblages generally decreases with increased anthropogenic alteration of natural flow patterns, which has contributed significantly to a decrease in both quality and quantity of salmonid spawning habitat (Poff et al. 1997; Poff and Zimmerman 2010). However, whereas numerous qualitative relationships have alluded to indirect benefits of extreme flow events, few such relationships are sufficiently well quantified or long-term for use in adaptive management (Naiman et al. 2008; Jager 2014; Quiñones et al. 2014; Brail et al. 2018). Moreover, assessments of the effects of flows have traditionally focused on discharge impacts over short time intervals (< 1 year), thus minimizing insight in anticipating longer-term effects (Holčík 1996; Walton et al. 2016). Indeed, Jager (2014) maintained that externally derived flow targets implemented without regard to specific mediating factors likely are suboptimal for resident fish.

Because flows in unregulated rivers in the western United States vary naturally in response to spring runoff from storms that historically occur relatively consistently during certain times of the year, populations of resident fish have generally evolved life history traits and habitat preferences adapted to natural pulsed-flow events (Rytwinski et al. 2017). Yet, pulse flows associated with highly managed flow regimes that occur outside the natural cycle can be problematic, as life history composition of fish assemblages historically adapted to regional habitat templates may be significantly altered downstream of dams in only a few decades owing to modifications in the timing of streamflow (Konrad et al. 2011; Mims and Olden 2013). Use of pulse flows to mimic natural hydrologic processes, mobilize sediments, habitat creation, or to elicit migration and spawning of fish is a common management strategy in highly regulated riverscapes (Peterson et al. 2017). Furthermore, seasonal variability in relative abundance and timing of migration of fish associated with annually managed flows allied with riverine restoration programs can be considerable (Platts and Nelson 1988; Holtby and Scrivener 1989; Bradford et al. 1997; Ham and Pearsons 2000; Bayley 2002). In California pulse flows were used in the Trinity River to minimize risk and spread of disease among adult upstream migrating Chinook Salmon (*Oncorhynchus tshawytscha*) in lower reaches of the Klamath River (Strange 2007), and to create juvenile rearing habitats for salmonids in the upper Trinity River (Quiñones et al. 2014; Beechie et al. 2015). Elsewhere, flow regulation in tributaries of the Sacramento River resulted in delayed spawning and smolt migration, which contributed to declines in salmon populations (Keith et al. 2008).

Use of flow management, especially if the timing of which deviates from a region's historical natural hydrograph, requires understanding of the mechanisms by which altered flow regimes influence fish migration (Hasler et al. 2014; Jager 2014), particularly in river systems inhabited by multispecies assemblages of anadromous salmonids. This need is a prerequisite to enabling placement of a broad suite of covariate non-flow factors into perspective, which is particularly relevant to flow management related to riverine restoration actions, as it enables resource managers to better understand and evaluate their methodology (Baril et al. 2018). Further, knowledge of native fish movements and out-migrant survival through flow management in large river systems is especially important in management and listing status of anadromous salmonids given reduction in native stocks in northern California that have experienced declines due to environmental and anthropogenic factors regionally and elsewhere (Sommer et al. 2014; Adams et al. 2017; NOAA 2018).

As such, the objectives of our study were threefold. First, we assess annual fluctuations in relative abundance in a diverse assemblage of resident non-anadromous and anadromous species of fish, and evaluate temporal fluctuations in patterns of local migration and run-timing characteristic of a highly anthropogenic flow-regulated riverine system to identify any concordant patterns among taxa as a potential function of altered streamflow. Second, we evaluate the relationship between annual and seasonal fluctuations in relative abundance of fish in relation to variation in average daily water temperature and flow volume. Third, using results of our analyses, we test the research hypothesis (H_1) that annually managed flow regimes (hydrographs) in a highly regulated river system implemented beginning in 2003 have altered the post-dam baseline pattern of local migration and run-timing in counts of both non-anadromous and anadromous species of fish inhabiting the upper Trinity River, relative to their species-specific "historical" post-dam migration patterns.

METHODS

Study area

Trinity River is in northwestern California and is the largest tributary of the Klamath River system (Figure 1). Construction of Trinity and Lewiston dams occurred in the early 1960s. Trinity Dam creates Trinity Lake, storing up to 3,022 m^3 of water. Lewiston Lake, formed by Lewiston Dam, is located 11.8 km downstream of Trinity Dam at river kilometer (rkm) 179.8, which serves as a re-regulating reservoir for flow to the Trinity River and diversion to the Sacramento River Basin, comprising the Trinity River Division of the Central Valley Project. Lewiston Dam is the uppermost limit of anadromous fisheries on the Trinity River. From Lewiston Dam, the Trinity River flows for approximately 180 kilometers before joining the Klamath River at the township of Weitchpec, California. The Klamath River flows for an additional 70 rkm before entering the Pacific Ocean near Klamath Glen. The upper Trinity River (mainstem) is the stretch from the confluence of the North Fork Trinity River to 63.1 km up stream to Lewiston Dam. Trinity River Hatchery (henceforth called "hatchery") is located immediately below Lewiston Dam and the Junction City Weir (henceforth called "weir") is located 135.8 rkm downstream from Lewiston Dam and the

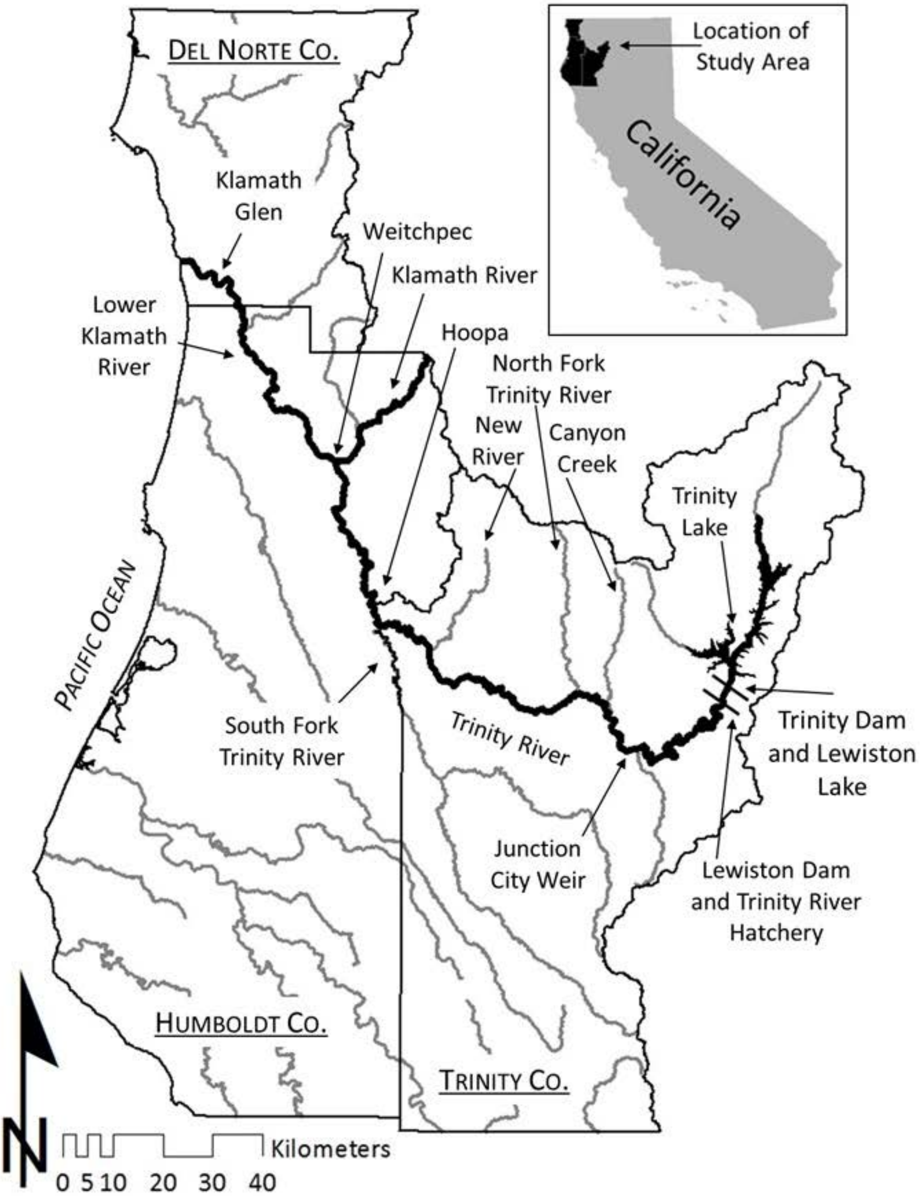

Figure 1. Map of the Klamath River, Trinity River, and other landmarks discussed in the text, encompassing the entire ranges of all species discussed herein. Area colored black in inset map is the study area pictured in main figure. Major rivers and streams that exceed 34 kilometers in length are colored gray.

hatchery. The upper 63.1 rkm of the Trinity River or "mainstem" ends at the confluence of the North Fork Trinity River and the Trinity River. This section of the river is the primary focus of restoration efforts by the Trinity River Restoration Program (TRRP).

Managed flows

The TRRP, created by the Record of Decision (henceforth called "ROD"; USBR 2000), outlined a plan for restoration of the mainstem of the upper Trinity River and its fish and wildlife populations (TRFES 1999). The Trinity River Mainstem Fishery Restoration Environmental Impact Statement was the basis for the ROD. The TRRP strategy for restoration included 1) flow management through manipulation of the annual hydrograph, 2) mechanical channel rehabilitation, 3) sediment management, 4) watershed restoration, 5) infrastructure improvements, 6) adaptive environmental assessment and monitoring, and 7) environmental compliance and mitigation. Since 2001, total restoration releases have included flows for 1) restoration, 2) Tribal Ceremonial Boat Dances, and 3) late summer pulse flows (Table 1, Figure 2). Ceremonial Tribal Boat Dance flows occur only in odd years in ROD flows and just prior to any pulsed flow augmentation in Pulse flow years. They are illustrated in each hydrograph and amount to < 0.6% of the total release into the Trinity River (Figure 2b and 2c; TRRP 2019). They are included herein as Pulse flows tier off the trailing ends of Ceremonial Boat Dance flow hydrographs when the latter occur.

Table 1. Attributes of ascending and descending limbs of hydrographs that characterized baseline PreROD, ROD, and Pulse flow-types for years 1995 to 2017. Rate of flow measured in cubic meters per second (m3/s) and flow release in hectare meters. For each hydrograph, a bench indicated a temporary holding steady of flow release volume and flattening of the hydrograph for at least one day. Rapidness indicated a steep and immediate increase or decrease in rate of flow, relative to a more prolonged or gradual increase or decrease in rate of flow. Abbreviations: NA = no data, shape of the ascending and descending limbs of the hydrographs: R = rapid, G = gradual, B = number of benches, and 2P = double peak.

Year	Water year-type	Low release magnitude (m^3/s)	Peak release magnitude (m^3/s)	Restoration release (hectare m)	Low release magnitude (m^3/s)	Date and duration to base-flow	Total days
Pre-ROD Flow (1995 - 2002)							
1995	NA	14	131	NA	27	25 Apr-22 May	28
1996	NA	9	144	NA	14	10 May-9 Jun	31
1997	NA	10	62	NA	13	2 May-2 Jul	62
1998	NA	47	192	NA	13	24 May-27 Jul	65
1999	NA	15	71	NA	13	8 May-18 Jul	72
2000	NA	9	66	NA	13	8 May-27 Jul	81
2002	normal	9	171	59540	13	27 Apr-25 Jun	28
Average		**16.1**	**119.6**	**NA**	**15.1**		**52.4**
Minimum		**9**	**62**	**NA**	**13**		**28**

Table 1. continued.

Year	Water year-type	Low release magnitude (m^3/s)	Peak release magnitude (m^3/s)	Restoration release (hectare m)	Low release magnitude (m^3/s)	Date and duration to base-flow	Total days
Maximum		**47**	**192**	**NA**	**27**		**81**
ROD Flow (2005 - 2011, 2017)							
2005	wet	8	197	79880	13	27 Apr-22 Jul	87
2006	extra wet	8	286	99900	13	16 Apr-22 Jul	98
2007	dry	8	135	55963	13	25 Apr-25 Jun	62
2008	dry	9	183	80016	20	22 Apr-15 Jul	85
2009	dry	8	125	54952	12	24 Apr-6 Jul	74
2010	wet	9	194	81003	12	22 Apr-2 Aug	102
2011	wet	7	329	89033	13	26 Apr-1 Aug	98
2017	extra wet	9	326	101536	13	22 Apr-11 Aug	112
Average		**8.3**	**221.9**	**80285.4**	**13.6**		**89.8**
Minimum		**7**	**125**	**54952**	**12**		**62**
Maximum		**9**	**329**	**101536**	**20**		**112**
Pulse Flow (ROD segment): 2003, 2004, 2012 - 2016)							
2003	wet	9	74	55272	12	29 Apr-22 Jul	85
2004	wet	9	176	80300	12	4 May-22 Jul	80
2012	normal	9	172	79817	13	4 Apr-26 Jul	114
2013	dry	8	125	55741	13	13 Apr-25 Jun	74
2014	critically dry	9	97	45701	13	21 Apr-26 Jun	67
2015	dry	9	241	55593	13	21 Apr-1 Jul	72
2016	wet	9	283	87429	13	20 Apr-2 Aug	105
Average		8.9	166.8	65693.3	12.7		85.3
Minimum		8	74	45701	12		67
Maximum		9	283	87429	13		114
Pulse Flow (Pulsed augmentation segment: 2003, 2004, 2012 - 2016)							
2003	wet	13	51	4194	13	23 Aug-18 Sep	27
2004	wet	16	485	4465	14	21 Aug-14 Sep	25
2012	normal	13	39	4811	13	12 Aug-20 Sep	40
2013	dry	13	74	2294	13	24 Aug-20 Sep	28
2014	critically dry	12	97	7993	13	15 Sep-25 Sep	11
2015	dry	19	83	5908	13	20 Aug-21 Sep	31
2016	wet	14	35	4835	13	24 Aug-28 Sep	36
Average		**14.3**	**123.4**	**4928.6**	**13.1**		**28.3**
Minimum		**12**	**35**	**2294**	**13**		**11**
Maximum		**19**	**485**	**7993**	**14**		**40**

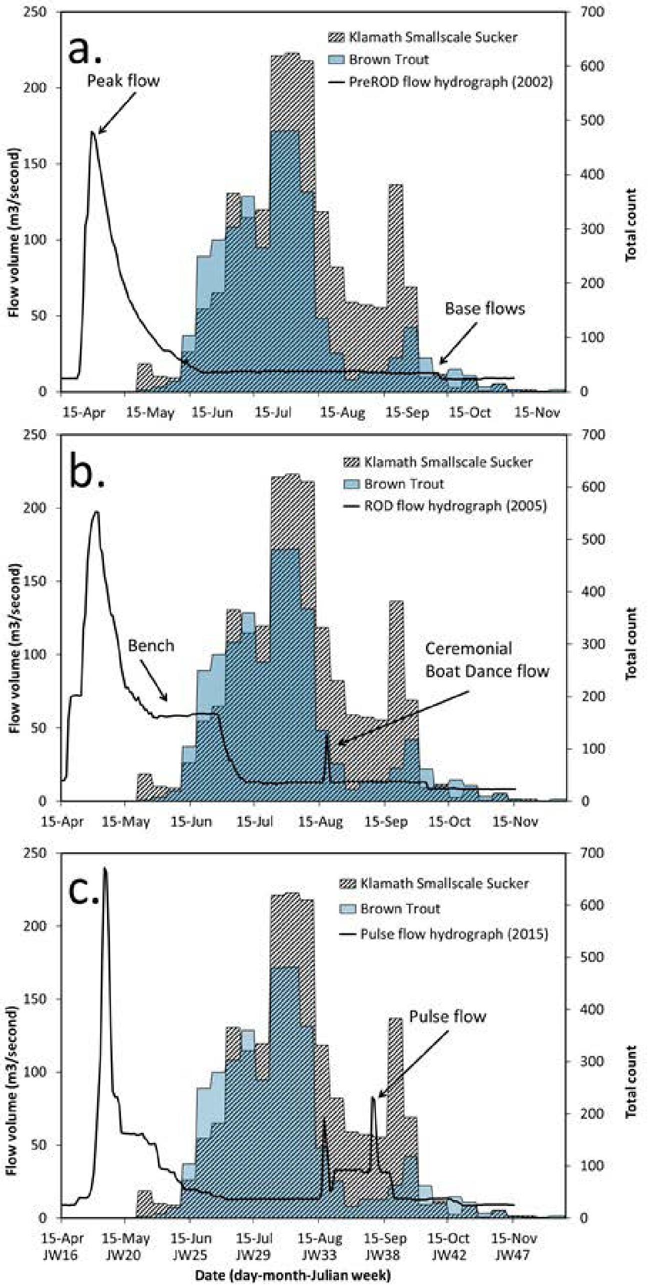

Figure 2. Examples of hydrographs representative of the three flow type superimposed onto the historical migration pattern of Brown Trout (1982-2017) and Klamath Smallscale Suckers (1993-2017); a) PreROD flow (2002), b) ROD flow (2005), and c) Pulse flow (2015) and its companion late summer pulsed augmentation flow. Tribal Ceremonial Boat Dance flows occur in odd years. Approximate Julian week (JW) superimposed below dates on x-axis.

To date, shapes of the ascending limbs of ROD flow hydrographs were mostly rapid, with few years in which there were benches all of which were associated with managed flows (Table 1). In contrast, shapes of the descending limbs of ROD flow hydrographs were generally gradual with numerous "benches" associated with virtually all managed flows. We designated benches in these hydrographs as indicating stabilization of water release for one or more consecutive days. There were two double peaked ROD flows (2016 and 2017). All Pulse flows had rapid ascending hydrographs and at least one bench. Similarly, all descending limbs of Pulse flows were rapid with at least one bench. Spring and summer base flow releases historically equate to 13 m^3/second. ROD flows generally occurred from late April to August, whereas conjoining Pulse flows mostly occurred from August to September (Figure 2). For the upper Trinity River, the actual timing, magnitude, and duration of each ROD flow and Pulse flow varied annually in hydrologic characteristics, cubic meters per second (m^3/s), and shape and duration of the hydrograph depending upon the specific intent of varied management actions. Average duration of ROD flows approximated 89.8 days (range 62.0 - 112.0 days) from mid-April to early August and averaged approximately 221.9 m^3/s (range 124.9 - 328.6 m^3/s) of flow at the top end of the hydrograph. Average duration of Pulse flows approximated 28.3 days (range 11.0 - 40.0 days) from mid-August to late September and averaged approximately 61.1 m^3/s (range 35.3 - 97.0 m^3/s) of flow at the top end of the hydrograph. For the same general monthly period, average duration of baseline PreROD flows approximated 52.4 days (range 28.0 - 81.0 days) from late April to late July and averaged approximately 119.6 m^3/s (range 62.3 - 192.3 m^3/s) of flow at the top of the hydrograph. Water summary data and typical flow release diagrams (hydrographs) teared to water-year type are available at the TRRP website (TRRP 2019).

Study design and sampling

To test H_1, we designated three annual flow groups (henceforth called "flow-types") 1) "baseline" PreROD flows (1982 - 2002), 2) ROD flows (2005 - 2011, 2017), and 3) Pulse flows (2003, 2004, 2012 - 2016, 2018) illustrated in Figure 2a, 2b and 2c, respectively). Late summer pulsed flows were intended to cue up-river seasonal migration of Chinook Salmon out of the lower Klamath River to reduce risk of the epizootic of the ciliate parasite *Ichthyophthirius multifiliis*, even though no quantitative studies post-2003 have definitively confirmed that augmentation by cold-water flows from the upper Trinity River have actually prevented another fish die-off in the lower Klamath River. Prior to 2003, there were no annually managed ROD or Pulse flows. Importantly, we note that each Pulse flow event was accompanied by a single ROD flow hydrograph (ROD flow plus Pulse flow), beginning in 2003. Thus, for each Pulse flow, effects of each pulsed augmentation are not completely separable or independent from effects of its companion pre-pulse ROD flow segment.

Data and samples analyzed herein derive from two sources. First, we obtained all counts of anadromous co-occurring "wild" and hatchery stocks of native Coho Salmon (*Oncorhynchus kisutch*), spring- and fall-run Chinook Salmon, and anadromous Rainbow Trout (*Oncorhynchus mykiss*) (henceforth called "steelhead") from adult (> 32 cm fork length) returns to the hatchery. Second, we obtained daily trap-counts of adult non-anadromous Brown Trout (*Salmo trutta*) and Klamath Smallscale Suckers (*Catostomus rimiculus*) from the weir because both species do not enter the hatchery, are not "focal management" taxa, and are only encountered and counted at the weir. Adult Brown Trout and Klamath Smallscale Suckers (> 32 cm fork length) are considered "by-catch" at the weir, as target species are anadromous salmonids, specifically spring-run Chinook Salmon. Sampling effort for all species based on size constraints at the weir has historically been relatively consistent from 1996 to 2018 (average = 55.1 trap days), except when weir operations are temporarily halted during ROD and Pulse flow years (beginning in 2003) until flows have subsided sufficiently to reinstate the weir (average trap-days post-2003 = 50.9). We provide detailed operation and efficiency estimates of the weir designed to assess only upriver movement of salmonids to the hatchery elsewhere (Sullivan and Hileman 2018).

Brown Trout and Klamath Smallscale Suckers in the upper Trinity River represent non-anadromous populations (Sullivan and Hileman 2018). Brown Trout are a piscivorous non-native species found in the Klamath Basin, introduced into tributaries in the lower Trinity River in 1893 to promote recreational angling (Dill and Cordone 1997; Sullivan and Hileman 2018). As such, this species has coexisted in sympatry with native anadromous salmonids in the Trinity River for over a century. Similarly, the Klamath Smallscale Sucker is native to the Trinity River. It is the most genetically unique phylogenetically and the least widely distributed geographically of all other species of suckers in the Klamath River Basin (Moyle 2002; Tranah and May 2006). Although both species display migratory behavior associated with availability of water and food, larval and juvenile development, water temperature, and spawning (Hohler 1981; Desjardins and Markle 2002; Hampton 2006; Pirrello 2011), information on extrinsic environmental factors that influence the pattern and timing of migration in response to altered flow regimes in both species of resident freshwater fish is relatively unknown.

Non-anadromous populations of Brown Trout and Klamath Smallscale Suckers in the Trinity River have acclimated or adapted to the Trinity River system for many years in sympatry with native anadromous species. Importantly, Brown Trout and Klamath Smallscale Sucker annual abundance, seasonal pattern and timing of migration, and response to changes in flow patterns derived from highly managed flow regimes involving pulsed augmentation flows are independent of any oceanic or marine influence. This condition is unlike anadromous species of co-occurring “wild” and hatchery stocks of native Chinook Salmon, Coho Salmon, and steelhead. Thus, for comparison with anadromous species we view Brown Trout and Klamath Smallscale Suckers as excellent “control” species for evaluating potential effects of managed hydrological variation within the upper Trinity River, which may provide insight into fisheries management of large, highly regulated riverine systems regionally or elsewhere. Importantly, that non-native brown trout are known to be piscivorous on juvenile salmonids is irrelevant to our assertion that they provide a valid “control” species, as steelhead (Naman 2008) and numerous terrestrial vertebrate species also readily consume both hatchery-produced and wild juvenile salmonids in the Trinity River (Sullivan and Hileman 2018). Yet no study has provided evidence of the relative degree of 1) predation by Brown Trout or 2) estimates of the availability and abundance of potential juvenile salmonid prey species in relation to other piscivorous taxa endemic or introduced into the Trinity River, including a recent bioenergetics model of Brown Trout predation in the Trinity River (Alvarez and Ward 2019). Further, understanding the basic pattern of migration and learning from the behavior of non-anadromous as well as anadromous resident species of fish in response to flow management is a prerequisite to enabling placement of a broad suite of covariate non-flow factors into perspective, which is particularly relevant to restoration actions, as it enables resource managers to better understand and evaluate their methodology and facilitated completion of the adaptive management process (Sullivan and Hileman 2019).

For Brown Trout (1982 - 2018, n = 3,614) and Klamath Smallscale Suckers (1993 - 2018, n = 5,156), we used the metric catch per unit effort (CPUE) in units of adult fish trapped per trap-day to estimate relative annual abundance and evaluate "population" trends over time (Sullivan and Hileman 2018). Estimates of CPUE derive from constant effort by-catch data collected at the weir (trap-counts). Although CPUE is not a measure of true abundance, it is an established indicator of relative abundance (Bonar et al. 2009; Arshad-Ul-Alam and Azadi 2015). Conversely, we used adult return hard-counts (henceforth called "counts") in our analysis of Coho Salmon (1990 - 2018, n = 153,872) and steelhead (1990 - 2018, n = 100,547). In contrast, adult return counts to the hatchery of coded wire tagged individuals (CWT) were used to evaluate known genetic race spring-run (1994 - 2018, n = 28,436) and fall-run (1994 - 2018, n = 56,749) Chinook Salmon. Importantly, counts of both spring- and fall-run coded wire tagged Chinook Salmon only represent an averaged marked subsample of approximately 20.7% of the total combined return to the hatchery of adult Chinook Salmon for each race (1994 - 2018, n = 41,1888), as currently only 25% of hatchery produced Chinook Salmon are coded wire tagged prior to release into the Trinity River below Lewiston Dam as juveniles (Sullivan and Hileman 2019). We used counts of coded wire tagged known race returns in our analysis to tease out definitive spring- and fall-run Chinook Salmon because there is frequently temporal overlap between returning adults of the two sympatric races in the autumn at the hatchery. Additionally, there is no other functional way to accurately determine racial segregation because there is no definitive external phenotypic difference between races of Chinook Salmon that would allow identification at the weir. Finally, counts of coded wire tagged known spring- and fall-run Chinook Salmon were used in lieu of estimates based on expansion equations (Kilduff et al. 2015; Sullivan and Hileman 2019).

Statistical analyses

All statistical tests performed used the R-suite of statistical programs (v3.5.2, R Core Team 2019). Prior to analysis univariate normality for annual fluctuations in the distribution of counts and CPUE estimates we conducted for all taxa, which were visually inspected by use of normalized (0.0, 1.0) quantile-quantile (Q-Q) and standardized residual plots. As expected, this assessment showed that all count and CPUE data were skewed significantly to the right, consistent with a Poisson distribution. A follow-on statistical evaluation by use of the Shapiro-Wilk's (W) test (McGarigal et al. 2000) similarly indicated that annual species-specific counts and CPUE data were not normally distributed (Brown Trout: W = 0.87, P < 0.01, n = 33; Klamath Smallscale Sucker: W = 0.88, P < 0.01, n = 24; CWT spring-run Chinook Salmon: W = 0.83, P < 0.01. n = 25; fall-run Chinook Salmon: W = 0.84, P < 0.01, n = 25; Coho Salmon: W = 0.88, P < 0.01, n = 29; steelhead: W = 0.82, P < 0.01, n = 29; Appendix I). Thus, all subsequent non-regression statistical analyses of count and CPUE data used non-parametric methods (McDonald 2014). For count and CPU data, we used the Spearman's rank correlation rho (r_s) 2-tailed test to calculate strength and direction of the relationship between two variables, expressed as a monotonic relationship, whether linear or not (Corder and Foreman 2014). Whereas the Pearson correlation coefficient (r_c) was used in all correlation analyses involving water temperature, flow, and other continuous metrics. The Wilcoxon signed-rank test (paired = "TRUE", zero method = "Pratt"; package "asht" v0.9.4) computed from two-sided probabilities using approximate normal variates (Z) for all planned multiple comparisons, was used to evaluated the hypothesis that the median difference between pairs of Julian week counts was zero among different flow-types for each taxon of fish (Hasler et al. 2014).

We obtained telemetered digital data for 1) average daily water temperature (ADWT, degrees centigrade [C°]) and 2) averaged daily flow volume (ADFV m^3/s) from the United States Bureau of Reclamation, Lewiston Water Quality Gauge (LWS), upper Trinity River at river-km 178.2 (UTM 516,634 m E and 4,507,678 m N, elevation 558 m), 1.7 rkm downriver from the Lewiston Dam and the hatchery (DWR 2018) for the sampling period 1994 to 2018 for which there were complete data for each variable. This gauge was the "standard" used in all National Environmental Protection Act (NEPA 1969) assessments and flow augmentation analyses of fluctuations in in-river average daily water temperature, specific to the upper Trinity River out of Lewiston Dam since 1997 (Magneson and Chamberlain 2014 and included references). Trends in seasonal count data were analyzed by use of Julian weeks (JW), defined as one of seven consecutive-day-sets of 52 weekly periods in a calendar year, beginning 01 January of each year. This procedure allowed inter-annual comparisons of identical weekly periods. Extra day in leap years was included in the ninth week. To determine if timing of seasonal migration in species-specific ROD and Pulse flow-types deviated from their baseline PreROD flow pattern, we calculated a Percent Deviation Index (PDI) from total counts (Sullivan and Hileman 2019):

PDI for ROD flows = %ROD flow count – %PreROD flow count
PDI for Pulse flows = %Pulse flow count – %PreROD flow count

Generalized additive models

We assessed annual trends in continuously distributed linear measures of ADWT and ADFV from 1994 through 2018, and seasonally by use of Julian weeks specific to the documented presence (counts) of each species as a function of species-specific migration or run-time schedules (JW21 - JW13). Generalized additive models (GAM, Package "mgcv" v1.8-28, Wood 2017) were used in regression of hatchery counts and weir CPUE data for all species as described in detail elsewhere (Hastie and Tibshirani 1990; Madsen and Thyregod 2011; Sullivan and Hileman 2019). Response curves generated from each GAM showed the relationship between the fitted function to the response scaled to zero. Statistics reported from each GAM were 1) F- or χ^2- statistics (approximate significance of smooth terms) including P-values and 95% confidence bands for spline lines (Nychka 1988), 2) adjusted regression coefficients for each model (R^2 adj.), 3) estimated residual degrees of freedom (Ref. d.f.), and 4) proportion of null deviance explained (Dev.Exp.). We used the Spearman's rank correlation coefficient as a follow-on procedure to assess strength and significance of trends in counts delineated by smooth terms because GAMs lack a statistical inference procedure and formal parameter of goodness of the fit, which makes interpretation of output potentially complicated (Diankha and Thiaw 2016). Because our count data were over-dispersed (Package "AER" v1.2-6), the negative binomial error-structure (family = "nb" [theta = NULL, link = "log']) was used in construction of GAM models to establish the relationship between response variables and the smoothed functions of predictor variables (Wood et al. 2016; Wood 2017; Lipp 2016). In contrast, the gamma error-structure (family = "Gamma" [link = "log"]) was used to assess error distributions of annual and seasonal (JW) fluctuations in ADWT and ADFV (Package "fitdistrplus" v1.0-14). The Akaike information criterion (QAICc, Package MuMIn v1.43.5) modified for overdispersed count data adjusted for small sample uncertainty was used to select the most parsimonious error distribution for each environmental attribute, as well as GAM models for comparisons between counts, and individual and combined water temperature and flow volume effects (Akaike 1973; Burnham and Anderson 1998). Whereas, a Gaussian error-structure (family = "gaussian" [link = "identity"]) was used to plot difference curves (Package "itsadug" v2.3) using GAM model predictions of the number of counts that deviated from the species-specific baseline PreROD flow-type as a result of both ROD and Pulse flows, because these data included both positive and negative numbers (Cox 2017). We set statistical significance for all analyses at $P < 0.05$.

Autocorrelation analysis of residuals derived from GAM analyses investigated the relationship of each time point to each previous time point in the distribution of consecutive annual counts in relative abundance for all species (Package "forecast" v8.3 and "stats" v3.6.0). Visual inspection of these results showed that all time-points were contained within approximate 95% confidence levels of significance for each correlation in the autocorrelation function correlograms (Appendix II). Additionally, follow-on Box-Pierce test (χ^2) tests and augmented Dickey-Fuller tests (SDF) evaluated the extent of stationarity of the time series models (Fuller 1976; Ljung and Box 1978). Each of these analyses indicated no evidence of non-zero autocorrelations in the in-sample forecast errors at any lag-point for any taxon (Coghlan 2019). Because we found no evidence against time dependency for any species, we concluded that annual counts of all species represented stationary series of relatively constant autocorrelation structure over time for the sequence of consecutive years analyzed herein. Importantly, a stationary time series likely will always occur for Trinity River Hatchery raised salmonids as numbers of hatchery released fingerlings and yearlings are based on egg-take allotments established in the 1980s to meet fixed mitigation goals of returning adult Chinook Salmon, Coho Salmon, and steelhead to the hatchery irrespective of annual hatchery escapement (Sullivan and Hileman 2019).

RESULTS

Annual fluctuations in relative abundance

Annual fluctuations in CPUE estimates in Brown Trout and Klamath Smallscale Suckers from the weir, hatchery counts of coded wire tagged known race spring- and fall-run Chinook Salmon, and hatchery counts of Coho Salmon and steelhead all fluctuated considerably on an annual basis (Figure 3a-3f). Peaks in relative abundance generally exhibited increasing trends beginning early in 2002. Plots of partial residuals showed that annual counts were non-linear and well defined by response curves (Figure 4a-4f), as all smooth terms were significant, and deviance explained ranged from 42.1% (Brown Trout, spring-Run Chinook Salmon) to 64.8% (steelhead). Additionally, 60.0% (n = 15) of all planned pair-wise correlations among species were significant and positively correlated indicating that most species exhibited similar annual patterns for the years that they shared in our sample (Table 2).

However, from 2003 to 2018 GAM regression showed that the strength of the relationship between year and counts and CPUE estimates was strong, significant, and negative for all non-anadromous and anadromous species irrespective of the reduction in years sampled. Concordance in these data indicate that all taxa have declined abruptly in relative abundance since 2003, irrespective of divergent life history strategies (Table 2). Importantly,

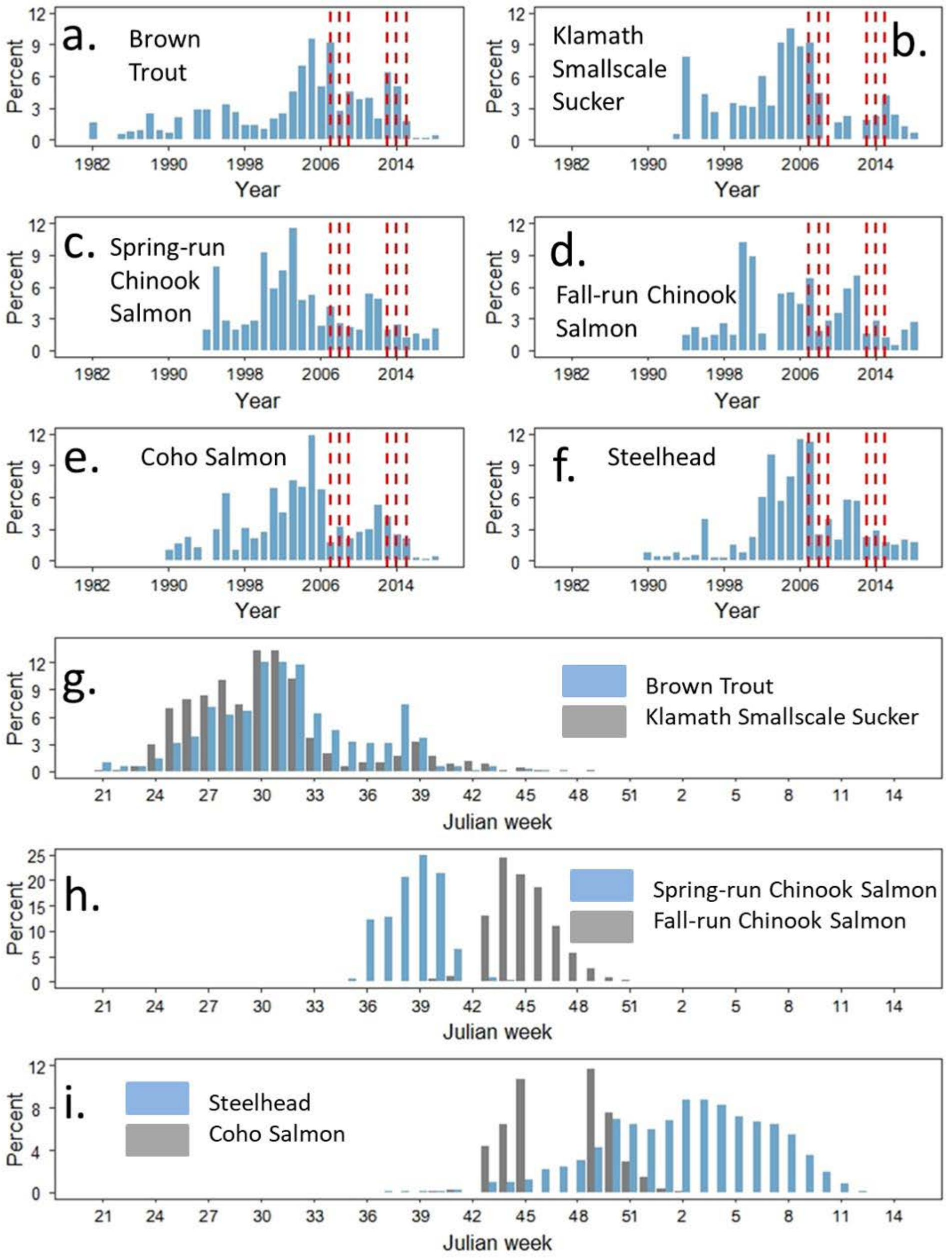

Figure 3. Bar charts of the percentage of annual and seasonal Julian week (JW) fluctuations in total adult counts of Brown Trout (a and g), Klamath Smallscale Suckers (b and g), spring-run Chinook Salmon (c and h), fall-run Chinook Salmon (d and h), Coho Salmon (e and i), and steelhead (f and i). Vertical dashed red lines reference two periods of three consecutive years of drought (2007-2009, 2013-2015).

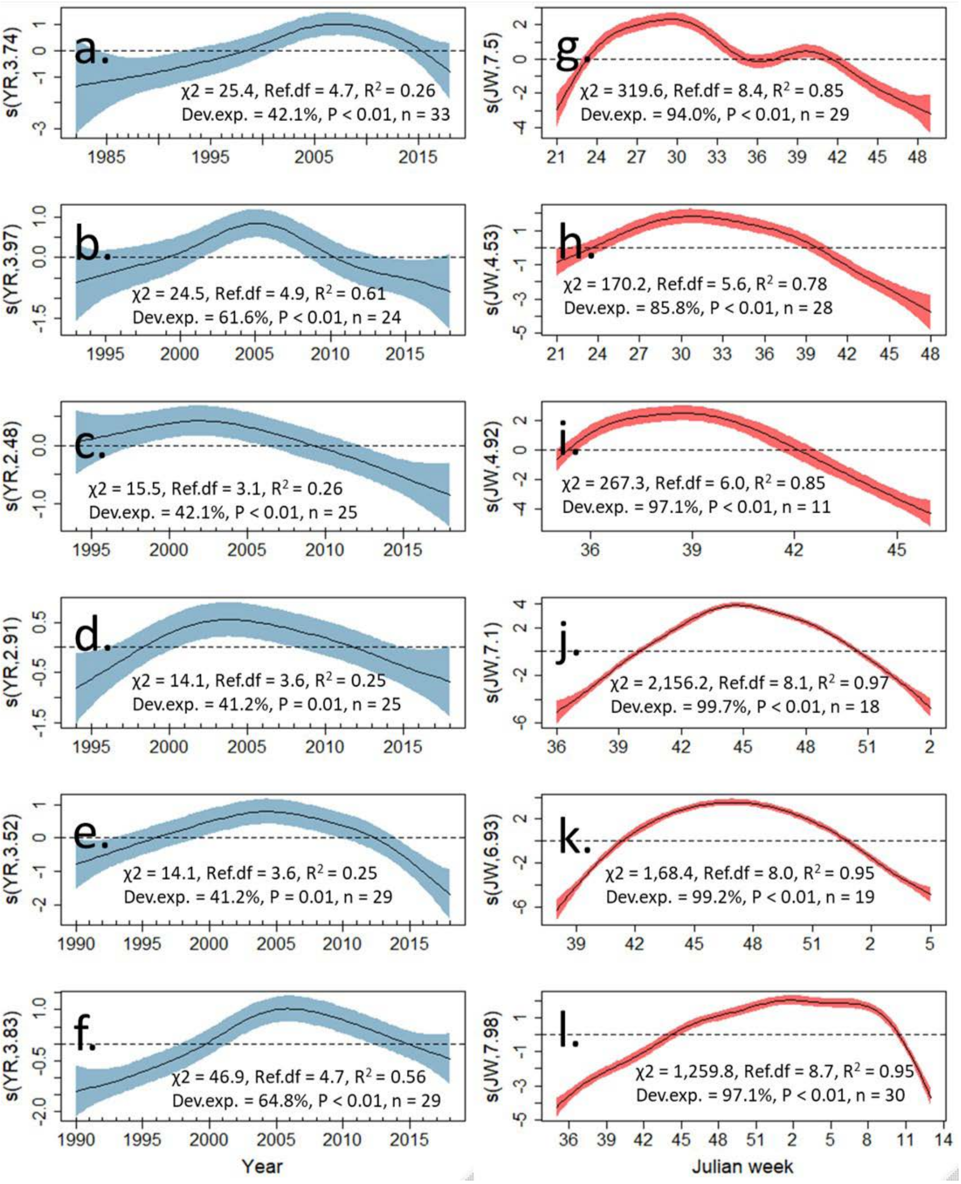

Figure 4. Partial residual response curves (centerline) generated from GAM regression of total annual counts and CPUE estimates (a - f), and seasonal Julian week (JW) counts (g - l) for each species of fish. Each plot shows the relationship of the fitted function to the response scaled to zero, including approximate 95% point-wise standard error bands for each curve of the factor level. Y-axes (log-scaled) are based on partial residuals indicating the relative influence of each year sampled (explanatory variable) on the relative abundance of counts (prediction). X-axes (independent variable/predictor) are labeled with the covariate name; whereas Y-axes (outcome/dependent variable) are labeled by the covariate name (cov) and estimated degrees of freedom (edf) of each of the smooths (i.e., s[cov,edf]); and smooths are "centered" to ensure model identity and sum to 0 over covariate values).

Table 2. Planed Spearman rank correlations Coefficients (rs) of 1) annual catch per unit effort (CPUE) and counts of all fish at the Trinity River Hatchery (hatchery) and Junction City (weir) for Brown Trout (BT, 1982 - 2018), Klamath Smallscale Sucker (KSS, 1993 - 2018), spring-run Chinook Salmon (SRC, 1994 - 2018), fall-run Chinook Salmon (FRC, 1994 - 2018), Coho Salmon (COS, 1990 - 2018), and steelhead (STH, 1990 - 2018); 2) CPUE and counts from the hatchery and weir from 2003 to 2018; and 3) seasonal Julian week (JW) variation in counts from 1994 to 2018. Correlations coefficients are below the diagonal and P-values are above the diagonal; P-values: * < 0.05, ** < 0.01, *** < 0.001.

Group	YR	BT	KSS	SRC	FRC	COS	STH
Years specific to taxa							
Year (n = 37)		0.04*	0.31	0.03*	0.82	0.97	0.01**
Brown Trout (n = 33)	0.36		0.05*	0.04*	0.01**	< 0.01***	< 0.01***
Klamath Smallscale Sucker (n = 24)	-0.22	0.40		0.13	0.56	0.06	0.01**
Spring-run Chinook Salmon (n = 25)	-0.44	0.42	0.32		< 0.01***	< 0.01***	0.06
Fall-run Chinook Salmon (n = 25)	0.05	0.53	0.13	0.63		0.02*	0.01**
Coho Salmon (n = 29)	0.01	0.69	0.39	0.63	0.47		< 0.01***
Steelhead (n = 29)	0.48	0.75	0.53	0.39	0.51	0.61	
Years and taxa (2003 - 2018)							
Year (n = 16)		< 0.01***	< 0.01***	< 0.01***	0.01**	< 0.01***	< 0.01***
Brown Trout (n = 16)	-0.76		0.12	< 0.01***	< 0.01***	< 0.01***	< 0.01***
Klamath Smallscale Sucker (n = 16)	-0.78	0.41		0.15	0.32	0.11	0.03*
Spring-run Chinook Salmon (n = 16)	-0.71	0.80	0.38		< 0.01***	< 0.01***	< 0.01***
Fall-run Chinook Salmon (n = 16)	-0.65	0.86	0.26	0.85		0.02*	< 0.01***
Coho Salmon (n = 16)	-0.78	0.73	0.41	0.70	0.56		0.01**
Steelhead (n = 16)	-0.80	0.79	0.55	0.79	0.84	0.65	
Julian weeks (JW21 - JW14) and years specific to taxa							
Julian week (n = 46)		0.01**	0.01**	0.07	0.95	0.74	0.02*
Brown Trout (n = 29)	-0.48		< 0.01***	< 0.01***	0.02*	< 0.01***	< 0.01***
Klamath Smallscale Sucker (n = 28)	-0.51	0.85		0.01**	< 0.01***	< 0.01***	< 0.01***
Spring-run Chinook Salmon (n = 11)	-0.57	0.96	0.71		0.02*	< 0.01***	0.07
Fall-run Chinook Salmon (n = 18)	0.02	-0.65	-0.80	-0.72		< 0.01***	0.87
Coho Salmon (n = 19)	-0.08	-0.93	-0.85	-0.93	0.73		0.87
Steelhead (n = 30)	0.44	-0.77	-0.85	-0.56	0.04	-0.04	

this continuing trend in declining stocks of sympatric non-anadromous and anadromous fish inhabiting the upper Trinity River initiated prior to the two periods of three consecutive years of regional drought as indicated by water year-type for 2007 through 2009 through 2013 to 2015 (Table 1; TRRP 2019).

Seasonal fluctuations in Julian week counts

Fluctuations in seasonal Julian week counts increased early in the migration or run-timing cycle then declined late in the season for all non-anadromous and anadromous species (Figure 3g-3i). Plots of partial residuals showed that counts associated with seasonal migration and run-timing were non-linear and well defined by response curves, as deviance explained was > 85.8% in all taxa (Figure 4g-4l). Initiation and termination of seasonal migration in non-anadromous Brown Trout and Klamath Smallscale Suckers ranged from mid-May through early December (JW21 - JW49). Whereas in anadromous salmonids initiation of seasonal run-timing ranged from late August to early January (JW36 - JW2) in spring- and fall-run Chinook Salmon, from mid-September to late March (JW38 - JW5) in Coho Salmon, and from late August through late March (JW35 - JW13) in steelhead. However, we note that initiation of annual hatchery counts does not start until the fish ladder opens at the beginning of September. Thus, based on count data presented herein seasonal migration in Brown Trout and Klamath Smallscale Suckers, and run-timing in steelhead were the most drawn-out migratory patterns of all the species evaluated.

Brown Trout and Klamath Smallscale Suckers overlapped significantly in their seasonal Julian week pattern of migration (Table 2). Regression analysis using GAM indicated that the timing of migration in Brown Trout passing through the weir equated to 51.3% of the deviance explained in timing of migration of Klamath Smallscale Suckers trapped at the weir during the same time period ($\chi^2 = 33.4$, Ref.df = 3.7, $P < 0.01$, $R^2 = 0.74$). Moreover, seasonal Julian week migration in both Brown Trout and Klamath Smallscale Suckers was significant and positively correlated with run-timing in spring-run Chinook Salmon but significant and negatively correlated with all other anadromous salmonids (Table 2). Intersection of species-specific run-timing in spring- and fall-run Chinook Salmon was minimal. However, historically some overlap between these genetically differentiated races occurs from mid-September through mid-November (Figure 3h, JW38 - JW46, Kinziger et al. 2013).

Coho Salmon and fall-run Chinook Salmon also exhibited a significant and positive relationship in run-timing with migration in fall-run Chinook Salmon equating to 51.4% of the deviance explained in Coho Salmon ($\chi^2 = 24.6$, Ref.df = 3.1, $P < 0.01$, $R^2 = 0.27$). Presence of steelhead in the upper Trinity River system encompassed run-timing of both Chinook Salmon and Coho Salmon. Yet, steelhead did not exhibit a similarly significant pattern of seasonal run-timing with any other anadromous species except marginally with early arriving spring-run Chinook Salmon; whereas the relationship in seasonal migration with both non-anadromous Brown Trout and Klamath Smallscale Suckers was highly significant but negative (Table 2).

Fluctuations in water temperature and flow volume

Regression analysis of raw environmental data using GAM reviled significant positive trends in annual fluctuations in ADWT, but significant negative trends in annual fluctuations in ADFV (Figure 5a). Yet the proportion of the null deviance explained in both variables was low and follow-on correlations indicated that the strength of the relationships between year and each variable was extremely weak (Table 3). Whereas GAM analyses of seasonal Julian week fluctuations in ADWT and ADFV identified significant negative trends in both environmental variables, particularly ADWT. However, for each comparison much larger percentages of the null deviance were explained by seasonal variation in ADWT and ADFV compared to annual variation in these two environmental attributes (Table 3, Figures 5b and 5c).

Fluctuations in water temperature and flow volume in relation to counts of fish

Against this background, there were no significant trends in counts or CPUE estimates of each species of fish in relation to annual fluctuations in ADWT or ADFV based upon results of GAM regression or ranked correlation analyses (Table 3). Conversely, counts of Brown Trout, Klamath Smallscale Sucker, and spring-run Chinook Salmon exhibited significant positive trends in relation to seasonal Julian week fluctuations in ADWT (Table 3, Figure 5b). This pattern was a function of increased species-specific counts associated with early spring and late summer patterns of migration, coincidental with relatively warm water temperatures from approximately mid-July to late August (JW30 - JW34).

In contrast, counts of steelhead showed a significant negative relationship with ADWT, as a function of progressively late fall and early winter run-timing in association with comparatively colder water temperatures beginning in late October (JW45) through the coldest water-months from January to early February (JW51 - JW5, Table 3, Figure 5b). In comparison, counts of Fall-run Chinook Salmon and Coho Salmon exhibited no significant trends in relation to seasonal Julian week variation in ADWT following ROD and Pulse flow-type events and a return to base-flows in late fall (Table 3, Figure 3h and 3i, Figure 5c). As relates to seasonal Julian week fluctuations in ADFV, only counts of Fall-run Chinook Salmon and Coho Salmon exhibited a significant negative relationship with this variable. Whereas the marginally significant and positive correlation between CPUE and ADFV in Klamath Smallscale Suckers appears to represent only a small segment of the terminal end of local migration in response to seasonal peaks in odd year Tribal Ceremonial Boat Dance flows and companion or individual Pulse flow events after 2003, which also appear to promote local migration in Brown Trout (Figure 2c, Figure 5c; Sullivan and Hileman 2018).

Additionally, the proportion of null deviance explained in seasonal Julian week variation in relative count abundance by ADWT was greater than when count data were regressed against ADFV for each taxon (Table 3). Further, the proportion of null deviance explained improved greatly by combining the two environmental attributes in all species. Combining environmental variables in a composite GAM model for spring-run Chinook Salmon was not possible as there were more coefficients than the number of Julian week samples. However, combining ADWT with ADFV in model regressions did not appreciably improve all measures of relative fit for each predictive species-specific model as measured by the QAICc information criterion (Table 2). For example: 1) seasonal and local migration in Brown Trout appeared more aligned with warmer water in association with pulsed flow augmentations; 2) run-timing in Coho Salmon appeared closely affiliated with a return to base-flows in the fall on post-pulsed augmentation flows; and 3) run-timing in steelhead appeared more closely aligned with an increase flows late in the season in conjunction with the onset of winter storms and accretion of cold water derived from watersheds of the upper Trinity River basin.

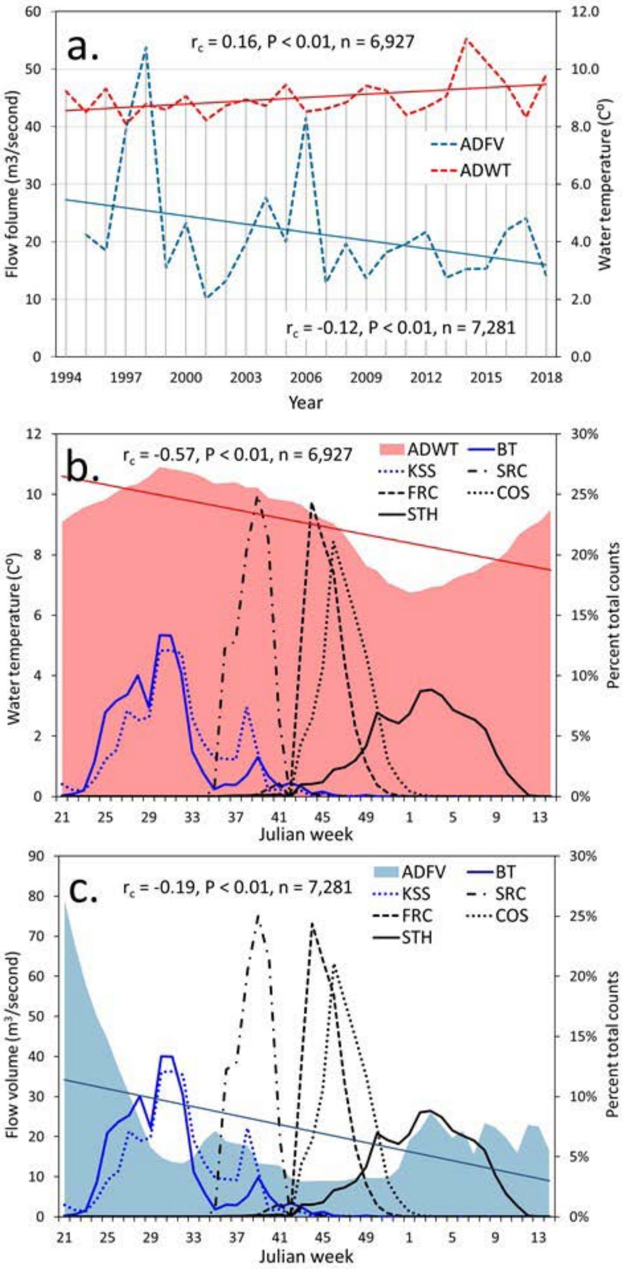

Figure 5. a) Mean values of annual fluctuations in average daily water temperature (ADWT) and averaged daily flow volume (ADFV) from 1994 to 2017 and for Julian weeks (JW) 21 to 14, which encompasses sampling dates for both hatchery and weir. Seasonal JW fluctuations in counts of salmon and steelhead and catch per unite effort (CPUE) for Brown Trout and Klamath Smallscale Suckers from 1994 to 2018 are superimposed onto mean values of b) ADWT and c) ADFV. Coho Salmon = COS, spring-run Chinook Salmon = SRC, fall-run Chinook Salmon = FRC, steelhead = STH, Brown Trout = BT, and Klamath Smallscale Sucker = KSS.

Table 3. Summary of approximate significance of smooth terms and statistics derived from generalized additive model (GAM) regressions of count and CPUE data for fish in relation to year (YR) and Julian week (JW), and raw data for averaged daily water temperature (ADWT) and flow volume (ADFV). Statistics reported for each GAM were 1) GAM F- or the GAM χ^2- statistic (approximate significance of smooth terms), P-value, 2) adjusted regression coefficient for the model (R^2 adj.), 3) estimated residual degrees of freedom (Ref.d.f.), 4) proportion of null deviance explained (Dev.Exp.), Akaike information criteria (QAICc), and parametric Pearson correlations (rc) or nonparametric Spearman rank correlations (rs) for each comparison; NA = no data.

Regression comparisons	GAM statistics (family = "Gamma")						Pearson correlation		
	GAM F	P-value	Ref.df	R^2-adj	Dev.Exp. (%)	QAICc	r_c	P-value	n
	Environmental variables vs. year (1994 - 2018) and Julian week (JW21 - JW14) for all raw data								
ADWT ~ YR	47.2	< 0.01***	9.0	0.06	5.6	24,653.5	0.16	< 0.01***	6,927
ADFV ~ YR	48.2	< 0.01***	9.0	0.06	11.5	55,847.1	-0.12	< 0.01***	7,281
ADWT ~ JW	1602	< 0.01***	9.0	0.67	67.4	17,533.4	-0.57	< 0.01***	6,927
ADFV ~ JW	190.8	< 0.01***	9.0	0.25	41.6	52,657.3	-0.18	< 0.01***	7,281
	GAM statistics (family = "nb")						Spearman rank correlation		
	GAM χ^2	P-value	Ref.df	R2-adj	Dev.Exp.	QAICc	r_s	P-value	n
	Annual counts or CPUE estimates vs. mean values of ADWT (1994 - 2018)								
Brown Trout YR CPUE ~ ADWT	0.2	0.70	1.0	0.04	0.6	97.7	-0.12	0.58	24
Klamath Smallscale Sucker YR CPUE ~ ADWT	0.7	0.41	1.0	0.03	2.8	110.3	-0.07	0.76	23
Spring-run Chinook Salmon YR counts ~ ADWT	3.3	0.07	1.0	0.01	10.2	384.5	-0.31	0.13	25
Fall-run Chinook Salmon YR counts ~ ADWT	1.9	0.17	1.0	0.01	6.5	424.0	-0.22	0.29	25
Coho Salmon YR counts ~ ADWT	0.4	0.54	1.0	0.03	1.1	473.9	-0.14	0.51	25
Steelhead YR counts ~ ADWT	0.6	0.42	1.0	0.03	2.0	455.2	-0.01	0.96	25
	Annual counts or CPUE estimates vs. mean values of ADFV (1995 - 2018)								
Brown Trout YR CPUE ~ ADFV	0.1	0.86	1.4	0.04	1.9	95.9	-0.01	0.98	23
Klamath Smallscale Sucker YR CPUE ~ ADFV	2.4	0.12	1.0	0.08	10.2	105.0	0.09	0.69	22

Table 3. continued.

Regression comparisons	GAM statistics (family = “Gamma”)						Pearson correlation		
	GAM F	P-value	Ref.df	R^2-adj	Dev.Exp. (%)	QAICc	r_c	P-value	n
Spring-run Chinook Salmon YR counts ~ ADFV	0.9	0.32	1.0	0.03	3.3	372.4	-0.06	0.76	24
Fall-run Chinook Salmon YR counts ~ ADFV	0.2	0.62	1.0	0.04	0.9	410.1	0.00	0.99	24
Coho Salmon YR counts ~ ADFV	0.1	0.92	1.0	0.05	0.1	455.8	0.07	0.74	24
Steelhead YR counts ~ ADFV	0.1	0.77	1.0	0.04	0.3	438.9	-0.24	0.25	24
Seasonal Julian week counts vs. mean values of ADWT (1994 - 2018)									
Brown Trout JW counts ~ ADWT	107.3	< 0.01***	5.5	0.58	77.8	298.4	0.83	< 0.01***	29
Klamath Smallscale Sucker JW counts ~ ADWT	95.1	< 0.01***	1.0	0.76	72.3	301.5	0.88	< 0.01***	28
Spring-run Chinook Salmon JW counts ~ ADWT	78.0	< 0.01***	3.4	0.67	83.5	202.2	0.62	0.04*	11
Fall-run Chinook Salmon JW counts ~ ADWT	305.2	< 0.01***	6.5	0.86	93.9	266.7	-0.02	0.94	18
Coho Salmon JW counts ~ ADWT	70.7	< 0.01***	4.8	0.70	67.8	337.1	-0.02	0.94	19
Steelhead JW counts ~ ADWT	147.9	< 0.01***	3.2	0.92	75.8	488.5	-0.93	< 0.01***	30
Seasonal Julian week counts vs. mean values of ADFV (1995 - 2018)									
Brown Trout JW counts ~ ADFV	37.6	< 0.01***	5.7	0.10	51.3	322.3	0.28	0.14	29
Klamath Smallscale Sucker JW counts ~ ADFV	73.2	< 0.01***	7.5	0.28	68.8	330.3	0.39	0.04*	28
Spring-run Chinook Salmon JW counts ~ ADFV	54.3	< 0.01***	3.1	0.81	81.4	195.5	0.46	0.15	11

Table 3. continued.

Regression comparisons	GAM statistics (family = “Gamma”)						Pearson correlation		
	GAM F	P-value	Ref.df	R^2-adj	Dev.Exp. (%)	QAICc	r_c	P-value	n
Fall-run Chinook Salmon JW counts ~ ADFV	148.2	< 0.01***	1.4	0.63	83.1	258.1	-0.93	< 0.01***	18
Coho Salmon JW counts ~ ADFV	59.1	< 0.01***	2.9	0.54	64.6	329.9	-0.79	< 0.01***	19
Steelhead JW counts ~ ADFV	0.6	0.45	1.0	0.03	1.8	528.1	0.10	0.62	30
Seasonal Julian week counts vs. combinations of environmental variables (1995 - 2018)									
Brown Trout JW counts ~ ADWT + ADFV									
ADWT	47.9	< 0.01***	2.9	0.69	83.5	303.6	NA	NA	29
ADFV	15.8	< 0.01***	4.4				NA	NA	
Klamath Smallscale Sucker JW counts ~ ADWT + ADFV		< 0.01***							
ADWT	93.3	< 0.01***	1.0	0.82	88.1	298.1	NA	NA	28
ADFV	24.4	< 0.01***	3.8				NA	NA	
Fall-run Chinook Salmon JW counts ~ ADWT + ADFV		< 0.01***							
ADWT	137.1	< 0.01***	4.0	0.88	98.1	249.7	NA	NA	18
ADFV	31.0	< 0.01***	1.9				NA	NA	
Coho Salmon counts JW counts ~ ADWT + ADFV		< 0.01***							
ADWT	394.6	< 0.01***	8.8	0.96	99.7	411.5	NA	NA	19
ADFV	653.1	< 0.01***	4.0				NA	NA	
Steelhead JW counts ~ ADWT + ADFV		< 0.01***							
ADWT	205.1	< 0.01***	1.0	0.38	81.2	495.0	NA	NA	30
ADFV	19.4	< 0.01***	2.3				NA	NA	

Seasonal fluctuations among flow-types in relation to Julian week counts

Results of Wilcoxon signed-rank tests for planned multiple comparisons showed a significant overall effect between all flow-types for some but not all species of fish (Table 4). For example, Brown Trout and Klamath Smallscale Suckers showed significant differences in counts between PreROD and Pulse flow-types and between ROD and Pulse flow-types, spring-run Chinook Salmon showed significant differences between PreROD and ROD flow-types, and steelhead showed significant differences between PreROD and ROD flow-types and between PreROD and Pulse flow-types. In contrast, fall-run Chinook Salmon and Coho Salmon did not show any significant differences between flow-types in the paired distribution of Julian week counts.

Comparative analysis of the effects of both managed flow-types on the baseline species-specific PreROD flow pattern of seasonal migration on resident species of fish showed that from 44.5% (Coho Salmon) to 66.3% (steelhead) of the cumulative counts (positive + negative counts) were affected by managed flow hydrographs from 2003 to 2018 (average = 56.2%, Table 5). For ROD-affected flow-types this varied from an addition of counts to the baseline PreROD flow pattern from 99.6% in steelhead to 0.9% in spring-run Chinook Salmon (average = 56.0%). For species-specific Pulse-affected baseline flows addition of counts varied from 31.2% in Coho Salmon to 98.9% in spring-run Chinook Salmon (average = 61.3%). In relation to total hard-counts from both hatcheries returns and the weir for the period 2003 to 2018, variation in the number of fish affected by managed flow-types fluctuated from 21.7% in coded wire tagged fall-run Chinook Salmon to 63.8% in coded wired tagged spring-run Chinook Salmon (Table 5). Thus, the total number of counts for all species affected both positively and negatively by managed flow-types from 2003 to 2018 was estimated to be approximately 237,506 individuals. However, recall that counts of coded wire tagged adult Chinook Salmon assessed herein represent only 21.7% (85,185/243,154) of the total hatchery return of all marked and unmarked fish of all age classes. Thus, for both spring-and fall-runs of this species the actual count of individual adult Chinook Salmon affected by managed flow regimes would be considerable larger, approximating a 4.43688 multiplier of the coded wire tag count for each genetic race comprising the total hatchery return from 2003 to 2018.

Deviation in counts from species-specific baseline flow patterns

Percent deviation indices generated for each taxon showed when and how managed flow-types deviated from their species-specific baseline PreROD flow patterns (Figure 6). For example, the PreROD migration pattern in Brown Trout deviated both positively and negatively from a reduction in counts at the ascending limb of the baseline PreROD flow owing to both ROD and Pulse flows. Yet both managed hydrographs added counts along the declining central and trailing segments of the baseline PreROD flow pattern. In Klamath Smallscale Suckers deviation away from the baseline flow pattern resulted from a decrease in counts by managed flows prior to the peak in the baseline, followed by an increase in counts mid-season and ending with a series of alternating increases (ROD flows) and decreases (Pulse flows) throughout the remainder of migration cycle. In spring-run Chinook Salmon Pulse flows added counts to the baseline pattern early in migration, followed by a decrease in counts by both ROD and Pulse flows throughout the balance of the baseline flow. Conversely, in fall-run Chinook Salmon Pulse flows added counts to the baseline early in the

Table 4. Wilcoxon signed-rank tests (Z) for planned multiple comparisons between pairs of Julian week counts among different flow-types (groups) for each taxon of fish; n = number of Julian weeks per flow-type used in each comparison; P-values: * < 0.05, ** < 0.01, *** < 0.001.

Taxon	PreROD vs ROD		PreROD vs Pulse		ROD vs Pulse	
	Z	*P*-value	*Z*	*P*-value	*Z*	*P*-value
Brown Trout (n = 29)	0.4	0.72	2.1	0.04*	2.5	0.01*
Klamath Smallscale Sucker (n = 28)	1.2	0.24	2.1	0.04*	3.1	< 0.00***
Spring-run Chinook Salmon (n = 11)	2.5	0.01**	1.6	0.12	1.4	0.16
Fall-run Chinook Salmon (n = 18)	1.1	1.00	0.0	1.00	0.9	0.40
Coho Salmon (n = 19)	1.3	0.22	1.3	0.24	1.6	0.11
Steelhead Trout (n = 30)	4.4	< 0.00***	3.8	< 0.00***	1.5	0.13

season declining at the end of run-timing; whereas ROD flows reduced counts mid-season but then increased counts late in the season. In Coho Salmon, however, deviation away from the baseline PreROD flow occurred primarily through addition of counts by ROD flows and to a lesser degree by Pulse flows early- to mid-season, the exact opposite of what occurred in fall-run Chinook Salmon, followed by a reduction in counts primarily from Pulse flows near the end of run-timing. Finally, in steelhead both ROD and Pulse flows added counts to the baseline PreROD flow pattern midway through the season, which continued throughout most of the remaining run-timing cycle, primarily in association with ROD flows. Species-specific Julian weeks in which counts were most commonly affected by ROD and Pulse flow-types are shown in Table 5.

Also apparent as a function of correlation analyses (Figure 6, Table 2), was the observation that managed flows imparted similar effects to baseline PreROD flow patterns of migration in non-anadromous Brown Trout and Klamath Smallscale Suckers, which have highly divergent life history strategies but are similar in timing of their seasonal migrations. Whereas alterations of baseline flow patterns in anadromous species by managed flows that have more similar life histories strategies compared to anadromous taxa, were highly dissimilar as a function of divergent run-time schedules (Mims and Olden 2013; Rytwinski et al. 2017), particularly Coho Salmon and steelhead. As such, Julian weeks in which baseline PreROD flow patterns were most frequently affected by managed flows were clearly a function of the timing of species-specific migration in non-anadromous taxa or run-timing in anadromous salmonids (Table 5).

Additionally, in spring-run Chinook Salmon, steelhead, Brown Trout, and Klamath Smallscale Suckers additions and deletions of counts to their corresponding baseline PreROD flow-type patterns were significant and positively correlated (Figure 6). These data indicate that for these species managed flow-types altered their companion baseline flows in similar ways along the seasonal spectrum of Julian weeks, albeit to different degrees. Conversely, in fall-run Chinook Salmon and Coho Salmon there was no significant correlation between ROD and Pulse flow-types in how hydrographs altered their corresponding baseline PreROD flow-types.

Finally, using species-specific difference curves GAM model predictions identified the specific Julian weeks that were significantly different statistically (at alpha = 0.05) between managed flow-types (Figure 7). This analysis closely mirrored the graphic illustrations

Table 5. Species-specific 1) total and percent of the cumulative counts (positive + negative) of fish for both flow-types (ROD and Pulse) that have affected the baseline PreROD flow pattern as a function of managed hydrographs; 2) total and percentage of cumulative counts of ROD and Pulse flow-types separately; 3) comparison of total and percent of affected counts relative to the total Trinity River Hatchery return counts from 1994 through 2018; and 4) Julian week (JW) sequences in which the PreROD flow pattern was most commonly affected and modified by managed flow-types since 2003.

Taxa	Cumulative count both flow-types			Cumulative count ROD flow-type			Cumulative count Pulse flow-type			Total hatchery/weir count (2003 - 2018)		Most common Julian weeks	
	n	Affected (%)	Pulse (%)	n	Add (%)	Delete (%)	n	Add (%)	Delete (%)	n	Affected (%)	Sequence	%
Brown Trout	1,249	62.0	38.0	775	68.4	31.6	474	35.7	64.3	2,459	50.8	28JW - 33JW	64.1
Klamath Smallscale Sucker	2,082	58.1	41.9	1,209	74.0	26.0	873	36.0	64.0	3,500	59.5	25JW - 36JW	68.7
Spring-run Chinook Salmon	10,292	49.9	50.1	5,140	0.9	99.1	5,152	98.9	1.1	16,140	63.8	36JW - 41JW	96.2
Fall-run Chinook Salmon	8,391	56.3	43.7	4,728	58.6	41.4	3,663	72.9	27.1	38,663	21.7	43JW - 49JW	91.2
Coho Salmon	54,931	44.5	55.5	24,439	34.3	65.7	30,492	31.2	68.8	96,201	57.1	43JW - 50JW	87.1
Steelhead	42,705	66.3	33.7	28,299	99.6	0.4	14,406	93.3	6.7	80,543	53.0	49JW - 6JW	71.0

derived by use of our percent deviation indices. For example, in non-anadromous species significant differences between ROD and Pulse flow GAMs for Brown Trout occurred JW26 and from JW29 to JW34 and for Klamath Smallscale Suckers from JW28 to JW38 (Figure 7a, 7b) In anadromous species there were no significant differences between managed flows in spring-run Chinook Salmon, but in fall-run Chinook Salmon significant differences occurred from JW41 to JW44 (Figure 7c, 7d). In Coho Salmon significant differences between ROD and Pulse flow-types occurred from JW45 to JW46 and from JW48 to JW50, and in steelhead from JW50 to JW8 (Figure 7e, 7f).

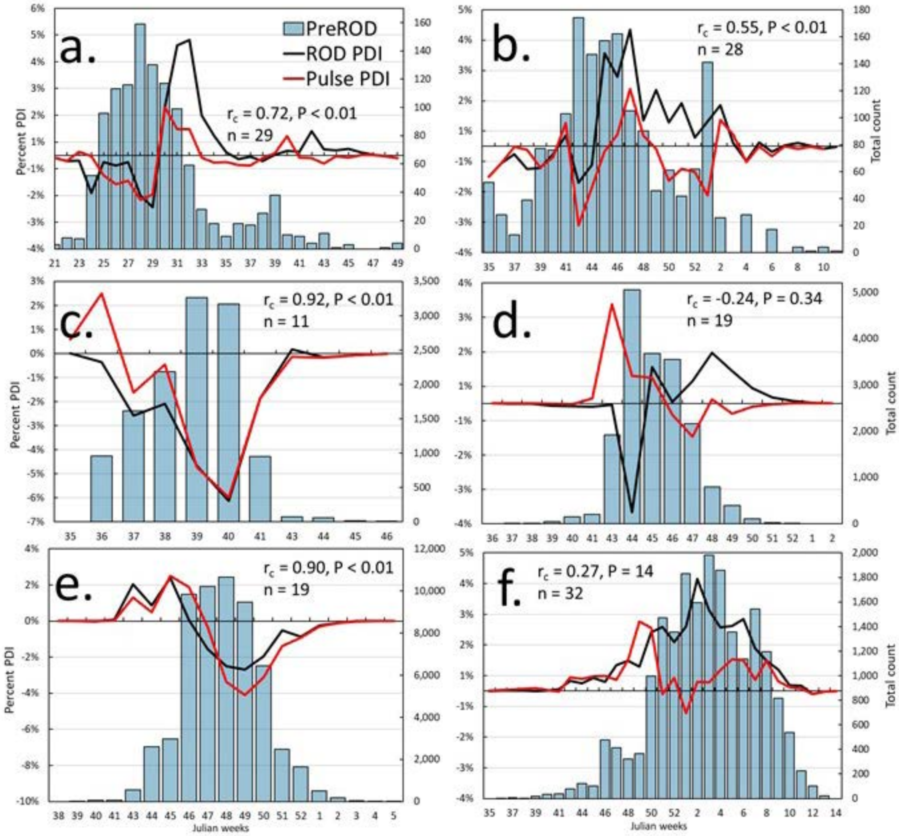

Figure 6. Line graphs showing seasonal fluctuations in total counts (positive + and negative) by Julian week for each species that deviated from the baseline PreROD flow pattern of migration (bar graphs), as a function of the Percent Deviation Index (PDI) in response to ROD and Pulse flows. Lines above or below the dashed black zero line on the y-axis added or subtracted fish from the baseline PreROD flow pattern in timing of migration or run-timing specific to each Julian week (x-axis). Pearson correlations (rc) and sample sizes are between each species-specific ROD and Pulse PDI; a = Brown Trout, b = Klamath Smallscale Sucker, c = spring-run Chinook Salmon, d = fall-run Chinook Salmon, e = Coho Salmon, and f = steelhead

DISCUSSION

Annual fluctuations in estimates of relative abundance

Natural flow regimes reflect inter-annual climate variability, as larger peak flows, longer duration recessions, and higher base-flows occur in wet years, and smaller, shorter, lower flows occur in dry years. Thus, inter-annual variation is a key attribute of functional riverine ecosystems for all life forms (Yarnell et al. 2015). Similarly, duration and magnitude of dry-season low flows are important drivers of lotic ecosystems and most native species adapt to these biologically stressful periods. Whereas, episodic disturbances from climatically driven high-flow events tend to reset successional stages in riverscapes and regulate aquatic food webs by decreasing abundance of predator-resistant primary consumers that support diverse food chains (Ward 1998; Power et al. 2013). Consequently, the magnitude, timing, and duration of natural flow events vary seasonally, depending upon regional climatic conditions, and between years depending upon fluctuation in patterns of global climate. When combined with spatial heterogeneity throughout the channel and floodplain, this inter-annual variability supports diversity in habitat conditions, recruitment, and refugia from competition, thus facilitating subsequent diversity in native species (Naiman et al. 2008; Viers and Rheinheimer 2011; Petts and Gurnell 2013).

In contrast, altered natural streamflow and highly variable flow regimes associated with dams and other anthropogenic activities exhibit reduced flow seasonality and variability (Poff et al. 2007; Carlisle et al. 2011), which generally increase short-term minimum flows while decreasing short-term maximum flows (Magilligan and Nislow 2005). Moreover, highly managed flows may alter the historical disturbance regime, rendering some biotic adaptations to these regimes obsolete while potentially favoring others. Reduced flow variability by dams has been associated with significant losses of native fish species while concurrently creating new niche opportunities often occupied by non-native species with life histories novel to the system or basin (Bunn and Arthington 2002; Olden et al. 2006). In our study, although fluctuations in counts of anadromous and non-anadromous species of fish fluctuated considerably on an annual basis, we show that all taxa decreased significantly and strongly from 2003 to 2017, more so than in any other sequence of years sampled. This sequence of dates coincides with establishment of the Trinity River Restoration Program in 2002 and subsequent “ROD flows” in combination with periodic pulse flow augmentations beginning in 2003. Additionally, since 2001, 38.9% of regional water-years had “dry” or “critically dry” designations, including two periods of three consecutive dry water-years associated with regional drought (Table 1; TRRP 2019). Yet the relative abundance of populations of anadromous and non-anadromous taxa we studied began declining prior to 2007 and continue to do so (Figure 3 and Figure 4).

Albeit some watersheds and associated streams in the upper Trinity River basin suffered from the effects of regional drought over the last several years (CDFW 2019), the mainstem Trinity River and its associated major tributaries did not (Canyon Creek, North Fork Trinity River, South Fork Trinity River, and New River; Figure 1). This was because management of annual hydrographs resulted in release of flows down the Trinity River throughout all drought-years and tributary accretion of water from this segment of the watershed was less relative to inflow from the major tributaries mostly below the mainstem Trinity River. Additionally, although a recent assessment of the effects of drought on critical habitat for nine

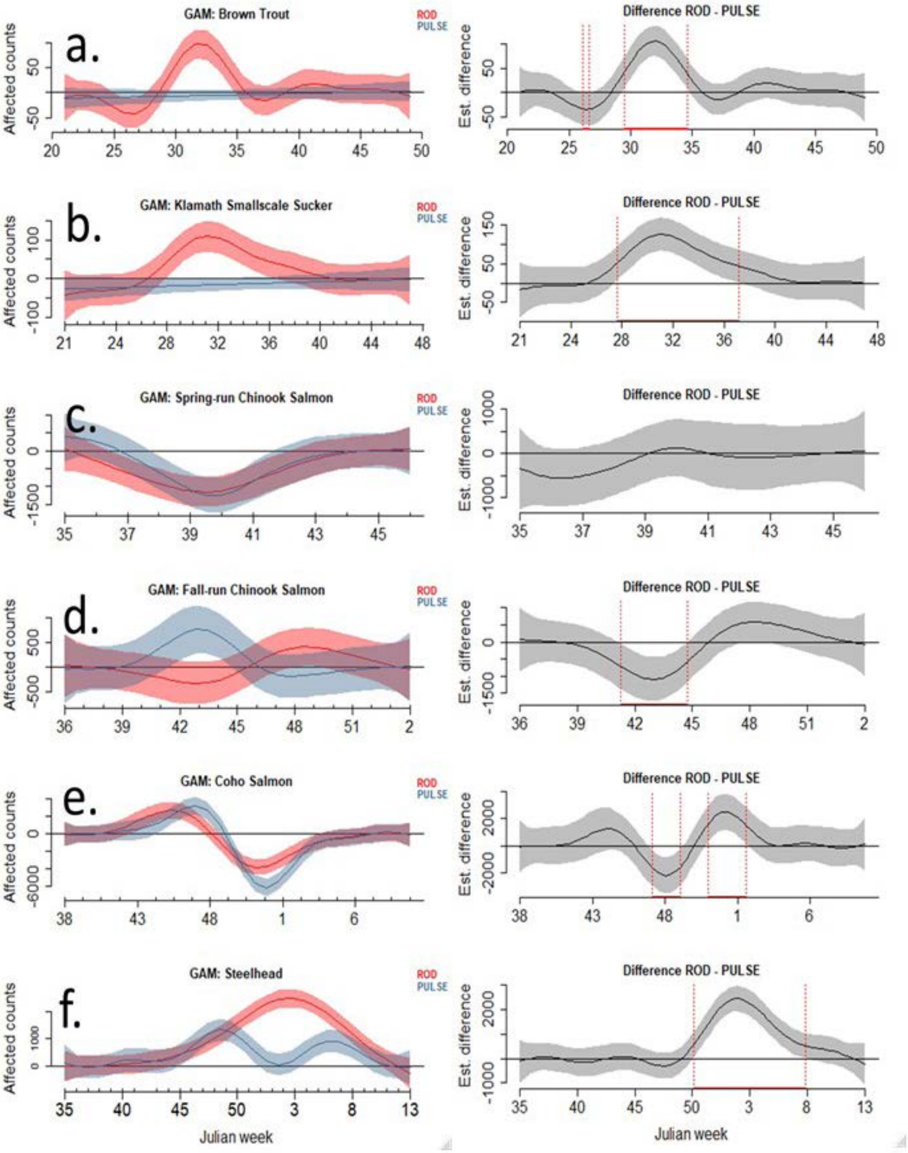

Figure 7. Species-specific GAM plots and their companion differenced plots showing differences between ROD and Pulse flow-types based on the number of counts (positive + negative) that deviated from the baseline PreROD flow-type. Red colored dashed vertical (y-axes) and solid horizontal lines (x-axes) identified Julian weeks that were significantly different ($P < 0.05$) between GAMs of each species-specific pair of ROD and Pulse flow-types, including approximate 95% point-wise standard error bands for each curve of the factor level. X-axes (independent variable/predictor) labeled with the covariate name. Scale of the y-axis in the difference smooth is the same as the link function (link = "identity") of the model because affected data included both positive and negative counts.

streams in watersheds within the upper Trinity River basin found significant reductions in streamflow, water temperatures remained suitable for survival of Coho Salmon and steelhead in four of the nine streams monitored, including Canyon Creek the northernmost and only major tributary of the mainstem upstream of the North Fork Trinity River (CDFW 2019). Yet this report provided no assessment of 1) the three larger tributaries of the Trinity River below Canyon Creek, 2) the effect on the mainstem Trinity River, or 3) the potential impact of in-basin regional drought on productivity derived from natural-origin spawning for any species of salmonid in the streams studied or in major tributaries of the mainstem Trinity River (CDFW 2019).

Similarly, whereas several environmental documents have determined no significant impact to populations of salmonids in the Trinity River from implementation of ROD flows or Pulse flows (USBR 2016 and references therein), there has been no quantified assessment of the potential effects of altered flow regimes on run-timing or impacts to female reproduction performance, relative to Pre-ROD baseline conditions, for any species of salmonid, whether hatchery- or natural-origin spawning. Moreover, whereas factors responsible for decreasing stocks of anadromous salmonids in both the Trinity and Klamath rivers frequently reference recent ocean conditions and regional drought (Dettinger and Cayan 2014; Diffenbaugh et al. 2015; Mann and Gleick 2015; Michel et al. 2015; Adams et al. 2017), yet to be fully documented is the degree to which these conditions pose threats to inland fisheries, as a function of changing climate (Sullivan and Hileman 2019). Importantly, Lawson et al (2004) and Michel (2018) emphasize that climatic dynamics that led to increases or decreases in precipitation over inland portions of the geographic range of anadromous salmonids may also influenced marine conditions in a manner not captured by marine productivity indices. For example, contrary to the popular belief that “the ocean did it,” recent telemetry-based information on hatchery-origin fish suggests that marine mortality may not be the primary source of variability in cohort size, and that out-migrant survival (freshwater seaward migration) associated with flow is likely more important (Michel 2018). The hypothesis that fisheries managers potentially have control over as much as 35% of the annual variability in production of hatchery populations of salmon, thus potentially buffering populations from negative effects of poor marine conditions, provides significant incentive to do a better job of monitoring flow dynamics during out-migration (Michel 2018). However, declines in the quality of the marine food web, which support anadromous salmonid life histories, could not have affected relative abundance in Brown Trout or Klamath Smallscale Suckers, because neither taxon exhibits anadromous migratory behavior, yet both species exhibited concordant patterns of annual decline in relative “synchrony” with all anadromous species described herein.

Seasonal fluctuations in migration in relation to flow type

Our study showed that seasonal variation in Julian week counts of all anadromous and non-anadromous species 1) appeared more attuned to timing of managed flows and water temperature than to flow volume, and 2) all species-specific baseline PreROD flow patterns were affected by managed flow-types in both addition or deletion of counts at some point in their seasonal Julian week migration or runtime schedules since 2003. Additionally, whereas several studies have yet to demonstrate a clear relationship between pulse flows and fish movement (Thorstad and Heggberget 1998; Thorstad et al. 2003; Hasler et al. 2014; Peterson et al. 2017), we found significant differences between ROD and Pulse flows in several taxa (Table 3, Figure 8). These findings suggest to us that pulsed flows likely represent an important additional and independent factor affecting the pattern and timing of migration, irrespective of their accompanying ROD flow segment, relative to a "purely" ROD flow hydrograph, which dominated most patterns we described.

Notably, not all taxa exhibited deviation away from their species-specific baseline PreROD flow pattern through reduction in counts early to mid-season and increasing counts late in the season, which resulted in displacement of the actual timing of migration in post-2003 flows to later in migration or run-timing. Instead, several species, including spring-run Chinook Salmon, Coho salmon, and steelhead demonstrated variations on this run-time theme. That both ROD and Pulse flows have altered the pattern and timing of migration, relative to the baseline PreROD flow condition, in both anadromous and non-anadromous species, which exhibit very divergent life histories and in a significant and concordant way, provides evidence in support of our hypothesis of significant difference in timing of local migration or run-timing in response to annually altered flow schedules. The observation that both anadromous and anadromous species respond behaviorally to altered flow regimes, is consistent with the recent suggestion that velocities and higher turnover rates of water associated with the magnitude and duration of additional water provided by pulse flows are likely more important than quality of additional cold water from the Trinity River intended to stimulate fish to move for prevention of potential disease outbreaks in the lower Klamath River (Strange 2010; Peterson et al. 2017). Likewise, Peterson et al. (2017) used a variety of environmental attributes to assess the relative influence of pulsed flow augmentation to explain the magnitude of daily trap-counts and proportions of fall-run Chinook Salmon in the Stanislaus River, California. They concluded that although managed pulse flows resulted in immediate increases in daily passages, the measured response was brief, representing only a small portion of the total run, relative to a stronger response between migratory activity and discharge levels. As relates to the upper Trinity River, we interpret these observations to be more reflective of the effects of implementing annual ROD flows as opposed to short-term pulsed flow augmentations. However, pulsed augmentations may be very influential in stimulating declining stocks of spring-run Chinook Salmon to migrate from the estuary at the mouth of the Klamath River into the upper Trinity.

Management implications

Although we show that both non-anadromous and anadromous species responded behaviorally to flow augmentation, a test of the hypothesis that managed flow regimes also effect reproductive performance post-2003 in anadromous salmonids necessarily requires information on adult female reproductive performance for both hatchery-spawning and natural-area spawning fish, particularly if ROD and Pulse flow augmentations continue indefinitely. As in iteroparious (multiple reproductive cycle life histories), Brown Tout, Klamath Smallscale Suckers, and steelhead flow-related impacts to multiple brood-year cohorts likely have significant implications for co-occurring semelparous (single reproductive cycle life history) salmonids, particularly those that overlap in run timing, most notably spring- and fall-run Chinook Salmon. Currently, none of the issues discussed above have been part of any long-term effects analysis to protect adult anadromous salmon in the lower Klamath River, even though flows designed to facilitate such protection originate in the upper Trinity

River (USBR 2016). As of 25 July 2016, there was no plan to address these issues for any species of adult salmonid in the upper Trinity River or as part of proposed environmental impact assessments (M. Paasch, personal communication, 2016).

Scientific and policy communities increasingly acknowledge the need for maintaining or restoring natural flow variability to sustain the ecological health of fluvial ecosystems (Rytwinski et al. 2017). Maintaining or "mimicking" features of a natural flow regime is paramount to any successful management strategy designed to conserve freshwater biodiversity (Marchetti and Moyle 2001; Viers and Rheinhemir 2011). To accomplish this goal requires an understanding of the mechanisms that structure riverine communities using a multiscale approach that recognizes the nested physical hierarchy of natural river systems (Fausch et al. 2002; Lowe et al. 2006). Our study suggests that further investigations into the effects of flow management on migration and life history strategies and requirements in sympatric multispecies assemblages of non-anadromous and anadromous species of fish inhabiting the Trinity River are warranted and necessary. For these reasons, we recommend the following management strategies:

1. Because life history differences among taxa are important for assessing responses to flow manipulations (Konrad et al 2011), it is necessary to implement long-term and carefully designed field studies using controls that test species-specific hypotheses in relation to life history requirements potentially influenced by physical elements of the riverine environment. This approach, combined with long-term data sets, modeling, and monitoring of the effects of flow management on lotic environs is essential in identifying the underlying mechanistic links that inform environmental flow standards specific to regional riverscapes (Konrad et al. 2011; Mims and Olden 2012).
2. Develop an integrated management strategy that includes comparative analyses and synthesis of potential impacts of managed flow regimes on timing of migration, population size, age structure, individual performance, composite reproductive output, and recruitment of both hatchery production and in-river spawning in populations of adult anadromous salmonids (Peterson et al. 2017; Rytwinski et al. 2017).
3. Initiate annual comparative monitoring of production in juvenile and adult age classes, annual and seasonal frequency distributions, and migration patterns before, during, and after flow augmentation. This action will help determine if a change in flow regimes acts either as a temporary stimulus or as a retardant to both upstream and downstream movements, and run-timing, particularly as it applies to survival through flow management and other associated beneficial riverine conditions during the out-migration season in populations of hatchery-origin salmonids.
4. Assemble and initiate inspection, coordination, and integration of historical flow related information with covering physical riverine attributes, and hatchery records, procedures, and production mandates with agency flow and operational mandates, in tandem with in-river restoration activities and collection of long-term biological data to provide insight into potential effects of planned management of flow augmentation on all fisheries resources.
5. As part of the overall coverage of the fluvial ecosystem and the fisheries resources supported, we recommend integrating non-anadromous species into monitoring and modeling analyses, as these taxa have historically not been a management priority for the upper Trinity River. Because these resident species are not affected by marine conditions, they potentially are better suited as potential "control" or "indicator" taxa for assessing year-round effects of

managed flows, restoration actions, and variation in other intrinsic environmental co-variates concurrently with studies on commercially valuable anadromous salmonids.

Adoption of such recommendations will allow a better understanding of the potential for managed flows in facilitating conservation of fisheries resources in connected and co-varying segments of regulated river systems (Hasler et al. 2014; Peterson et al. 2017); thus enabling practical flow management based on hypotheses-driven study designs applied to priority issues (Watts et al. 2010; Viers and Rheinhemir 2011). Such actions are an integral part of any coordinated science-based adaptive management program, which was in large measure the original vision of the Record of Decision outlining a plan for restoration of the Trinity River and its populations of fish and wildlife (USBR 2000). Recommendations developed herein are particularly relevant given: 1) fluctuations in influential ocean conditions, 2) climate change and associated regional drought (Rupert et al. 2017a,b), and 3) increased environmental degradation and pollution of watersheds from illegal growing of marijuana throughout the Trinity River basin (Welsh 2011; Kilduff et al. 2015; Murad et al. 2018). Immediate, however, are the social, political, and scientific pressures to ‘legitimize’ rivers as water users continue to aggressively focus on compromises in water-flow management and policy in California and elsewhere, to meet both human and ecosystem water needs through provisioning of “environmental flows” (Arthington et al. 2006; Arthington et al. 2010; Konrad et al. 2011). Given the growing momentum in recognition of the need for ecologically sustainable water management, lack of scientific information often cited by resource agencies, is no longer a valid excuse for failure to endorse application of such approaches to ensure maintenance of healthy and productive aquatic ecosystems, and sustainability of resident riverine fisheries (Viers and Rheinhemir 2011; Mims and Olden 2013). Inevitably, these issues will become more problematic as climate change predictably progresses in step with the insatiable anthropocentric demand for water (Tockner et al. 2010).

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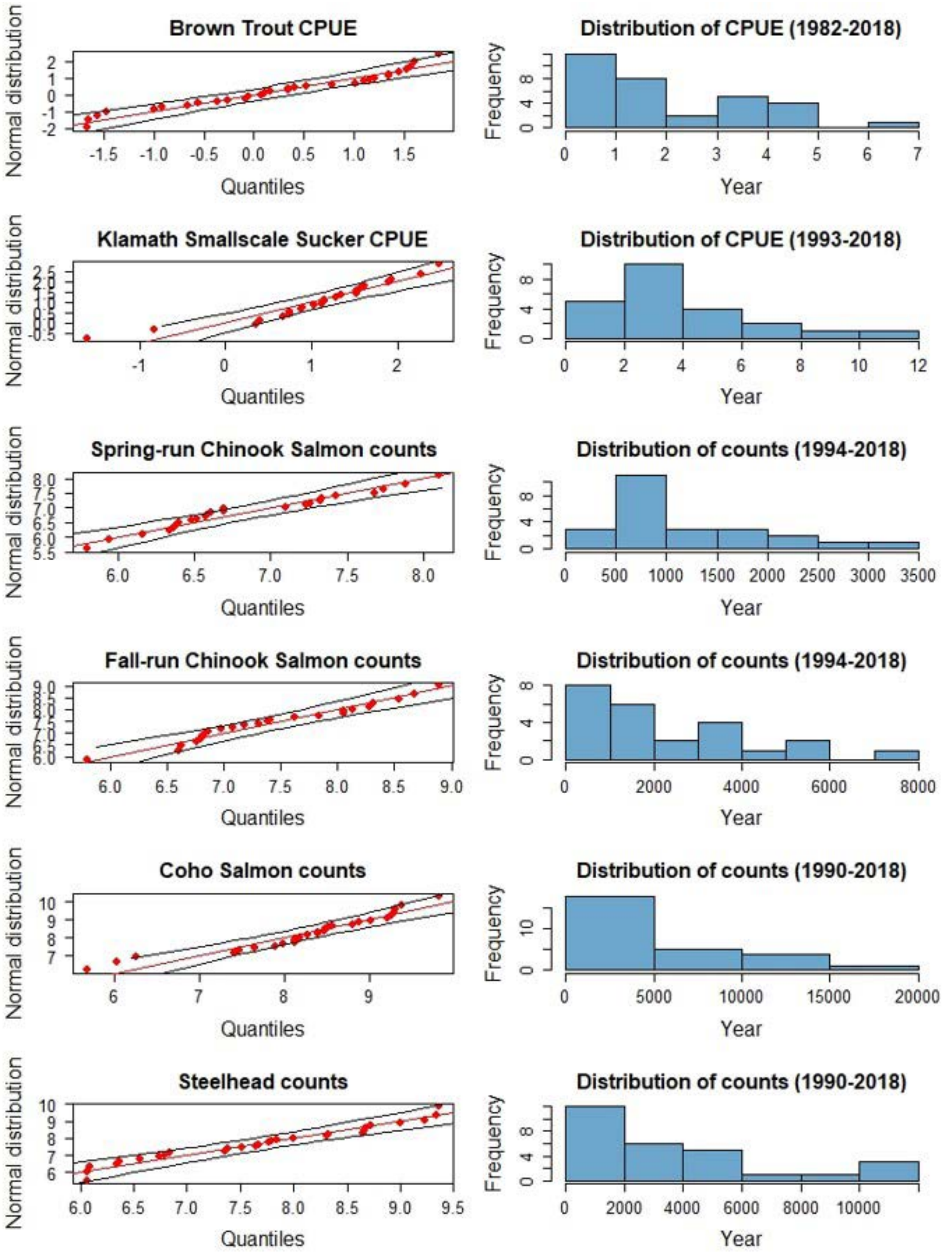
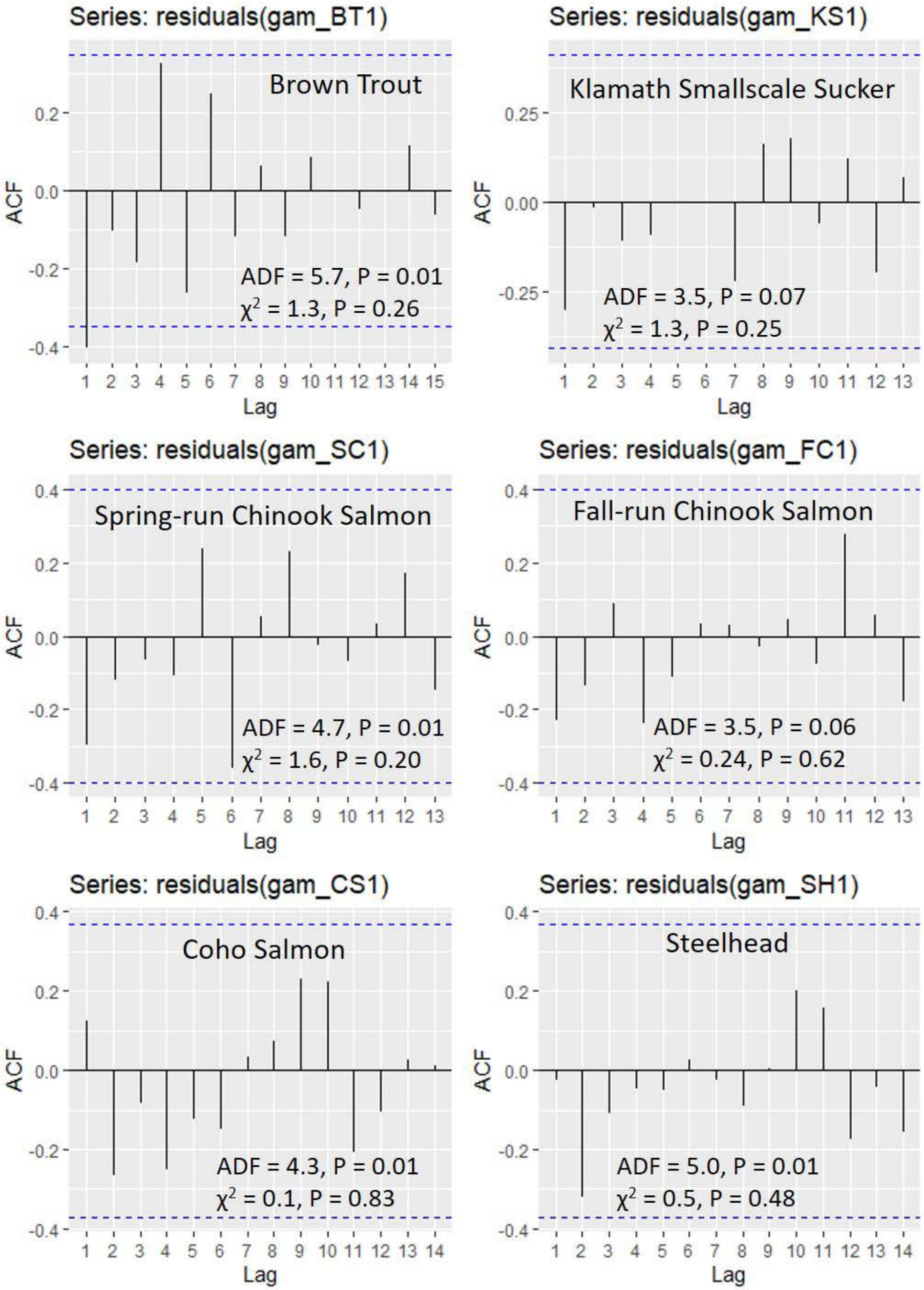
Appendix I. Normal quantiles plots and histograms of the frequency distributions catch per unit effort (CPUE) and counts of annual data for all species of fish. Dashed black lines represent 95% confidence limits for the fitted normal quantile plots for each count variable. Relative normality is assumed if all red points fall approximately along the reference solid black line.



Appendix II. Results of the autocorrelation analyses of residuals derived from generalized linear model (GAM) analyses of annual catch per unit effort (CPUE) and counts of all species of fish, including follow-on Box-Pierce ($\chi2$) and augmented Dickey-Fuller tests (SDF).



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Feasibility of hydroacoustic surveys of spawning aggregations for monitoring Barred Sand Bass populations off southern California

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For fishes that migrate to specific locations to spawn within large aggregations at predictable times, fishery independent surveys of the abundance, distribution, and population structure of adult fish at spawning aggregation sites can provide valuable data for fisheries monitoring and assessments. We tested the feasibility of using high resolution, split-beam sonar to estimate the distribution, abundance, and group sizes of Barred Sand Bass (*Paralabrax nebulifer*) at their primary spawning aggregation site off Huntington Beach, California, in July 2010 and July 2012. We established an *in-situ* target strength distribution for Barred Sand Bass using tethered fish, collected hydroacoustic data opportunistically over the entire spawning grounds, and validated acoustic data with concurrent video surveys and rod and reel sampling of fishes present within the survey area. The modal target strength of Barred Sand Bass was determined to be -35 dB and was distinct from other fish species present. Groups of Barred Sand Bass averaged 30 individuals in abundance and ranged from 2 to 1,711 individuals, with the vast majority of the groups containing less than 10 individuals. Groups of Barred Sand Bass were most abundant in the water column between 5 and 10 m below the surface over bottoms depths of 20 to 30 m, resulting in a negative relationship between group size and depth. Due to the sand bottom habitat of the spawning site, the tendency for fish to aggregate to spawn in the water column during predictable periods, and the low diversity of other fish species present at the spawning site during the peak spawning months, hydroacoustic surveys of primary spawning aggregation sites represent an efficient, practical method for regional population monitoring and fishery assessments of Barred Sand Bass.

Key words: Barred Sand Bass, fishery independent assessment, hydroacoustics, spawning aggregations

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Many marine fishes migrate to form large spawning aggregations that are predictable in time and space, which support very productive commercial and recreational fisheries (Erisman et al. 2017). These aggregations represent a paradox of sorts, as the same aspects that facilitate efficient reproduction make them such ideal targets for fisheries (i.e. large biomass of fishes concentrated at known sites and times) also allow them to be easily and rapidly overfished. For that reason, aggregation fisheries tend to follow a "boom and bust" cycle in which a few years of immense harvest levels are often followed by rapid declines in catch and stock abundance (Sadovy de Mitcheson and Erisman 2012). Widespread declines in spawning aggregations and their fisheries have stimulated increases in targeted efforts to mitigate the negative ecological, social, and economic impacts associated with overfishing them (Nemeth 2005; Aburto-Oropeza et al. 2011; Hamilton et al. 2011; Heppell et al. 2012).

The successful management of aggregation fisheries is predicated on the ability to accurately and rapidly identify changes in the status of the stock or aggregations so that regulatory agencies can respond in a timely manner, which can prove challenging when conventional fisheries monitoring techniques are incongruent with the dynamics of spawning aggregations. Conventional fisheries-dependent (e.g., catch-per-unit effort) and fisheries-independent (e.g., visual censuses) protocols both tend to rely on density-based estimates as proxies for monitoring changes in stock abundance, which can be problematic for assessments of certain spawning aggregations, because the density of fish within aggregations may remain stable even as the total abundance of fish and the aggregation area declines (Erisman et al. 2011). This issue is referred to as hyperstability in fisheries science and can result in the overestimation of population biomass and delayed responses to population declines (Rose and Kulka 1999). Also, while visual censuses work for assessing aggregations located in well-delineated areas of reef in clear, shallow (< 30m) waters of the tropics, they may be less efficient for surveying aggregations in temperate, offshore areas where visibility is poor and fish are widely dispersed across large areas (Colin et al. 2003; Heyman et al. 2017).

Hydroacoustic surveys – here defined as active acoustic surveys with an echosounder – are advantageous for assessing fish populations due to their ability to quickly and efficiently cover large areas, record data instantly over nearly the entire water column, and minimization of the selectivity and observer biases that can be associated with other methods (Trenkel et al. 2011; Yurista et al. 2014). As they are non-invasive and can be conducted over a wide range of depth and visibility conditions, hydroacoustic surveys are suitable for many ecosystems and environments (Murphy and Jenkins 2010). However, hydroacoustic surveys are most commonly used to assess homogenous pelagic fish populations in areas with low diversity, as estimation of target strength (TS) for a given species – a critical step in calculating fish density, abundance, and biomass – is confounded by the presence of other species, size, and position in the water column (Simmonds and MacLennan 2005; Zenone et al. 2017). Thus, rigorous ground truthing with complementary methods is essential for drawing inferences about a given species with hydroacoustics (McClatchie et al. 2000; Simmonds and MacLennan 2005). With ground truthing, hydroacoustic surveys have been established as a useful method of assessing spawning aggregations of fishes (Fudge and Rose 2009; Rose and Leggett 1987; Kloser et al. 1996; Rowell et al. 2017; Egerton et al. 2018; Michaels et al. 2019). Moreover, acoustic surveys may be ideal for robust, quantitative estimates of the density, distribution, and abundance of fishes that aggregate to spawn in the water column, which greatly reduces potential biases associated with the close association of fish with bottom substrate (Egerton et al. 2018).

Barred Sand Bass (*Paralabrax nebulifer*) is a coastal marine fish that ranges from Santa Cruz, California south to Baja California Sur, Mexico, including Guadalupe Island (Kells et al. 2016). Juveniles and adults occupy a variety of different habitats including kelp beds and sand flats on the open coast to inland harbors and bays (Allen et al. 2006). Relatively sedentary and rarely found more than 3 m above the substrate during non-spawning times, Barred Sand Bass form spawning aggregations up in the water column in waters 15 – 40 m deep over soft bottom areas (Turner et al. 1969; Feder et al. 1974; McKinzie et al. 2014; Teesdale et al. 2015). Seasonal patterns in reproduction are consistent across the species' range, with gonadal maturation beginning in April to May and spawning occurring from late June through early September with a clear, strong peak in July and August (Bautista 2014; Jarvis et al. 2014b; Erisman et al. 2017). Based on the collection of ovulated eggs from actively spawning females and vertical movement patterns of tracked fish, spawning is thought to occur in the water column during the mid-day and afternoon hours (Oda et al. 1993; McKinzie et al. 2014). Barred Sand Bass eggs and larvae are pelagic, drifting in open water, and juveniles appear in shallow water from late summer to early winter (Love 1996).

Barred Sand Bass are one of the most commonly caught game fish in southern California, where they have represented a major source of revenue for the local commercial passenger fishing vessel (CPFV) fleet for more than five decades (Schroeder and Love 2002; Dotson and Charter 2003; Jarvis et al. 2014a). The regional recreational fishery for Barred Sand Bass occurs almost exclusively at five sites that collectively represent the main locations of their summer spawning aggregations: Imperial Beach, San Onofre, the Huntington Flats, Santa Monica Bay, and the Ventura Flats (Figure 1). Barred Sand Bass consistently ranked among the top five species in the southern California marine recreational fish catch since the 1970s and represented the most important recreational fishery in the region from the late 1980s to the early 2000s (Oliphant 1990; Jarvis et al. 2014a). However, persistent fishing of their spawning aggregations combined with unfavorable environmental conditions for larval recruitment resulted in severe fishery and population declines in the mid-2000s that have not yet recovered (Erisman et al. 2011; Miller and Erisman 2014; Jarvis et al. 2014a).

The California Department of Fish and Wildlife (CDFW) implemented regulatory changes in 2013 that reduced the daily bag limit from 10 to 5 fish per angler and increased the minimum size limit from 12 to 14 in (30.48 to 35.56 cm) total length (TL) as a means to stimulate recovery of the regional stock and fishery. However, there is a lack of fishery-independent data on the abundance of Barred Sand Bass in southern California and thus a need to create long-term monitoring program to create a fishery independent index of abundance to assess how the stock responds to changes in management regulations, annual variations in environmental conditions, and fishing pressure.

Recently, Davis et al. (2019) compared two fishery-independent survey methods and determined that underwater visual census (UVC) and baited remote underwater videos (BRUVs) were both effective for a long-term monitoring study of Barred Sand Bass at the edges (ecotone) of inshore natural and artificial reefs in southern California where they are known to occur during the non-spawning season. We contend that monitoring of the spawning aggregations that occur away from reefs, over soft bottom habitats, would be the ideal way

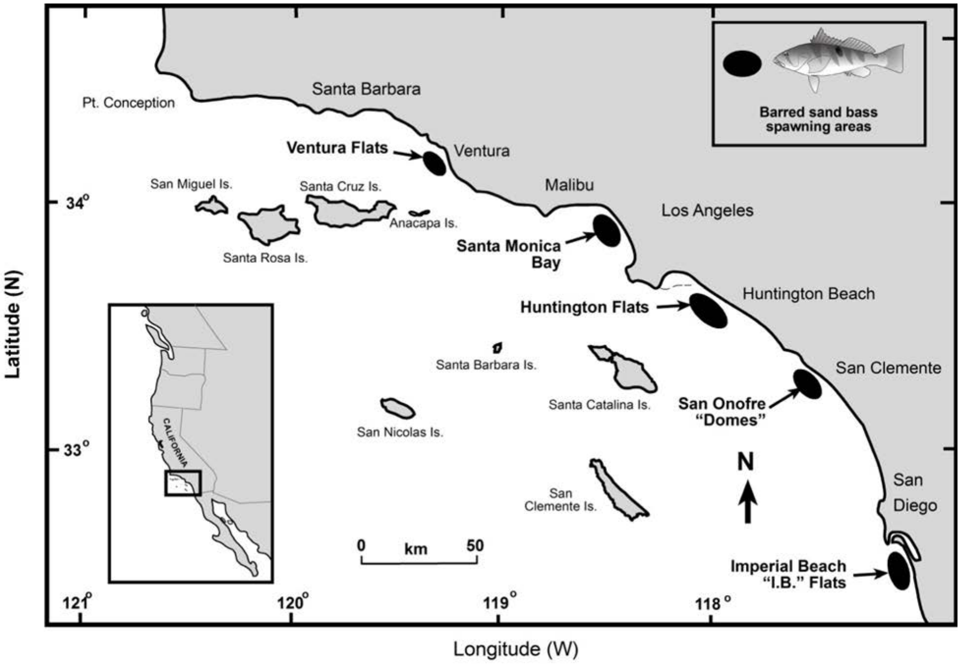


Figure 1. Locations of the five major spawning areas of Barred Sand Bass occupied each year from June to August, historically peaking in July.

to monitor adult abundance and biomass over time. These sites contain the largest numbers of adults and thus are more representative of the regional population of Barred Sand Bass.

For the present study, we explored the feasibility of using active acoustics to survey spawning aggregations of Barred Sand Bass to generate information on adult densities, abundances, and distributions for use in regional monitoring and fishery assessments. Given the spawning behavior of Barred Sand Bass (i.e., aggregate in the water column over sand bottom habitats), the challenging environmental conditions that restrict diver surveys (strong currents, poor visibility, boat traffic) at spawning aggregation sites, and the predictable timing and locations of spawning aggregations, we hypothesized that acoustics would represent an efficient and non-invasive way to survey an entire aggregation site in a systematic, repeatable, and logistically feasible manner. Here we present the results of this preliminary study to test this hypothesis and discuss potential approaches for creating a long-term monitoring protocol for Barred Sand Bass spawning aggregations in the region.

METHODS

Site description

We conducted hydroacoustic surveys on 15, 16, 22, and 23 July 2010 and 18, 19, and 20 July 2012 off Huntington Beach, CA, USA (between 33° 41.0' N, 118° 08.0'W and 33° 39.0' N, 118° 05.0'W) to describe the spatial distribution and group sizes of Barred Sand Bass spawning aggregations. Active acoustic transects were performed across the Huntington Flats area, which is a large, low-relief sandy habitat that occurs between 3-5 km off the coast of Huntington Beach, California (Figure 1) with a depth range of approximately 15-30 m. This area is adjacent to two shallow water oil platforms to the north, with its northwest limit surrounded by a scattered network of artificial reefs, collectively known as Bolsa Chica Artificial Reef. Northwestward of the artificial reef is an area commonly used as an anchorage by large commercial freight vessels, which is just southward of an area known to local anglers as the "Sand Bass Junction." It is well known among the local sport angling community that Barred Sand Bass can be found along the Long Beach and Los Angeles Federal Breakwaters for most of the year, but during the summer months, they are easiest to catch in large numbers on the Huntington Flats in the mid to upper water column (McKinzie et al. 2014).

Acoustic data collection

Data collection employed the use of a BioSonics® DT-X portable split-beam echosounder (206 kHz) integrated with Garmin™ GPS detection. The opening angle of the beam emitted from the circular transducer was 6.8°. We acquired data digitally on VisAcq® acoustic acquisition software on a Panasonic® Toughbook laptop computer. The ping rate was set to 5/s, and the pulse duration was set to 1.0 ms. Calibration of the echosounder was performed on each survey event using a -41.5 dB tungsten carbide sphere according to the standard methods of Foote (1987). We pole-mounted the echosounder on the port side gunwale aboard the R/V Yellowfin, a 24 m research vessel, and transects were conducted opportunistically throughout the Huntington Flats area. Data were recorded over approximately 20 km on each survey day. All surveys were conducted at a speed of 6 knots, and occurred from 0800-1500 h each survey day, as this period was centered on the time of day when Barred Sand Bass are likely to be actively spawning (Oda et al. 1993; Bautista 2014).

Target strength characterization

Based on our rod and reel collections, spawning adult Barred Sand Bass in this region are largely uniform in size (290 ± 29 mm SL), so it was not necessary to develop a target-strength (*TS*) to length relationship. Instead, the target strength distribution of representative individuals was characterized in three principle ways, through 1) rod and reel sampling of specific sonar targets (Figure 2), 2) video confirmation of target species, and 3) tethering of specimens lowered into the sonar cone. We conducted ground truthing of acoustic data on a subset of groups detected by the echosounder. When a school was detected on sonar, video was captured after short time delay, by the Deep Blue Pro Color Underwater Video Camera being towed 10 m directly behind the transducer at the approximated depth of the school.

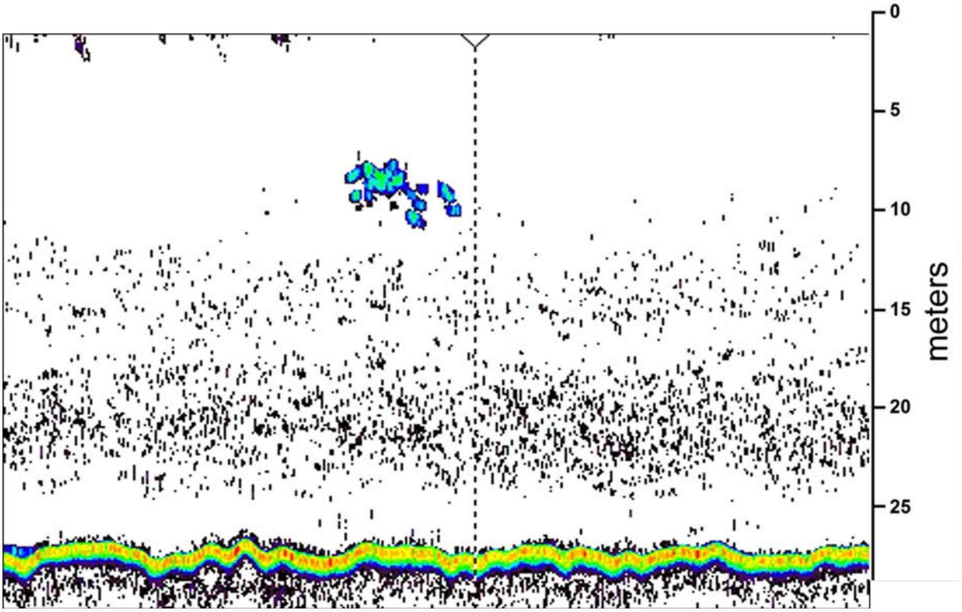


Figure 2. Sonar target verified as Barred Sand Bass aggregation in water column by rod and reel sampling (6–10 m depth); 22 July 2010.

This video was subsequently analyzed to determine the species composition of schooling and surrounding fishes. To account for the influence of gear bias, we also conducted angling as a complementary method of ground truthing. The morphology of these groups was noted to further assist analysts with identification of Barred Sand Bass for the schools that were not ground truthed with camera or angling. *In situ TS* characterizations were performed on 22 and 23 July 2010 and again on 6 August 2012. In separate trials, we collected live Barred Sand Bass and Pacific Mackerel (*Scomber japonicus*) by rod and reel and tethered to a hookless ganion and allowed to swim at different orientations within the acoustic beam.

Acoustic data analysis

We performed all acoustic data analyses in Echoview ® v7.0. Surface noise caused by wave action and bubbles was excluded from the analysis, and a one-meter exclusion zone was created to exclude backscatter from the seabed and the acoustic ‘dead zone’ that occurs above the seabed. Time varied gain corrections of 40log(R) for *TS* and 20log(R) for s_v, known as the volumetric backscattering coefficient, were applied.

Aggregations were identified manually by analysts, and echo integration was performed to generate estimates of fish density within each school, following this formula:

$$\frac{s_v}{\sigma bs}$$

Where s_v is the volumetric backscattering coefficient (a measure of the total acoustic energy in a volume of water), and σb is the cross-sectional backscattering coefficient (a measure of the acoustic energy that can be attributed to a single target). We integrated all schools using a value for σbs generated by converting a TS of -35 dB, which was the modal TS of Barred Sand Bass in the region based on TS characterization experiments (Fig. 3). σbs is related to TS by the following relationship:

$$\sigma bs=10log_{10}(TS)$$

Because this species has been suggested to increase vertical activity during spawning events (McKinzie et al. 2014), the average depth was manually recorded for each target. Group size was then determined by extrapolating the number of individual targets in the group.

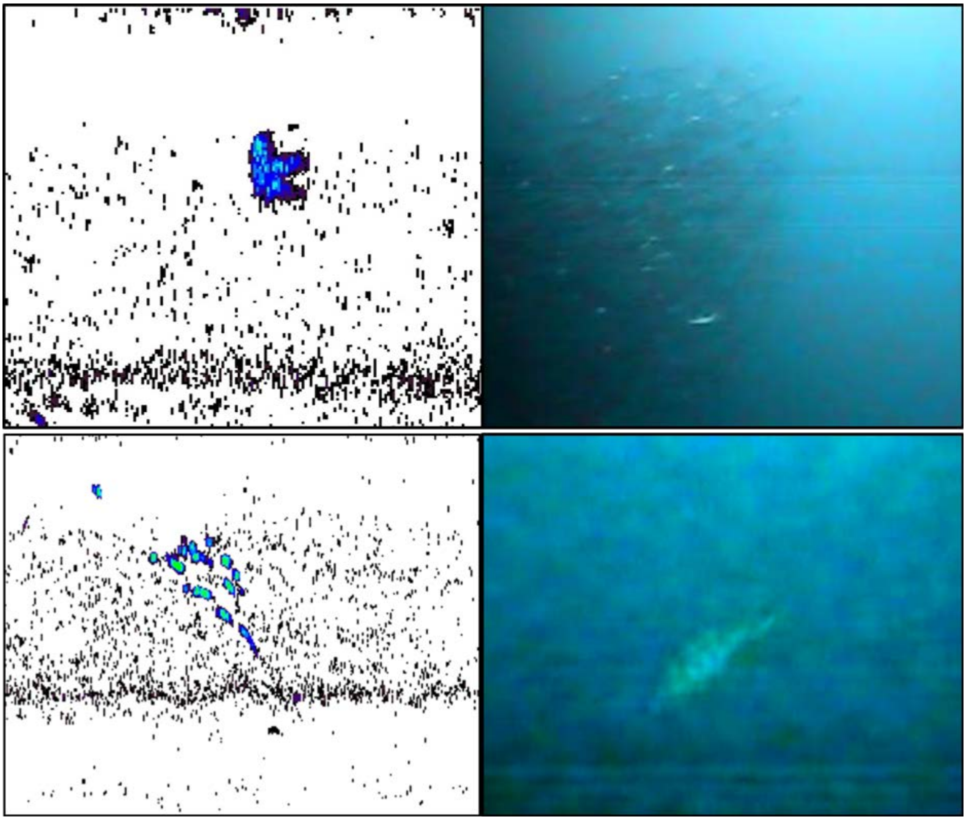


Figure 3. View of sonar record of a school of Northern Anchovy (top) and an aggregation of Barred Sand Bass (bottom) with enlarged still frames from video camera towed 10 m behind the superimposed for both cases; 23 July 2010; 1054–1056 hrs. Video stills of successful verifications were courtesy of Charles Valle, California Department of Fish and Wildlife.

RESULTS

Approximately three hours of video recording conducted on July 23, 2010 yielded four successful video verifications of sonar targets as Barred Sand Bass and one as a school of Northern Anchovy (*Engraulis mordax*). The first successful verification occurred between 1054 and 1056 h when first a school of Northern Anchovy and then a loose aggregation of Barred Sand Bass were detected by both sonar and the video camera which was towed 10 m behind the sonar cone (Figure 3). In this case, video revealed at least 6 individuals in the group where sonar detected 11. The distance of the camera from the sonar transducer, the low visibility, and the escape response of Barred Sand Bass accounted for this difference. Three additional video target verifications of Barred Sand Bass in the water column occurred at 1127, 1132 and 1345 h the same day. On July 22 and 23, 2010, specimens of both Barred Sand Bass and Pacific Mackerel were collected by rod and reel and tethered to a hook-less ganion. This apparatus was lowered into the sonar cone along the port side of the research vessel and staged at several depths while the Biosonics unit continuously recorded (Figure 4). These activities served to accurately calibrate the range, frequencies, and mean target strengths of both species that were numerically dominant in the sampling area. Utilizing sonar recordings of the targets verified by tethering combined with underwater video, the mean *TS* of Barred Sand Bass was determined to be approximately -35 ± 4 dB (Figure 5). The mean *TS* of Pacific Mackerel was -48 ± 5 dB and Northern Anchovy, -56 ± 10 dB with virtually no TS overlap with target species.

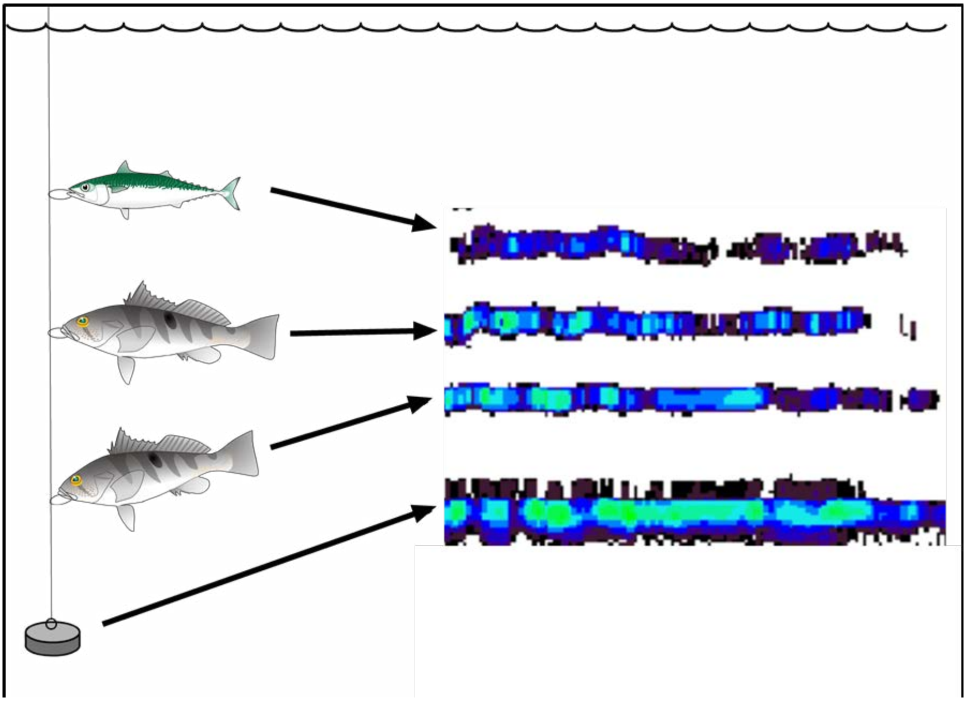
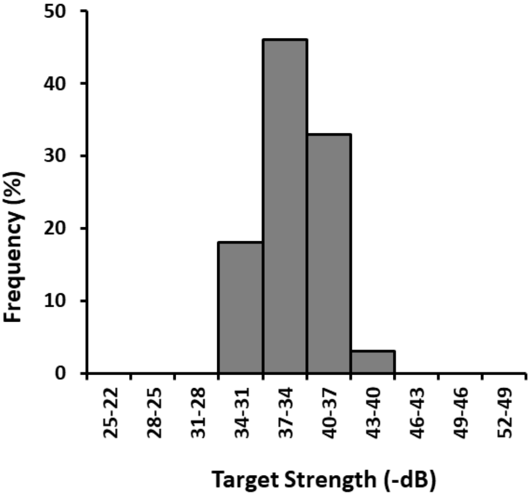


Figure 4. Sonar recording of tether apparatus for ground truthing *TS*; 23 July 2010; (top to bottom) one Pacific Mackerel, two Barred Sand Bass, and 1 kg weight.

Figure 5. Frequency of occurrence of target strength scores for tethered Barred Sand Bass on August 5, 2012 at a frequency of 206 kHz.



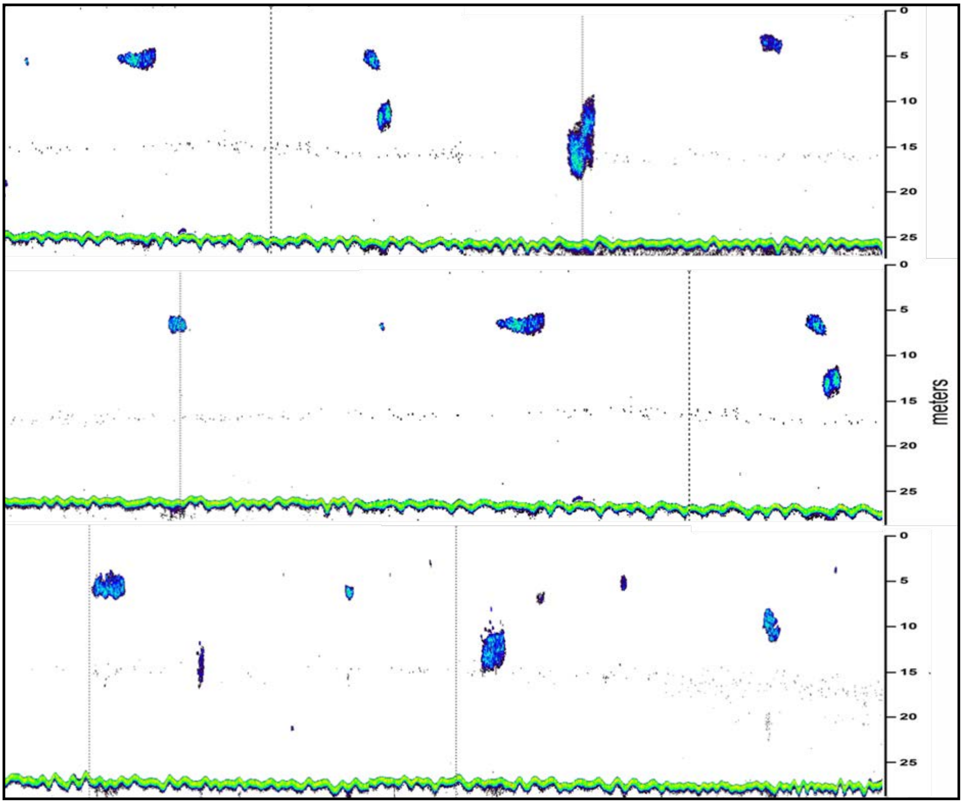


Figure 6. A collage of separate echograms taken from alongshore transects run from July 18 to 19, 2012 showing examples of the various sizes, configurations, and depths of Barred Sand Bass aggregations identified by Echoview ® 5.2 software based on a modal target strength of -35 dB. Depth scale is 1–25 m.

Aggregating groups of Barred Sand Bass were identified in the water column between 0900 and 1500 h during all seven sampling days covering July of both 2010 and 2012. A collage of separate echograms taken from alongshore transects run from July 18-19, 2012 showed examples of the various sizes, configurations, and depths of Barred Sand Bass aggregations. Targets ranged from small through large asymmetrical, globular groups to large, spheroidal aggregations (Figure 6).

During the four-day period of July 2010 sampling, 145 groups of Barred Sand Bass were identified in the water column between 1000 and 1500 h. These groups ranged in relative, estimated size from 2 to just over 1,700 individuals (median = 55). The three-day period of July 2012 sampling yielded a total of 117 groups of Barred Sand Bass were identified in the water column between 1000 and 1500 hrs. Groups ranged in size from 2 to 350 individuals (median = 10) with most of the groups containing less than 10 individuals. Groups of Barred Sand Bass were distributed throughout the water column principally between 5 m depth and the bottom (20 – 25 m) in both summers. However, the largest groups were found almost exclusively between 5 and 10 m depth resulting in significant, negative correlation ($y = -1.582 ln(x) + 17.808$, $R^2 = 0.196$, df = 237, $p << 0.001$) between group size and depth (Figure 7). Based on bathythermograph readings, the depth distribution of the largest groups corresponded closely with the thermocline present most sampling days and indicated that fish were aggregating at temperatures between 16 and 17 °C.

DISCUSSION

Our results indicated that sonar is a feasible and efficient means to assess the distribution and group size of Barred Sand Bass in spawning aggregations. Due to the relatively low diversity of pelagic fishes in the region and behavior of Barred Sand Bass, standardized hydroacoustic surveys would be a viable means to assess the biomass and abundance of spawning Barred Sand Bass in this region.

Barred Sand Bass are known to increase their vertical space usage as they commence spawning activity (McKinzie et al. 2014). However, a major factor to consider when performing active acoustic surveys is that the recorded data are "snapshots" of where fish happened to be at the exact moment when they were insonified. Typically, *Paralabrax* species should be higher in the water column when they are reproductively active, but they should also vary their depths during momentary vertical spawning rushes (Erisman and Allen 2006; Miller and Allen 2006). The Barred Sand Bass is a bottom-associated species rarely found above 3m from the seafloor, primarily inhabiting soft bottom habitats that are associated with ecotone (Love et al. 1996; Mason and Lowe 2010). Therefore, any vertical activity away from structure during the summer months could suggest spawning and/or spawning-related behaviors. However, it could also indicate other behaviors, such as feeding or temperature preferences.

Our findings clearly showed that the largest groups of Barred Sand Bass occurred up in the water column at depths between 5 and 10 m in July of 2010 and 2012. These depths are usually above the prominent thermocline during July off the Huntington Flats. Using acoustic telemetry, McKinzie et al. (2014) described the activity space size and association with seafloor and thermocline were compared for the spawning and non-spawning season Barred Sand Bass at the same location as our study. They found that non-spawning season fish showed affinity with sand/reef ecotone while remaining about 2 m off the seafloor. Spawning season individuals displayed two patterns of behavior, one indicative of spawning and another of resting behavior. Resting individuals tracked during spawning season behaved similarly to fish tracked during the non-spawning season, using smaller activity space areas while associating with reef structures and the seafloor. Presumed spawning individuals utilized sand habitats, using significantly larger activity spaces during the day than at night while associating with the thermocline and making repeated vertical dives toward the seafloor.

The acoustic data processing procedure employed in this study was undertaken to maximize processing efficiency and minimize the influence of confounding factors as much as possible. Integration of schools using a fixed value reduced the influence of multiple

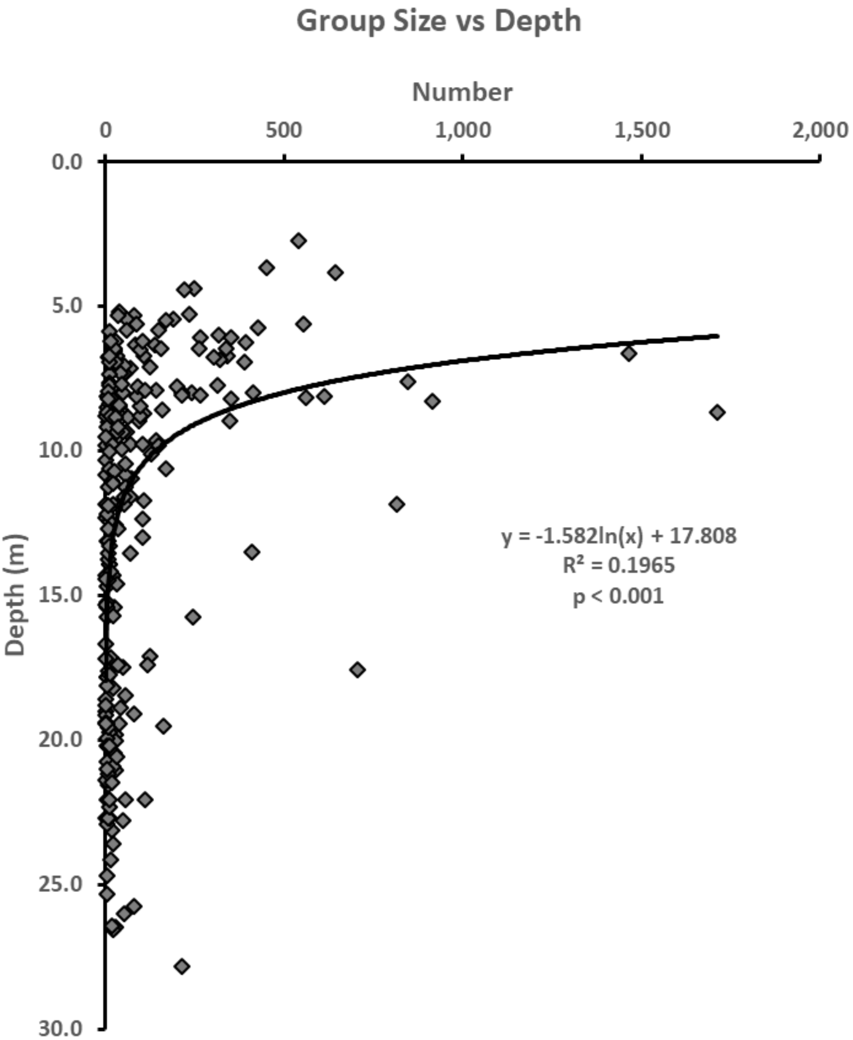


Figure 7. Frequency of group size (# individuals) of Barred Sand Bass aggregations determined from 2010 and 2012 over the Huntington Flats**.**

echoes (i.e. invalid *in situ TS* estimation due to insufficient separation of targets), which can significantly affect density and biomass estimation (Sawada et al. 1993; Yule et al. 2013). It should be noted that ground truthing was not conducted coincidently with all surveys, and therefore it is possible that individuals or schools of other species with similar *TS* to Barred Sand Bass (e.g., Kelp Bass; *Paralabrax clathratus*) may have been counted as Barred Sand Bass, despite the ecological unlikelihood of this (Young 1963; Mason and Lowe 2010). As the diversity of pelagic fishes in this area is limited, with Barred Sand Bass, Northern Anchovy, and Pacific Mackerel making up 73% of pelagic fishes observed in the present study, we view this as unlikely. Further, we found differences in modal *TS* between Barred Sand Bass, Northern Anchovy, and Pacific Mackerel, which were likely driven by differences in body size and swimbladder morphology (Simmonds and MacLennan 2005). Thus, the likelihood of overlap in *TS* was limited. This conclusion was further supported by the angling, tethering, and video ground truthing surveys, from which Barred Sand Bass were found to form monospecific schools and not to associate with other fishes outside of schools. In future studies, the potential for counting other fishes as Barred Sand Bass could be minimized using coincident ground truthing methods, such as the deployment of a towed camera system adjacent to the transducer on a glider or the deployment of a self-rotating video system (Koenig and Stallings 2015) in locations where large groups or aggregations are detected by the echosounder. Both techniques would allow acoustically-derived estimates of abundance, density, and biomass of Barred Sand Bass to be adjusted based on their relative abundance to other fishes present.

With standardized transects that adequately cover the area in which Barred Sand Bass Spawn (i.e. degree of coverage ≥ 6 based on Aglen 1989) and the addition of the processing of single targets or tracked fish within the *TS* range of Barred Sand Bass, a similar approach could be taken to estimate the biomass and abundance of Barred Sand Bass in the region. There are, however, multiple viable alternatives. For example, after selection of appropriately sized Elementary Distance Sampling Units, echo integration with *in situ TS* could be performed over the entire transect as long as the Nv and/or M% indices are calculated to identify multiple echoes (Sawada et al. 1993; Yule et al. 2013). This approach would require ground truthed knowledge of the relative abundance of species in the area for apportioning of density and biomass, and/or filtering of the data such that only targets within the *TS* range of Barred Sand Bass are integrated. Alternatively, echo counting could be used for single targets, but the integration of schools could have been performed with the distribution of *TS* shown from the *TS* characterization experiments, or with the distribution of *in situ TS* found immediately around the perimeter of the school, instead of the single *TS* value we used. Further, fish tracks (i.e. sequences of single targets belonging to the same fish) could be detected and used for echo counting instead of single targets for more conservative estimates. If cost-effective, systematic hydroacoustic surveys of Barred Sand Bass are to be conducted in this region in the future, it would be beneficial for future studies to compare the results from these alternative methods to facilitate better understanding of the influence of data processing choices on abundance and biomass estimates.

Recommendations for continued studies

Our recommendations for continued studies of Barred Sand Bass aggregations off southern California are as follows. Hydroacoustic assessments of Barred Sand Bass populations should be conducted on a weekly basis during July and August (8 weeks per year) to investigate temporal variations in the distribution, biomass, and abundance of fish at the aggregation site by week, month, and year and in relation to spawning activities, environmental (temperature, thermocline) and fishing (catch, CPUE) data collected on a concurrent basis at the site. However, two additional surveys should be conducted each year (total = 10 survey days per year). The first should be conducted to test and calibrate the equipment in preparation for the surveys, and the second will serve as a contingency day if any of the eight survey days experience technical or other issues that compromise the study design and subsequent analyses. Notably, all survey trips should be divided into a morning segment and an afternoon segment based on the time it takes the research vessel to complete a survey over the entire aggregation area. All surveys should be conducted using a sizeable, stable vessel as a platform. The vessel's on-board sonar must be turned off during the survey runs to avoid acoustic interference with the Biosonics unit. Acoustic surveys should cover depths between 15 and 30m over an approximately 15 km^2 area on the major spawning aggregation site, Huntington Flats, off southern California (Figure 8). For each weekly survey at Huntington Flats, the scientific team should conduct four 5 km sonar transects alongshore in each of two, time segments (0800-1200; 1300-1700 h). Sonar targets, depth, and GPS readings should be recorded continuously over the entire cruise track for each day. Echoview ® 7.0 (or higher) software should be used to estimate abundance, biomass, and

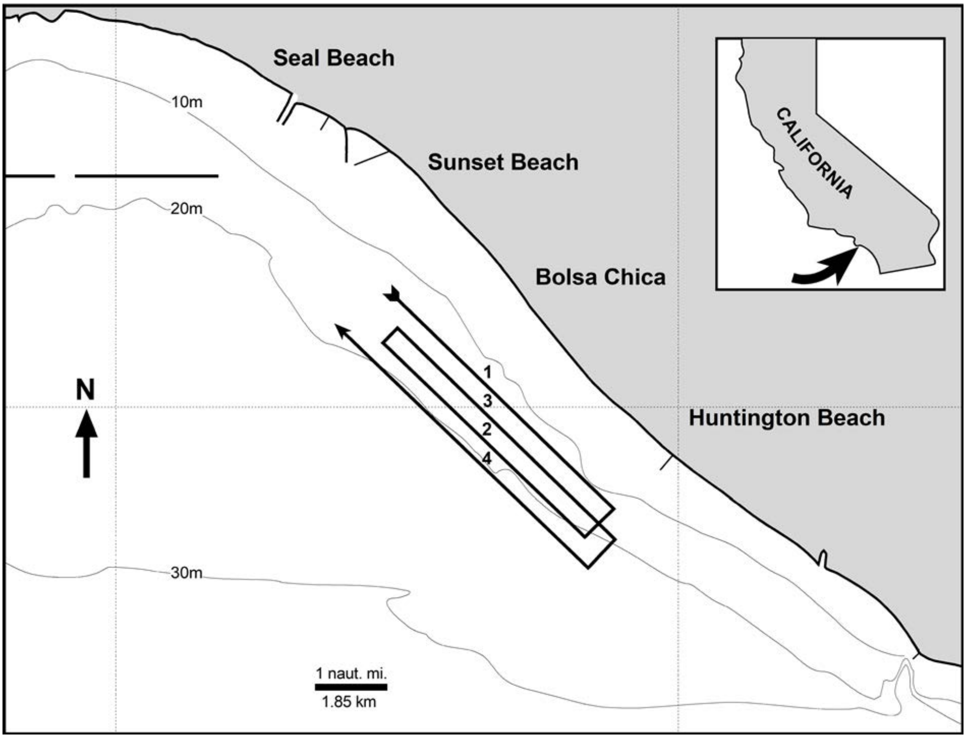


Figure 8. Proposed sampling track for future hydroacoustic surveys off Huntington Beach. Each week of the spawning season, four hydroacoustic transects (4 alongshore segments) should be run each in the morning (0800–1200 h) and afternoon (1200–1600 h) using a BioSonics DT-X sonar unit (centered around the major Barred Sand Bass spawning activity).

the vertical and horizontal distribution (density) of Barred Sand Bass aggregations from each survey. Results from each survey should be compared to characterize variations in the above parameters in relation to time of day, lunar day, month, and location on the survey grid. Moreover, data should then be compared across year to assess inter-annual differences in aggregation dynamics.

Prominent physical and chemical parameters should be closely monitored for each location on each sampling date. Measurements on temperature, salinity, dissolved oxygen, and pH should be taken near the surface and at each 2 m to the bottom using a Hydrolab CTD sensing unit (or equivalent) which should be aboard a smaller, support vessel. A vertical temperature profile should allow the researchers to determine thermocline depth from each survey. Researchers should also record data from various sources on tidal regimes, moon phases, current, wind (speed/direction), wave (height/direction), precipitation, air (barometric) pressure, and upwelling indices. Satellite infrared imagery data should also be obtained in order to examine large-scale temperature and current regimes in the study areas.

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Author Contributions

Conceived and designed the study: LA, BE, CW
Collected the data: LA, CW
Performed the analysis of the data: LA, BE, DB, CW
Authored the manuscript: LA
Provided critical revision of the manuscript: BE, DB, CW

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Hematology of mountain lions (*Puma concolor*) in the Sierra Nevada, California, USA: effect of sex, season, or location?

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There is a paucity of published hematological data for wild, free-ranging mountain lions (*Puma concolor*). We collected such information from mountain lions occurring at mid-elevations to increase available baseline information. We captured and sampled 43 individuals in a remote part of the eastern Sierra Nevada, Inyo and Mono counties, California, USA, and present descriptive statistics and reference intervals for hematological variables of mountain lions occupying that rural area. We tested for differences between males and females, and between winter (when mule deer [*Odocoileus hemionus*] were abundant in diets) and summer (when smaller prey were most common in diets). Male mountain lions exhibited a greater percentage of bands (i.e., immature neutrophils) than did females. Although the mean percentage of segmented neutrophils during winter was lower than during summer, that difference disappeared when a potential outlier was removed. Mean hematocrit among mountain lions sampled at 1,200–1,800 m elevation in the Sierra Nevada was higher than that of animals sampled at sea level in Florida, but lower than that of animals sampled exclusively at elevations >2,100 m in Colorado. Mean concentrations of red blood cells and hemoglobin also were higher for Sierra Nevada mountain lions than for animals sampled in Florida. These results are consistent with expectations for animals residing at different elevations and emphasize the value of establishing baseline information for populations existing under disparate ecological conditions.

Key words: altitude, comparative hematology, cougar, geographic variation, hematology, mountain lion, puma, *Puma concolor*, reference interval

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Aside from humans, the mountain lion (*Puma concolor*) is the most widely distributed terrestrial mammal in the western hemisphere (Logan and Sweanor 2001; Williams 2018). It is a highly adaptable predator, a source of human-wildlife conflict, and a keystone species that garners much public interest (Torres et al. 1996; Bleich and Pierce 2005; Torres 2005; Rominger et al. 2006; Jenks 2018; Williams 2018; Bleich 2020). Despite increases in ecological research, there remains a paucity of published physiological data, including hematological values and their potential variation among populations of wild, free-ranging mountain lions (Pierce and Bleich 2003). The few reports in the professional literature are based on data from captive (Currier and Russell 1982; Hawkey and Hart 1986; Pospisil et al. 1987), wild (Currier and Russell 1982; Dunbar et al. 1997; Foster and Cunningham 2009), or captive and wild animals in combination (Currier and Russell 1982).

Mountain lions have a broad geographic range that extends from northern British Columbia in Canada, to Patagonia in southern Argentina and Chile (Young and Goldman 1946; Williams 2018). These cryptic felids occupy a diversity of ecosystems across their distribution (Pierce and Bleich 2003; Cross 2017; Williams 2018), and previous investigators have emphasized the value of comparing hematological or serum chemistry variables among mountain lions occurring under various ecological conditions (Dunbar et al. 1997; Pierce and Bleich 2003; Bleich et al. 2019). Susceptibility of mountain lions to pathogens associated with domestic felids and the potential for spillover at the urban-wildland interface (Paul-Murphy et al. 1994; Foley 1997; Bevins et al. 2012; Kellner et al. 2018), combined with the role of ecological features in facilitating pathogen transfer (Kozakiewicz et al. 2018), make it especially useful to establish baseline physiological data from a variety of locations or environmental settings (Carver et al. 2016).

The Sierra Nevada is a massive mountain range extending 640 km in a north-south direction, attains elevations >4,400 m, and separates the San Joaquin and Sacramento valleys to the west from the Great Basin to the east (Storer and Usinger 1968). The east-facing slope of the Sierra Nevada is sparsely inhabited by people (<2 persons/km^2), and is among the least densely populated regions of California (Duncan 1993). Mountain lions occupy the eastern Sierra Nevada year-round where they prey primarily on mule deer (*Odocoileus hemionus*; Bleich and Taylor 1998; Pierce et al. 1999, 2000a, 2000b; Villepique et al. 2011). A migratory segment of the deer population in the region generally moves northward to higher elevations or westward through high mountain passes during spring, but rejoins the resident segment on lower-elevation winter ranges during autumn (Kucera 1992; Pierce et al. 1999; Monteith et al. 2011). As a result, the localized density of mule deer in the eastern Sierra Nevada is highest during winter (November–April) and reaches its nadir during summer (May–October). Mountain lions exhibited a functional response to density of mule deer on winter ranges, as evidenced by a marked increase in the frequency of deer remains in lion feces during winter and an increase in the frequency of smaller mammals in lion feces during summer (Villepique et al. 2011).

The seasonal difference in diet (Villepique et al. 2011), combined with the potential for dietary differences between male and female mountain lions (Pierce et al. 2000b), provided the opportunity to compare 17 hematological variables for animals captured at moderate elevations (~1,200 m –1,800 m) during winter or summer, and between males and females, and to establish reference intervals for a genetically defined population of these apex predators (Ernest et al. 2003; Gustafson et al. 2018). We also compared selected hematological variables for mountain lions in our study area with those for wild, free-ranging mountain lions occurring exclusively at sea level (Dunbar et al. 1997) or at high (>2,100 m) elevations (Currier and Russell 1982) elsewhere in North America.

METHODS

Study area

We concentrated our efforts in a 450 km^2 area in or proximate to Round Valley (37°25'N, 118°36'W) in Inyo and Mono counties, California. Round Valley (mean elevation ~1,500 m) long has been recognized as a critically important mule deer winter range (Loft and Bleich 2014), and general descriptions of the vegetation and topography are provided by Storer and Usinger (1963). The winter mule deer population in Round Valley declined substantially from approximately 6,000 animals (~13/km^2) in 1985 to about 1,000 animals (~2/km^2) in 1991. In 1992 the population began to increase slowly, and trended upward through the remainder of our investigation (Pierce et al. 2012). The mean number of mountain lions occupying the winter range declined from 6.1 in the winter of 1992-1993 to 0.6 in the winter of 1998-1999, lagging the decline of the deer population by about 7 years (Pierce et al. 2012; Pierce and Bleich 2014).

Animal capture and laboratory analyses

We captured mountain lions for ecological, behavioral, and genetic investigations from 1991 to 2004 and obtained blood samples for hematological analysis during 1993–2004. We followed guidelines published by the California Department of Fish and Game (Jessup et al. 1986) and then-current animal care and use protocols of the American Society of Mammalogists (*ad hoc* Committee on Acceptable Field Methods in Mammalogy 1987; Kirkland 1998). Additionally, our capture protocol and research plan were approved by the Institutional Animal Care and Use Committee at the University of Alaska Fairbanks (Pierce 1999).

We immobilized animals with Telazol® (tiletamine HCl and zolazepam HCl; Fort Dodge Animal Health, Fort Dodge, IA) after they were bayed by hounds (Young and Goldman 1946) or captured with foot snares (Logan et al. 1999); we also sampled one individual caught accidentally in a leg-hold trap set legally for other species, as described by Andreasen et al. (2018). Following immobilization, we covered the eyes with a blindfold and restrained each animal with hobbles, obtained morphometric information and body weight, and conducted a thorough physical examination. We collected whole blood (50 cc) from the medial saphenous vein and transferred it immediately to appropriate vacutainer tubes. We transported blood samples directly from the field (≤4 hr) to Northern Inyo Hospital, Bishop, California, where they were processed upon arrival (Vitros Chemistry System®, Ortho Clinical Diagnostics, Raritan, NJ); funds were not available for processing through a commercial veterinary laboratory (Bleich et al. 2019). Prior to release we fitted each mountain lion with a VHF or GPS telemetry collar (Bleich et al. 2000). At least one investigator remained with each study animal until it became mobile and had departed the capture site.

Statistical analyses

We sampled ten individuals >1 time, enabling us to use Mann-Whitney tests to compare variables between males and females, and between animals captured during winter or summer. Where we detected no statistically significant difference ($P > 0.05$) between sexes or between seasons, we pooled variables prior to further analysis. Where we detected such differences, we present values for the applicable category (sex, season) both separately and pooled.

We used Reference Value Advisor (Greffre et al. 2011), an Excel Spreadsheet add-in, to estimate descriptive statistics, reference intervals, or both, for 17 hematological variables. Where no plausible explanation existed for an outlier, we retained it for analysis (Greffre et al. 2009). Where sample sizes were insufficient to estimate reference intervals, we report only descriptive statistics and minimum and maximum values (Friedrichs et al. 2012).

We also compared mean values of hemoglobin (Hb; g/dL), red blood cells (RBC; #/µl), and hematocrit (Hct; %) of mountain lions captured at mid-elevations in the Sierra Nevada with those previously reported for mountain lions occurring exclusively at sea level in southern Florida, USA (Dunbar et al. 1997), and for a population occurring exclusively at high elevations in the Rocky Mountains of Colorado, USA (Currier and Russell 1982). To facilitate comparisons of these erythropoietic variables, it was necessary to estimate standard deviation (Higgins and Green 2011) and 95% confidence intervals of Hct provided by Currier and Russell (1982). We then used Welch's approximate t (Zar 1984) to test for differences in Hb, RBC, and Hct among these populations.

RESULTS

Intrapopulation comparisons

We report hematological results for 43 unique mountain lions (20 ♂, 23 ♀); descriptive statistics and reference intervals are based on sample sizes ranging 34 to 55. We sampled five animals twice, three animals three times, one animal four times, and one animal five times (median time between repeat captures = 18 months [range 4–38 months]). Although data for RBC were normally distributed, distributions of other analytes were non-Gaussian and asymmetrical, and we used a nonparametric method (Greffre et al. 2011) to estimate reference intervals for those analytes.

With two exceptions, we found no differences in analytes for sex or season (Table 1). Males ($\bar{x} = 3.15 \pm 5.186$ [SD]) exhibited a higher ($U_A = 494.5$, $Z = -2.24$, $P = 0.025$) percentage of bands (immature neutrophils) than females ($\bar{x} = 1.48 \pm 4.908$). Additionally, the percentage of segmented neutrophils was lower ($U_A = 437$, $Z = -2.27$, $P = 0.023$) for lions sampled during winter ($\bar{x} = 69.24 \pm 15.146$) when compared with animals sampled during summer ($\bar{x} = 77.88 \pm 8.015$), but when we excluded a suspected outlier that difference was no longer significant.

Interpopulation comparisons

Welch's approximate t revealed that mean Hct was greater ($t_{57} = 5.011$, $P < 0.001$) in mountain lions at high elevations (>2,100 m) in the Rocky Mountains ($\bar{x} = 46.9 \pm 2.81$)

when compared to mountain lions at intermediate elevations (1,200–1,800 m) in the Sierra Nevada ($\bar{x} = 41.95 \pm 5.424$), and both values were greater ($t_{44} = 12.201$, $P < 0.001$ and $t_{111} = 6.026$, $P < 0.001$, respectively) than in mountain lions sampled at sea level in Florida ($\bar{x} = 36.4 \pm 5.300$). Mean RBC in mountain lions at intermediate elevations ($\bar{x} = 8.76 \pm 0.690$) was also greater ($t_{104} = 7.340$, $P < 0.001$) than in mountain lions at sea level ($\bar{x} = 7.64 \pm 1.030$); comparative data for mountain lions at high elevation were not available. Mean Hb was higher ($t_{63} = 4.709$, $P < 0.001$) among mountain lions sampled at intermediate elevations ($\bar{x} = 15.16 \pm 4.467$) than among those captured at sea level ($\bar{x} = 12.21 \pm 1.700$), but comparative data for animals captured exclusively at high elevations again were unavailable.

Variability of Hct in the Sierra Nevada ($CV = 12.9$) was similar to that for animals sampled at sea level in Florida ($CV = 14.6$), and data from both of those areas exhibited far more variation than did lions sampled in the Rocky Mountains of Colorado ($CV = 6.0$). Variation in Hb was greater among mountain lions occupying the Sierra Nevada ($CV = 29.4$) than among mountain lions captured in Florida ($CV = 13.9$). This pattern was reversed, however, for RBC among lions sampled in Florida ($CV = 13.5$) and those in California ($CV = 7.8$).

DISCUSSION

Our results contribute to the published hematological reference values for mountain lions, and are consistent with results from other species that occur at different elevations above sea level, and likely represent local adaptations in RBC, Hb, and Hct (Mortola and Wilfong 2017). Our results also demonstrate the importance of obtaining hematological reference intervals from wild animals living under a variety of environmental conditions, which can influence pathogen dynamics or disease ecology (Kozakiewicz et al. 2018), rather than assuming that reference intervals from a single location are universally representative (Dunbar et al. 1997; Pierce and Bleich 2003; Bleich et al. 2019), or extrapolating reference intervals obtained from captive animals to wild populations (Allwin et al. 2019).

External physical examination and body weight (Roelke 1987; Dunbar et al. 1997), body conformation (our subjective index to body condition; see also Coon et al. 2019), and coat condition (Charlton et al. 1998) indicated that mountain lions captured in the Sierra Nevada and included in these analyses were healthy and in good condition. Further, none exhibited evidence of serious injury or heavy infestation by external parasites, either of which can confound interpretation of hematological values (Arlian et al. 1988; Serieys et al. 2013). Although we were not able to examine our study animals for serological evidence of pathogen exposure, none presented clinical signs of chronic disease at time of capture, a result that is consistent with the low prevalence of pathogens reported for mountain lions inhabiting the Sierra Nevada (Girard et al. 2012; Foley et al. 2013).

We compared variables between males and females, and between summer and winter, when mountain lions experienced differing ecological conditions and diets. With two exceptions, we found no differences by season or sex among hematological variables (Table 1). Anemia or poor condition among mountain lions has been attributed to an abundance of small prey in diets (Roelke 1987). Following removal of an outlier, however, no difference existed in percent segmented neutrophils—or mean value of any other hematological variable—among mountain lions during the period of mule deer abundance on winter ranges and the remainder of the year when small mammals increased substantially in diets (Villepique et al. 2011). Differences between males and females with respect to percent bands (immature neutrophils) might be more thoroughly investigated with a larger sample or stratification by additional covariates.

Hematocrit, RBC, and Hb increase in relation to the elevation above sea level at which a population exists (Adolph 1972; Luft 1972; Jain 1993) and are erythropoietic adaptions to effective oxygen concentrations (Mortola and Wilfong 2017). Oxygen concentration varies with altitude, and elevational differences among the three study areas were substantial, ranging from sea level to >2,100 m. Mean Hct for animals captured at mid-elevations in the Sierra Nevada was significantly less than that for mountain lions residing exclusively at high elevations (>2,100 m) in the Rocky Mountains, and significantly greater than for mountain lions existing at sea level on the Florida peninsula (Table 1); given elevational differences among these areas, these results are consistent with adaptations to ambient effective oxygen concentration in each area, and possibly other unique attributes of local habitat (Mortola and Wilfong 2017). Similar results were observed for mean Hb and RBC among the three populations.

Coefficient of variation for Hct of mountain lions occurring at sea level in Florida was similar to that for the *CV* of those captured at mid-elevations in the Sierra Nevada, but both were far greater than for wild mountain lions occurring at high elevations in the Rocky Mountains. Greater variability in Hct for Sierra Nevada lions, when compared with those from Colorado, may be the result of more variable life history strategies among Sierra Nevada lions: some individuals resided year-round near 1,500 m, while others spent part of each year at greater elevations (Pierce et al. 1999). At sea level, variability in Hct was thought to reflect differing body condition and health status between the two populations studied by Dunbar et al. (1997).

The *CV* for Hb of animals sampled in the Sierra Nevada was more than twice that for mountain lions existing at sea level. This result was consistent with the substantial range in elevations occupied by individuals occupying that mountain range, and likely reflected differences in seasonal use of habitats or individual life history strategies; no seasonal differences in habitat use were reported among mountain lions occurring in Florida. Our determination that the *CV* for RBC of mountain lions at sea level was greater than that for individuals occurring at higher elevations was inconsistent with expectation, but may be spurious. Alternatively, variances in health status, stress levels, physical exertion, or hydration during capture events (Dunbar et al. 1997) may have contributed to this unanticipated result.

Descriptive statistics and reference ranges reported for mountain lions inhabiting the eastern Sierra Nevada were obtained over 10 years, and data reported by Currier and Russell (1982) or Dunbar et al. (1997) predated our investigation by several years. Advances in laboratory equipment potentially introduced some variability in results, a caution previously raised by Dunbar et al. (1997), and differences in capture or handling protocols may have affected analytical results (Maceda-Veiga et al. 2015). In addition, some analyzers may, on occasion, confuse red blood cells and platelets of felids, and can yield suspect values (Duncan et al. 1994). Ideally, blood samples collected for determination of Hct, RBC, and Hb would have been obtained and preserved in the same manner, and would have been analyzed on identical laboratory equipment by the same technician (Maceda-Veiga et al. 2015). Compliance with such constraints, however, is virtually impossible in field settings involving a cryptic species that occurs at low densities across a broad geographic area, or when samples are obtained over an extended period. Despite our inability to comply with these caveats, however, mean RBC reported by Dunbar et al. (1997) for mountain lions in Florida fell within the reference interval for mountain lions from the Sierra Nevada, and

Table 1. Hematological values for mountain lions occurring at moderate elevations (1,200 m–1,800 m) in the Sierra Nevada, Inyo and Mono counties, California, 1993–2004. Reference intervals and the 90% CI around the upper and lower reference limit were estimated according to Greffre et al. (2011). Where sample sizes were inadequate to estimate a reference interval using nonparametric methods, we present only the mean and SD, median, and range of values (Friedrichs et al. 2012).

Analyte (unit)	*n*	Mean	SD	Median	Range	Reference Interval	90% CI Lower Limit	90% CI Upper Limit
WBC ($\times10^3$/dL)	55	11.49	7.831	9.1	3.3–46.2	4.02–40.72	3.30–5.24	24.00–46.20
Bands ♀+♂ (%)[a]	54	2.31	4.706	0.0	0–20	0.0–20.0	0.0–0.0	11.0–20.0
Female (%)	27	1.48	4.098	2.7	0–20	—	—	—
Male (%)	27	3.15	5.186	1.0	0–20	—	—	—
Seg. Neutrophils (%)[b]	54	71.96	13.843	73.5	2–94	19.63–93.25	2.0–55.0	90.62–94.00
Winter (%)	37	69.24	15.146	72.0	2–94	—	—	—
Summer (%)	17	77.88	8.015	76.0	67–91	—	—	—
Seg. Neutrophils (%)[c]	53	73.28	9.970	74.0	49–94	50.40–93.30	49.00–56.10	90.65–94.00
Lymphocytes (%)	54	20.37	13.961	17.5	1–94	1.37–75.63	1.0–5.0	38.00–94.00
Monocytes (%)	54	4.28	2.864	4.0	0–11	0.0–10.63	0.0–0.0	9.00–11.00
Eosinophils (%)	54	1.24	2.649	0.0	0–14	0.0–12.13	0.0–0.0	5.63–14.00
Basophils (%)	54	0.11	0.372	0.0	0–2	0.0–1.63	0.0–0.0	1.00–2.00
RDW (%)	37	24.69	6.383	21.5	18.7–39.0	—	—	—
MPV (fL)	34	10.49	1.023	10.4	7.1–12.0	—	—	—
RBC (#/µl)[d]	39	8.76	0.690	8.8	7.27–10.20	7.31–10.14	6.97–7.71	9.84–10.46
Hemoglobin (g/dL)	55	15.16	4.467	14.6	5.30–45.10	7.68–34.68	5.30–13.10	17.04–45.10
Hematocrit (%)	55	41.95	5.424	42.5	12.00–50.90	20.48–50.18	12.00–36.80	48.20–50.90

Table 1. continued.

Analyte (unit)	*n*	Mean	SD	Median	Range	Reference Interval	90% CI Lower Limit	90% CI Upper Limit
MCV (fL)	39	38.36	1.828	49.0	45.00–53.00	—	—	—
MCH (pg)	39	16.98	1.235	16.7	15.80–21.80	—	—	—
MCHC (g/dL)	39	35.15	2.747	34.5	33.30–48.40	—	—	—
Platelets ($\times 10^3$/dL)	38	338.61	154.394	347.0	16.00–810.00	—	—	—

[a] Males and females differed (U_A=494.5, Z=-2.24, P=0.025)

[b] Seasons differed with outlier included (U_A=437, Z=-2.27, P=0.023)

[c] Annual estimate with outlier excluded

[d] Box-Cox transformation not performed; data were normally distributed

mean RBC from the Sierra Nevada fell within the 10th and 90th percentiles for mountain lions sampled in Florida.

Reference intervals commonly are based on values obtained from individual animals. In this investigation we sampled 4 individuals on ≥2 occasions and, as a result, the population-specific reference values reported are based on a combination of intra-individual and inter-individual variation (Greffe et al. 2009). Resampling occurred under a variety of ecological conditions, and results likely reflected individual responses to differing environmental or physiological conditions. Animals captured more than once experienced variation in weather, prey availability and its effect on diet composition, reproductive status, age, and capture-related stressors, each of which are factors that can affect physiological variables (Ellervik and Vaught 2015) and are representative of conditions encountered by all mountain lions inhabiting the eastern Sierra Nevada at some point in their lives. Assessment of multiple samples from individuals can be beneficial in that they may increase the precision with which properties of those animals are estimated (Hurlbert 1984), and multiple samples from individual mountain lions were included in the population-specific reference intervals reported by Currier and Russell (1982) and Dunbar et al. (1997).

Our ability to stratify our samples by additional variables, such as age or reproductive status, that could influence hematological values and still yield meaningful descriptive statistics or reference intervals was limited. Foster and Cunningham (2009), however, noted lower mean Hct among neonatal mountain lions when compared to that for adults. Conversely, Dunbar et al. (1997) reported higher mean Hct in juveniles than in adults. Mean Hct for mountain lions occupying the Sierra Nevada could be biased downward if Hct of young that are ≥6 months old typically is less than that of adults. Juveniles reaching that age are weaned, however, and are feeding largely on prey killed by their mothers (Pierce and Bleich 2003); thus, the potential for any such bias is unexpected.

Our results augment the current paucity of published hematological values for a secretive carnivore and are consistent with local adaptations in RBC, Hb, and Hct among wild individuals occurring at different altitudes. Further, we confirm the value of obtaining reference intervals from wild animals living under a variety of environmental conditions that may influence pathogen dynamics or disease ecology (Kozakiewicz et al. 2018), rather than assuming that reference intervals from a single location are universally representative (Dunbar et al. 1997; Pierce and Bleich 2003; Bleich et al. 2019). Moreover, our data represent baselines against which to compare future changes as the urban-wildland interface expands, and the probability of contact between mountain lions and domestic felids increases.

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Author contributions

Conceived and designed the study: VCB, BMP, HBE
Collected the data: VCB, BMP, JTV
Performed the analysis of the data: VCB, JTV
Authored the manuscript: VCB, BMP, HBE, JTV
Provided critical revision of the manuscript: VCB, BMP, HBE, JTV

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Migration and seasonal ranges of the Eastern Tehama deer herd in northern California

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We investigated the movements and seasonal ranges of deer from the Eastern Tehama deer herd in northern California, USA. Twenty-eight adult female black-tailed deer (*Odocolieus hemionus columbianus*) were captured and fitted with GPS collars during 2013–2015. Average annual migration distances between summer and winter ranges was approximately 69 km. Deer used a variety of seasonal ranges including fall and spring stopovers during migration. Summer ranges averaged 3.3 km^2, winter ranges averaged 2.7 km^2, and fall and spring stopovers averaged 1.6 km^2 and 1.1 km^2, respectively. Fall migration (duration) averaged 30 days and spring migration averaged 21 days. The deer spent approximately 87% and 67% of the migration period at fall and spring stopovers, respectively. This study reinforces the importance stopover site use during migration. Conservation actions to benefit this herd should not only be focused on summer and winter ranges but also stopovers and migratory corridors which will require landscape-scale collaborations.

Key words: black-tailed deer, California, Eastern Tehama deer herd, migration, *Odocoileus hemionus columbianus*, seasonal ranges, stopovers

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Migration is an important part of the life history of many ungulate species (Mysterud et al. 2001; Bolger et al. 2008; Bischof et al. 2012; Fryxell and Holt 2013). In many areas, long-distance migrations of ungulates are being altered by human population growth, barriers to movement, habitat loss and modification, and climate change. These alterations are likely to result in population declines and a functional loss of migration (Berger 2004; Bolger et al. 2008; Sawyer et al. 2009; Lendrum et al. 2013; Sawyer et al. 2013; Monteith et al. 2018; Wyckoff et al. 2018). In 2018, the U.S. Secretary of the Interior signed Secretarial Order

3362, which directed the U.S. Department of the Interior to collaborate with state fish and wildlife management agencies to improve habitat quality on winter ranges and migration corridors used by big game species, including antelope (Antilocapra americana), elk (Cervus canadensis), and mule deer (Odocoileus hemionus) in the western U.S.

An integral part of migration behavior is use of seasonal ranges for rest, nutritional replenishment, reproduction, and predator avoidance (Monteith et al. 2011; Sawyer and Kauffman 2011; Middleton et al. 2013; Sawyer et al. 2013; Monteith et al. 2018; Wyckoff et al. 2018). The identification and characterization of seasonal ranges (including fall and spring stopover sites) is of great importance as these sites are vital to migratory ungulates (Sawyer et al. 2005; Monteith et al. 2011; Sawyer and Kauffman 2011; Bischof et al. 2012; Sawyer et al. 2013; Wyckoff et al. 2018). Mule deer migration in California has been well documented in the southern Sierra Nevada Mountains (Loft et al. 1989; Kucera 1992; Nicholson et al. 1997; Monteith et al. 2011) and to a lesser extent black-tailed deer in the Klamath-Trinity Mountains in northwestern California (Loft et al. 1984; Bowyer et al. 1998; Wittmer et al. 2014). However, relatively little is known about the migratory behaviors and seasonal ranges of California's largest migratory population of deer, the Eastern Tehama deer herd (ETDH) in northern California, USA (CDFG et al. 1981).

The ETDH is highly valued by the public for recreational uses and has declined in number over the past several decades (CDFW, unpublished data), which has resulted in a loss of recreational opportunities (26% reduction of hunting tags over the last 20 years; CDFW unpublished data), wildlife viewing opportunities, reduced contributions to local economies, and increased public concern regarding the status of the ETDH. The ETDH decline is thought have resulted from anthropogenic factors (e.g., land management activities and fire suppression) which have decreased habitat quality (CDFG 1998). Although several telemetry studies have been conducted on the ETDH (CDFW unpublished data), migration stopover sites had not previously been investigated. CDFW has long recognized the importance of stopover sites as key foraging sources for the ETDH, as stated in the Eastern Tehama deer herd management plan (CDFG 1981):

"Holding areas [i.e., stopovers] on intermediate range are of extreme importance to deer since it's there deer delay on their migrations between seasonal ranges. Deer heavily utilize these areas during the spring while awaiting forage development on the summer range. Deer also feed heavily on acorns within these types during the fall migration. Holding sites must be more accurately delineated for management purposes."

As stated above, several previous studies using telemetry collars were conducted on the ETDH and provided information that allowed for coarse-resolution identification of summer and winter ranges and some migration routes, but those collars did not include GPS technology, and the resulting data lacked the accuracy, resolution, and sample sizes to accurately delimit migration routes and important seasonal habitats used by the ETDH..

We initiated this project to identify areas of seasonal importance to deer in the ETDH. Our objectives were to: (1) document and characterize seasonal ranges and spring and fall stopover sites of the ETDH, and (2) quantify timing and duration of migration, including use of stopover sites. Project results will provide CDFW with information needed to prioritize areas for habitat conservation (e.g., conservation easements, fee title purchase, and management recommendations for both public and private lands) and enhancement, and will be used to update management planning for the ETDH.

METHODS

Study area

We conducted this study in northern California, in portions of Tehama, Plumas, Lassen, Shasta, and Butte counties (40.169 N, -121.560 W), occupied by migratory individuals in the ETDH. The study area encompassed 6,580 km^2 (Figure 1). Deer in the ETDH generally use low-elevation winter range, high-elevation summer range, and stopovers at intermediate elevations.

Winter range.—The winter range of the ETDH is in eastern Tehama and north-central Butte counties, in the western foothills of the southern Cascade and northern Sierra Nevada Mountains. The habitat types are primarily blue oak (*Quercus douglasii*) woodlands, annual grasslands, blue oak-foothill pine (*Pinus sabinina*) woodlands, and montane hardwoods (in the creek canyons) (Mayer and Laudenslayer 1988). Common woody plant species include blue oak, foothill pine, interior live oak (*Quercus wislizeni*), and numerous shrubs including wedgeleaf ceanothus (*Ceanothus cuneatus*), birch-leaf mountain mahogany (*Cercocarpus betuloides*), and manzanita (*Arctocstaphylos* spp.). Annual grassland habitats are primarily composed of introduced annual grasses and forbs including wild oats (*Avena* spp.), brome grasses (*Bromus* spp.), and redstem filaree (*Erodium cicutarium*). Common terrestrial wildlife species in the area include wild pig (*Sus scrofa*), coyote (*Canis latrans*), bobcat (*Lynx rufus*), and mountain lion (*Puma concolor*). Elevations range from 107 m near the valley floor to 820 m in the upper elevations of the winter range. Temperatures range from an average low of 3.7° C to an average high of 15.6° C from October–April (when deer are present). Most of the 85.5 cm of precipitation per year, falls from October–April (mean accumulation from 1995–2016, weatherbase.com, Manton, CA).

Summer range.—The summer range is located in the southern Cascades and northern Sierra Nevada Mountains in eastern Tehama, western Plumas, northeastern Butte, northwestern Lassen, and southeastern Shasta counties. Important habitat types include Sierran mixed-conifer forest, wet meadow, white fir (*Abies concolor*) forest, and montane chaparral (Mayer and Laudenslayer 1988). Primary tree species are white fir, ponderosa pine (*Pinus ponderosa*), incense cedar (*Calocedrus decurrens*), sugar pine (*Pinus lambertiana*), and California black oak (*Quercus kellogii*). Common shrub species include mountain whitethorn (*Cenanothus cordulatus*), snowbrush ceanothus (*Ceanothus velutinus*), bush chinquapin (*Castanopsis sempervirens*), and willow (*Salix* spp.). Common wildlife species in the area include black bear (*Ursus americanus*), coyote, mountain lion, and bobcat. Gray wolf (*Canis lupus*) may also be present at very low densities. Elevations range from 1300 m near the transitional point between conifer and hardwood-dominated habitats to 3100 m near Lassen Peak. We did not conduct captures in Lassen Volcanic National Park (due to permitting issues), which contains most of the highest elevation summer range. Temperatures range from an average low of 4.6° C to an average high of 25.8° C from May–September (when deer are present). The area receives approximately 81 cm of precipitation per year, with most falling as snow from December–March (mean accumulation from 1995–2016, Western Regional Climate Center, Chester, CA).

Stopover sites.—Typical habitat types of stopovers include ponderosa pine, Sierran mixed-conifer forest, and montane hardwood-conifer. Although both ponderosa pine and Sierran mixed-conifer forests are conifer-dominated, several oak species including California black oak, Oregon white oak (*Quercus garryana*), and canyon live oak (*Quercus chrysolepis*)

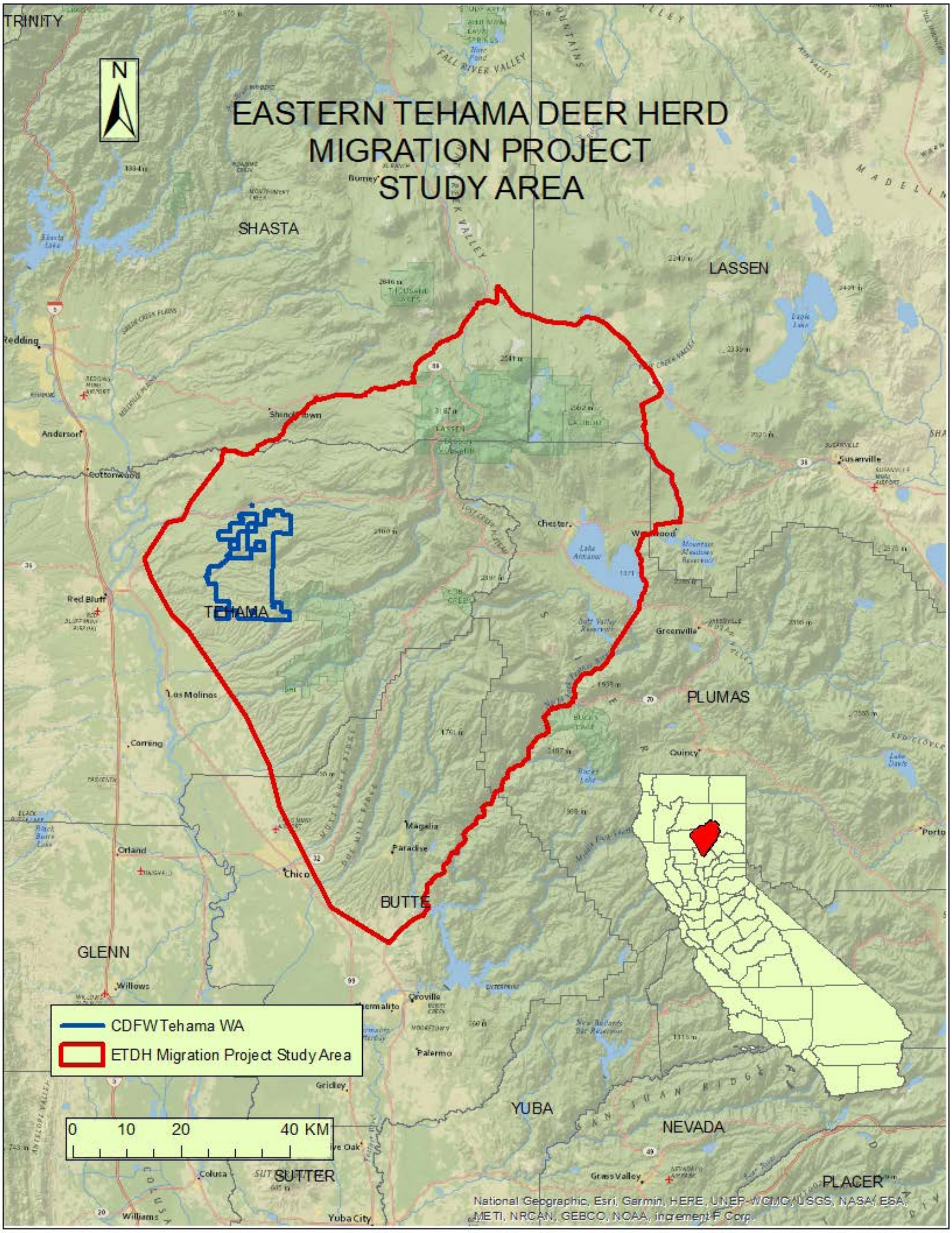

Figure 1. Study area for the Eastern Tehama deer herd migration study 2013–2016.

also occur in those forests and are important forage species during fall migration (mast and foliage) (CDFG 1981).

Captures

We conducted two capture efforts each year from 2013–2015. We captured deer during migration in April and May and on summer range in late July and August. We did not capture in June and early July to avoid the parturition period and any potential complications to does or fawns resulting from handling does during late stages of pregnancy or early lactation (Casady and Allen 2013).

All deer were chemically immobilized via free-range darting using a combination of Telazol® (tiletamine HCI and zolazepam HCI, Fort Dodge Animal Health, Fort Dodge, Iowa, USA) and xylazine HCI (Anased, LLOYD Laboratories, Shenandoah, Iowa, USA) at maximum dosages of 4.4 mg/kg (2.0 mg/lb) and 2.2 mg/kg (1.0 mg/lb), respectively. Tolazoline (LLOYD Laboratories, Shenandoah, Iowa, USA) was used as the antagonist for xylazine and was administered at a dosage of approximately 6.6 mg/kg (3.0 mg/lb) at least 80 minutes post immobilization (CDFW Wildlife Restraint Handbook 2012). Immobilization drugs were administered by CDFW staff with advanced training in chemical immobilization and in consultation with a wildlife veterinarian from the CDFW Wildlife Investigations Laboratory (WIL). The use of immobilization drugs was consistent with the CDFW Policy on the Use of Pharmaceuticals in Wildlife.

During captures, each deer was fitted with a store-onboard GPS collar (G2110B, Advanced Telemetry Systems Inc., Isanti, Minnesota, USA) equipped with VHF and mortality sensors. Collars also included a drop-off mechanism.

Monitoring

GPS collars were programmed to collect a location every four hours (six locations per day) and automatically release one year after deployment. We attempted to locate all collared deer for survival monitoring and to assess collar function using VHF telemetry, from the ground or fixed-wing aircraft, at least once a month while collars were active. After release, collars were recovered, and location data was downloaded.

Data analysis

GPS location data were analyzed using ArcGIS software (ArcMap 10.6.1, Environmental Systems Research Institute, Redlands, California, USA). Home ranges were estimated for individual deer using Brownian bridge movement models (BBMM: Horne et al. 2007; Nielson et al. 2013; Nicholson et al. 2016) in R (R Development Core Team 2019). Before determining utilization distributions (UDs), we separated locations by seasonal range based on a visual inspection that approximated the date when a deer left its summer or winter range to begin migration (Bunnefeld et al. 2011). Stopovers were also delineated by visually separating clusters of GPS locations within the migration and running BBMM analysis for each cluster. Summer home range, winter home range, and stopover sites were then delineated by generating the 95% isopleth upon the individual UDs.

RESULTS

Capture

Thirty female deer were captured from May 2013–August 2015 (Table 1). We affixed GPS collars to 28 of the captured deer (one doe died during capture processing and one yearling doe was too small to collar). Captures during spring migration were not as successful as post-parturition summer captures, as below normal precipitation during the study influenced the timing and predictability of spring migration.

Table 1. Dates and number of does captured from the ETDH 2013–2015.

Date	Number of Deer Captured
May 2013	2
July 2013	8
May 2014	3
July 2014	7
April 2015	5
August 2015	5

Telemetry

Between May 2013–June 2016, we collected 39,203 locations from GPS-collared deer (*n* = 26; two collars were not recovered) (Figure 2). Fix rate success by collar (i.e., proportion of collar fix attempts that successfully resulted in obtaining a GPS location) ranged from 53–94% ($\bar{X}$ = 83%, *SE* = 1.78). The proportion of fixes by collar that were 3-dimensional (i.e., ≥4 satellites used to determine location) ranged from 38–85% ($\bar{X}$ = 74%, *SE* = 2.01). Three-dimensional fixes are assumed to be more accurate than 2-dimensional fixes (Di Orio et al. 2003). GPS collars generally collected locations as scheduled on summer and winter ranges and stopovers; however, during migration the frequency of locations was reduced on some animals due to long distance movements through closed canopy areas (Rempel et al. 1995; Di Orio et al. 2003). The mean number of GPS locations collected for individual deer that completed both fall and spring migrations was 1,841 (range 1,363–2,057, *SE* = 31.73). The GPS collar collection interval (four hours) was too long to conduct BBMM analysis on an entire migration sequence because it over-approximated the width of the migration route. Therefore, migration routes were delineated by connecting successive GPS fixes from beginning to end of the migration sequence.

Sizes of home ranges and stopover sites

Seasonal home ranges were broken down into four classifications: summer, fall stopover, spring stopover, and winter (Figure 3). Only deer that had ≥2 months of location data for summer or winter range were included in the analysis. Summer range areas for collared does ranged from 0.85–9.93 km^2 ($\bar{X}$ = 3.29, *SD* = 2.52, *n* = 19). Winter range areas ranged

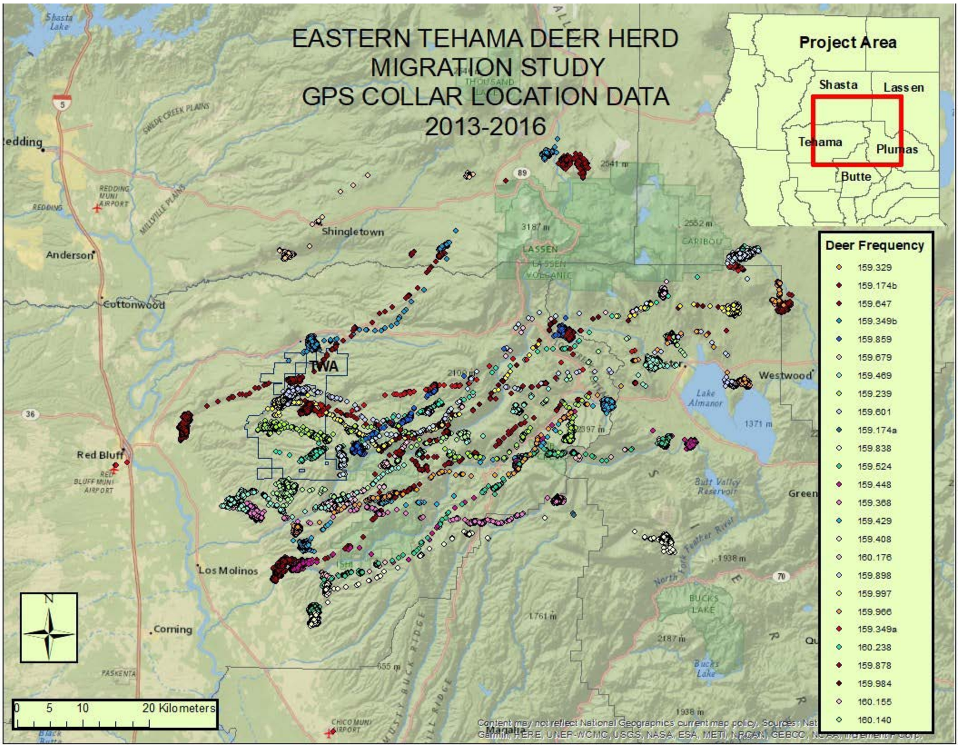

Figure 2. GPS location data for 26 female deer in Tehama, Plumas, Butte, Lassen, and Shasta counties, CA, USA, 2013–2016.

from 0.89–8.42 km^2 ($\bar{X}$ = 2.67, *SD* = 1.67, *n* = 21). Fall stopovers ranged from 0.18–4.65 km^2 ($\bar{X}$ = 1.64, *SD* = 1.43, *n* = 23) and spring stopovers ranged from 0.10–4.67 km^2 ($\bar{X}$ = 1.06, *SD* = 0.99, *n* = 36). In some instances, does used more than one fall and spring stopovers during one migration which we analyzed independently. The mean number of fall and spring stopovers for individual does was 0.95 (*SD* = 0.50, range 0–2) and 1.28 (*SD* = 0.57, range 0–2), respectively (Table 2).

Seasonal range elevation, habitat types, and land ownership.—Mean elevation of summer ranges was 1650 m (*n* = 28, range 1300–1900 m). Typical habitat types within summer ranges were Sierran-mixed conifer forest, white fir forest, wet meadow, and montane chaparral. The majority of the of the collared does summer ranges were on USDA Forest Service (USFS) lands (54%) followed by private timberlands (31%) and other private lands (15%). Mean elevation of winter ranges was 460 m (*n* = 25, range 200–825 m), and typical habitat types were blue oak woodlands and blue oak-foothill pine woodlands. The majority of the winter ranges were on private ranches (58%), followed by CFDWs Tehama Wildlife Area (TWA) (21%), Gray Davis Dye Creek Preserve (13%), and USFS (8%). The mean elevation of spring and fall stopovers were 1073 m (*n* = 36, range 640–1675 m) and 1240 m (*n* = 23, range 775–1465 m), respectively. Typical habitat types were Ponderosa pine forest, Sierran-mixed conifer forest, and montane hardwood conifer forest. Land ownership of the

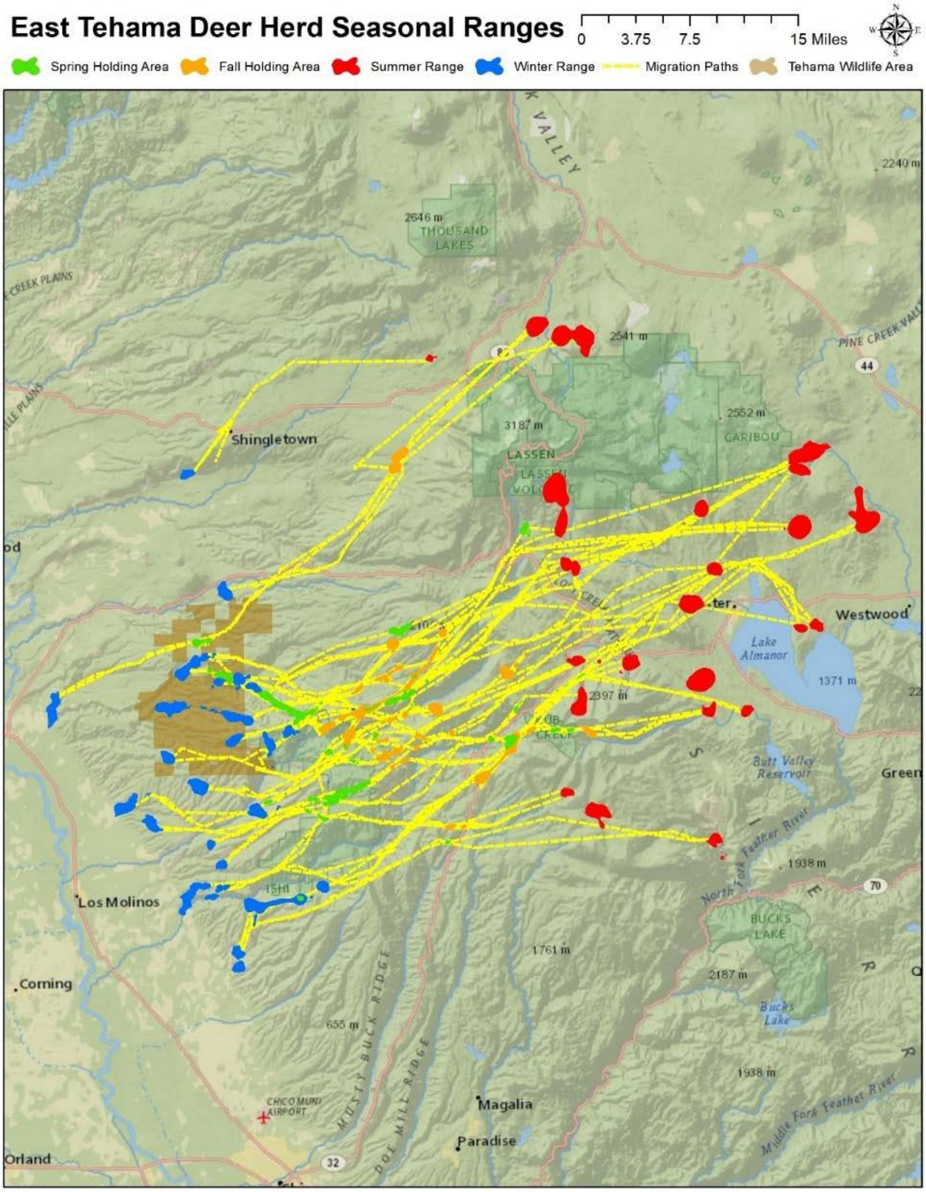

Figure 3. Seasonal ranges and approximate migration routes of collared does from the ETDH 2013–2016.

fall stopovers was private timberlands (55%) and USFS (45%). Ownership of the spring stopovers was USFS (72%), private timberlands (22%), and TWA (6%).

Migration corridors primarily followed the major east-west creek canyons, as indicated by previous telemetry studies (CDFG, unpublished data). Mill, Deer, and Antelope Creeks were most frequently used by collared does (33%, 25% and 13%, respectively). Typical habitat types include montane riparian and valley foothill riparian.

Table 2: Seasonal home ranges (km^2) of 21 does from the ETDH 2013–2016.

Deer #	Summer range	Winter range	Fall stopover	Spring stopover
3	0.9	2.1	4.2	2.1,0.4
4	2.5	2.9	2.4	0.5,0.7
5	4.8	2.9	0.6	0.6
6	5.7	3.4	*	2.2,4.7
7	4.6	5.2	3.1	3.4
9	2.2	3.2	4.7	0.2
10	2.4	2.0	1.3	1.7
11	8.2	1.8	1.7	0.3
12	1.4	0.9	0.8	1.7
13	2.4	1.2	1.2	1.8,1.6
14	1.4	1.5	0.3	1.2
15	0.8	1.6	0.4	0.9,0.4
16	1.9	1.9	*	0.2
19	1.2	2.5	1.0	*
20	1.8	1.4	0.7,0.2	*
21	1.2	1.9	0.4	*
22	1.5	1.6	*	0.1
23	4.5	3.8	0.3,2.3	0.3,0.4
24	4.3	8.4	0.7	0.4
27	*	2.5	1.9	*
28	9.9	3.7	1.6	1.4

*did not use a fall or spring stopover area or collar dropped prior to arrival on seasonal range

Migration distances

Twenty-three collared does made at least one migration from summer range to winter range and 21 migrated from winter range to summer range. Migration distance ranged from 38–101 km ($\bar{X} = 68.5$, $SD = 14.4$). Most deer used both fall ($n = 20$) and spring ($n = 19$) stopovers during migration. Spring stopover sites tended to be more westerly ($P < 0.001$) and at lower elevations ($P = 0.049$) than fall stopover sites. Mean distance from the summer range to fall stopovers was 35 km (range 11–60 km). Mean distance from fall stopover sites to winter ranges was 28 km (range 11–57 km). Mean distance from winter range to spring stopover area was 23 km (range 9–44 km). The mean distance from the spring stopover area to summer range was 40 km (range 7–65 km).

Migration timing

Fall migration.—The largest number of collared does left the summer range during the third week of September (*n* = 11) and the median date of departure was 19 September (*SD* = 10 days). The earliest a doe began fall migration was 30 August and the latest was 12 October. Collared deer arrived at fall stopovers between 5 September and 7 October, with the most common arrival time being the fourth week of September (*n* = 9). The median fall stopover arrival date was 23 September (*SD* = 9.9 days). Deer spent an average of 26 days (range 5–50 days) at fall stopovers before moving to winter ranges. Two does did not use a fall stopover site, including a doe that began its migration on 12 October and arrived on the winter range on 20 October after travelling almost 60 km. Most collared deer arrived on winter ranges around the fourth week of October (*n* = 9) and the median date of arrival was 22 October (*SD* = 11.2 days). The earliest arrival on winter range was 25 September and the latest was 3 November (Figure 4). The average number of days moving (i.e., not at a stopover) during migration was 5.1 (range 2–8 days). Total migration time from when a deer left summer range to arrival on winter range (including use of the fall stopovers) averaged 30 days (*n* = 23, range 7–51 days).

Spring migration.—The largest number of collared does left the winter range during the second or third week of April (*n* = 9) and the median departure date was 13 April (*SD* = 12.9 days). The earliest a doe began spring migration was 14 March and the latest was 30 April. Collared deer arrived at spring stopovers between 15 March and 2 May, with the most common arrival time being the third week of April (*n* = 9). The median arrival date was 18 April (*SD* = 14.6 days). Only one doe did not use a spring stopover. Does spent an

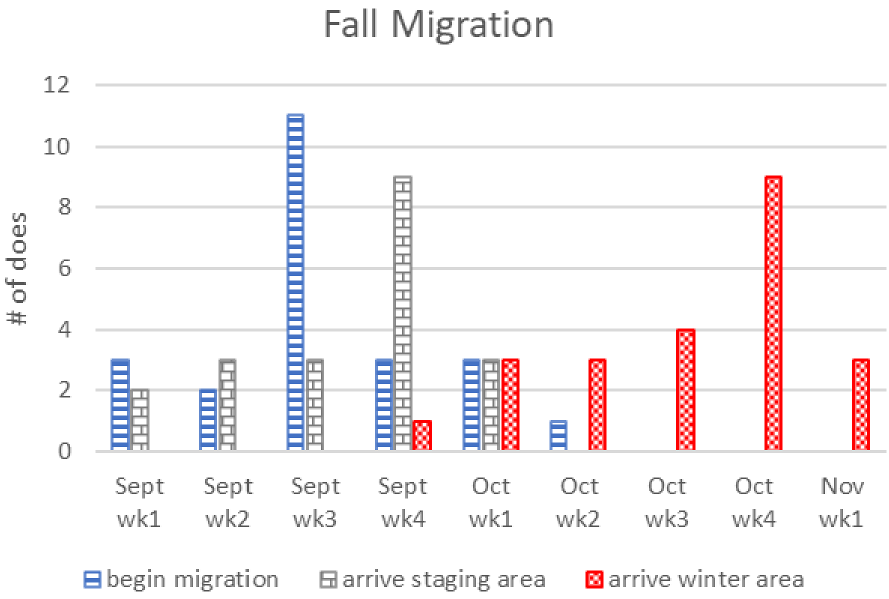

Figure 4. Fall migration timing, by week, of 23 collared does from the ETDH 2013–2015.

average of 14 days (range 3–37 days) at stopovers and most arrived on the summer range during the first or second week of May (*n* = 8) and the median date of arrival was 2 May (*SD* = 13.1 days). The earliest summer range arrival was 5 April (during 2015, an extremely low snow year) and the latest was 25 May (Figure 5). The average number of days moving during migration was 5 (range 3–8 days). Total migration time averaged 21 days (*n* = 16, range 3–53 days).

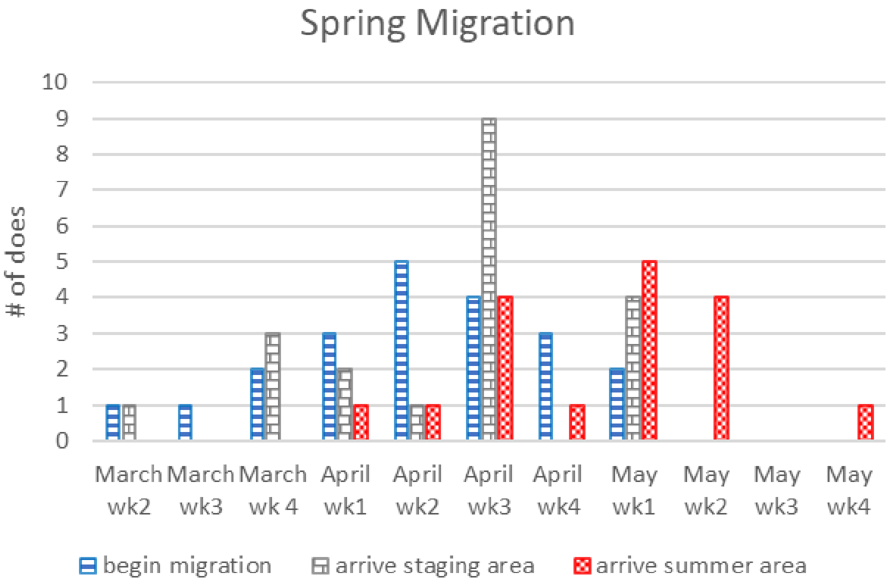

Figure 5. Spring migration timing, by week, of 21 collared does from the ETDH 2014–2016.

Discussion

Deer in the ETDH migrate long distances from summer to winter ranges compared to many deer herds throughout California (Longhurst et al. 1952; CDFG 1981). Migration distance for ETDH collared does in the study averaged 69 km and the longest distance a collared doe migrated was 101 km. Additionally, a doe marked in a CDFW study in central Plumas County (outside the typical ETDH summer range), migrated approximately 125 km to ETDH winter range (CDFW unpublished data). In contrast, Loft et al. (1984) recorded an average migration distance of 21 km (*n* = 16, range 11–35) for black-tailed does in Trinity County. Wittmer et al. (2014) found even shorter migration distance of 5–10 km for black-tailed does in the Mendocino National Forest. Although ETDH migration distances are shorter than migrations of deer in other western states (Sawyer and Kauffman 2011; Sawyer et al. 2016), ETDH deer encounter similar challenges to migration from various anthropogenic factors (Berger 2004; Bolger et al. 2008). Sawyer et al. (2016) found longer distance migrants had a higher exposure to anthropogenic mortality factors (i.e., highways and fences), however, reduced time on winter range by long-distance migrators may alleviate competition for limited forage. This suggests that there may be fitness trade-offs between migration strategies (long vs. short distance). We surmise that the longer distances between summer and winter ranges for the ETDH relative to those for herds in northwestern California are likely a function of differing elevation gradients and relief in each area.

Summer range areas for ETDH collared does averaged 3.29 km^2 and were slightly larger than winter ranges which average 2.67 km^2 and were larger than two other studies of black-tailed deer in northern California. Using local convex hull (95% isopleth), Wittmer et al. (2014) found average seasonal home range size for deer during summer were 0.61 km^2 and winter were 0.86 km^2 in Mendocino National Forest. In northwestern California, Loft et al. (1984) estimated 1.55 km^2 for summer ranges in Trinity County using the minimum convex polygon (MCP) method. However, in southern California, Nicholson et al. (1997) used both adaptive kernel (AK: 95%) and MCP methods to estimate average summer and winter range size of mule deer. Both methods showed larger average summer (AK = 5.54 km^2, MCP = 3.15 km^2) and winter range sizes (AK = 13.57 km^2, MCP = 7.67 km^2) than ETDH animals. The relative habitat quality and productivity of each study area (e.g., northwestern California conifer forest vs. arid southern California mountain ranges) may largely explain differences in home range sizes (Relyea et al. 2000).

Stopover sites have been extensively studied due to their importance in migratory ecology of mule deer (Kucera 1992; Sawyer et al. 2009; Monteith et al. 2011; Sawyer and Kaufman 2011). Most research into stopover ecology suggests that stopovers play a key role in the migration strategy by allowing individuals to migrate in concert with plant phenology and maximize energy intake rather than speed (Monteith et al. 2011; Sawyer and Kauffman, 2011; Bischof et al. 2012; Lendrum et al. 2014; Aikens et al. 2017). Sawyer and Kaufman (2011) found that mule deer in central Wyoming spent approximately 95% of the migration period at stopovers. Stopovers also appear important to the ecology of the ETDH, as collared does averaged approximately 87% (26 days) and 67% (14 days) of the migration period at fall and spring stopovers respectively. Loft et al. (1984) found similar periods of delay at spring stopovers in black-tailed deer in Trinity County (16 days). Sawyer and Kaufman (2011) also found high fidelity to stopover sites across season and years and concluded that the protection of stopover sites may provide an effective conservation strategy for migratory mule deer. In the ETDH, stopovers were often located on USFS lands and private timberlands. Management strategies for migratory sites should differentiate between stopover sites and movement corridors to be most effective (Sawyer et al. 2009).

Due to variability in weather patterns during the study period (2013–2016), including drought conditions during most of the study, our migration timing results may not be representative of a “normal” precipitation year. These conditions may have also affected our ability to capture deer during spring migration (migration timing predictability). Snowfall averages from Chester, CA in the heart of the summer range were 96%, 92%, and 50% below average during our study period (2013–2014, 2014–2015, 2015–2016; Western Regional Climate Center, Chester, CA).

Although the majority of collared does wintered on private ranches, our results emphasize the value of CDFW’s 190 km^2 Tehama Wildlife Area to the ETDH. The property has long been considered an important wintering area for the herd (Longhurst et al. 1952) and was specifically acquired in 1942 to protect deer winter range from being overgrazed by livestock. While TWA represents only about 9% of the total winter range for the ETDH, 46% (n = 11) of the collared does either wintered on or moved through TWA. Additionally, two does from a CDFW study in Plumas County wintered on or moved through TWA.

Anthropogenic factors can have a detrimental effect on mule deer migration and habitats (Monteith et al. 2018; Wyckoff et al. 2018). Stopovers for the ETDH are often located in areas of private timberlands managed primarily to maximize marketable lumber. Silvicultural systems used on private timberlands in California are diverse, and the effects of different systems on deer habitat quality and behavior are not well known. However, some silviculture practices (post-harvest herbicide use to control shrubs) can be detrimental to black-tailed deer forage quality (CDFG 1998; ODFW 2008; Ulappa 2015). Other types of human disturbance on stopovers (energy and residential development) have been shown to diminish use of stopovers thus increasing speed of migration (Wyckoff et al. 2018). Currently, the USFS administers a large portion of the ETDH summer range, fall and spring stopover sites, and to a lesser extent, winter range. Additional wildlife habitat restoration and improvement projects and forest management projects could be implemented on USFS and other lands to increase the quality and quantity of stands supporting valuable browse and forb species. While much of the herd's winter range is privately owned ranchlands, public lands such as TWA and the adjacent Gray Davis Dye Creek Preserve should be considered for additional deer habitat improvements. Although the TWA Vegetation and Fuels Management Plan (CDFW 2013) recommends prescribed burning to improve wildlife habitat, a lack of resources has limited the implementation of wildlife habitat improvement projects on TWA. Kie and Boroski (1995) recommended altered livestock grazing periods and stocking levels on TWA to benefit deer. Those recommendations are currently being implemented and continue to be assessed for effectiveness.

In conclusion, the ETDH is unique with respect to its migration and seasonal range use compared to other deer herds in California. The ETDH long-distance migrations may only be eclipsed by a few herds in the southern Sierra Nevada Mountains in California (CDFW unpublished data). Continued research into ungulate migration in California is crucial to conserving the migratory function of many herds. Seasonal ranges important to the ETDH and other herds, including stopover sites, need to be further studied and assessed to determine potential actions to improve habitats (Sutherland 1998; Sawyer and Kauffman 2011). The conservation of migratory ungulates is particularly challenging because entire regional landscapes must be managed in order to conserve migrations (Bolger et al. 2008). Effective conservation of the ETDH and other herds will require landscape-scale collaborations involving multiple parties and interests and the effective application of science, policy, and planning.

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Author contributions
Conceived and designed the study: SCH
Collected the data: SCH, PJF
Performed the analysis of the data: SCH
Authored the manuscript: SCH
Provided critical revision of the manuscript: PJF

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First record of pughead deformity in the threatened Clear Lake Hitch

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Clear Lake Hitch, a potamodromous minnow endemic to Clear Lake, CA, USA and its tributary creeks, is listed as threatened under the California Endangered Species Act (CESA), and is currently under petition for listing under the federal ESA (CBD 2012). We have encountered two Clear Lake hitch with pugheadedness, a cranial deformity. Based on literature review, we believe pugheadedness has not previously been documented in Clear Lake Hitch or any other fish species within Clear Lake or its watershed, though interviews with researchers and anglers would be helpful in solidifying this claim. Within California, the deformity has been documented in the closely related California Roach (Leidy 1985), as well as in Rainbow Trout (Croker 1931), and Brown Rockfish (Adams and Ryan 1982).

Pugheadedness is a skeletal deformity resulting in a characteristically steep forehead, bulging eyeballs, and a reduced upper maxillary. In severe cases, the mouth may be unable to close (Schmitt and Orth 2015). Skeletal abnormalities are exceedingly rare among wild fish, usually under 1% of the total population in undisturbed ecosystems (Dahlberg 1970, Berra and Au 1981). Surveying fish for deformities in an undisturbed freshwater creek, Berra and Au (1981) collected 2771 Bluntnose minnows (*Pimephales notatus*), of which 18 were pugheaded (0.65%). Dahlberg (1970) reported incidences of .05% and .25% for two estuarine species in the Chesapeake Bay. Among heavily disturbed environments, the rate of pugheadedness may be greater; in a heavily polluted stretch of the Rhine river, 2.6% of bream (*Abramis brama*) encountered were pugheaded (Sloof 1982).

The maxillary and cranial deformities characteristic of pugheadedness present early in development, and many individuals with the condition likely die as embryos (Morgan et al. 1981). Individuals that survive to adulthood may have reduced foraging efficiency (Schmitt and Orth 2015) or inefficient respiration due to impaired buccal pumping (Lijalad and Powell 2009).

Over three summers of an ongoing study of the habitat and status of Clear Lake Hitch (*Lavinia exilicauda chi*; Feyrer et al. 2019), we have encountered two pugheaded Clear Lake Hitch. The first was captured 27 June 2017 in a gill net set at 0749 hours in the Upper Arm of Clear Lake, CA (39.035, -122.905). It measured 257 mm standard length and weighed

185 grams (Figure 1). The second individual was captured 25 June 2019 in a gill net set at 1510 hours, also in the Upper Arm of Clear Lake, CA (39.029, -122.097). It measured 236 mm and weighed 235 grams (Figure 2). The upper maxilla of both individuals was severely reduced, giving the appearance of a protruding lower jaw and rendering the mouth unable to close. Both individuals displayed several visible tears on the caudal and anal fins, possibly caused from entanglement in the gill net, but displayed no other obvious deformities or external parasites.

The individual captured in 2017 appeared emaciated and was substantially lower in weight than expected based on a length-weight relationship generated from 643 Clear Lake Hitch observed during our study (Figure 3). The individual captured in 2019 appeared relatively normal in weight (Figure 3; Data are available from Steinke et al. (2018); https://doi.

Figure 1. Pugheaded Clear Lake Hitch collected in 2017, length 257 mm, weight 185 g.

Figure 2. Above, non-deformed Clear Lake Hitch, length 240 mm, weight 290 g. Below, pugheaded Clear Lake Hitch collected in 2019, length 236 g, weight 235 mm.

org/10.5066/P9A03OI6). This could be due to a variation in the etiology of pugheadedness, or differing levels of respiration or foraging impairment.

While causes of pugheadedness are not fully understood, most evidence points to epigenetic causes triggered by environmental factors (Schmitt and Orth 2015). However, no direct evidence links our observation of the pugheaded individual to any environmental condition. However, many factors linked to morphological deformity are present in Clear Lake, including pollution, periodic low concentrations of dissolved oxygen, and high temperatures (CBD 2012; Feyrer et al. 2019). Pollution, specifically heavy metal contaminants such as mercury and selenium, has been implicated in the development of skeletal deformities (Bengtsson et al. 1985; Lemly 1992). The operation of the Sulphur Bank Mercury Mine, now a U.S. Environmental Protection Agency Superfund site, has subjected Clear Lake to severe mercury contamination. Since the mine's closure in 1957, erosion of tailing piles and leaching from drainage pits have continued to add mercury to the system (Rueda et al. 2008). Gassel et al. (2005) found a methylmercury concentration of 0.16 ppm in Clear Lake Hitch tissue, the lowest concentration of any Clear Lake species sampled. Before the species was listed, the EPA suggested children and women of childbearing age limit their consumption of Clear Lake Hitch, to one serving a week (Gassel et al. 2005). All fish in the lake are subject to similar recommendations.

Clear Lake is also contaminated with numerous pesticides, both those applied directly to the lake to control aquatic weeds, and those used in the surrounding vineyards and orchards which contaminate runoff that drains into the watershed. Copper, organophosphates, and organochlorine are among the most commonly used agricultural pesticides in Lake County (CBD 2012); all of these are documented teratogens at acute doses (Eisler 1997; Sabra and Mehana 2015).

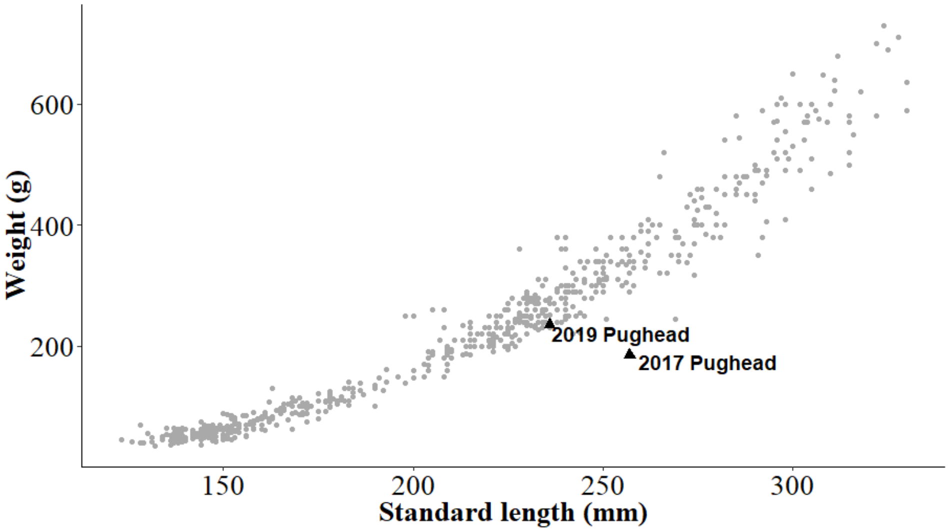

Figure 3. Length-weight relationship of Clear Lake Hitch collected from Clear Lake from 2017 to 2019 showing that the pugheaded individual encountered in 2017 was substantially lighter in weight than expected. Data are available from Steinke et al. (2018).

Highly eutrophic, Clear Lake is prone to noxious algal blooms and outbreaks of hypoxia that sometimes cause fish kills (Goldman and Wetzel 1964; Richerson et al. 1994). Water quality monitoring concurrent with fish sampling efforts (Feyrer et al. 2019; Steinke et al. 2019) found a hypoxic "dead zone" similar to those found in the Gulf of Mexico and the Chesapeake Bay, covering a large area of the lake bottom. Low dissolved oxygen has been linked to bodily anomalies (Berra and Au 1981; Slooff 1982), and Schmitt and Orth (2015) suggested that repeated encounters of pugheaded Blue Catfish in the Chesapeake Bay may be linked to hypoxia caused by eutrophication. Shallow water and hot summers also lead to elevated water temperatures in Clear Lake. In aquaculture operations, high temperatures have been linked to larval pugheadedness (Morgan et al. 1981).

Over three years and 644 Clear Lake Hitch observed, only two pugheaded specimens were encountered, an incidence of 0.31%. Comparing this rate to those in other populations is difficult due to differing sample sizes, methods, and species. However, our observed rate is lower than both the incidences reported by Sloof (1982), and Berra and Au (1981), but higher than those reported by Dahlberg (1970). Our observed incidence may be indicative of the overall rate of pugheadedness within the population, but higher effort and a larger sample size is needed to confirm this. Additionally, many deformed larvae have low survivability (Koo and Johnson 1970), and the rate of pugheadedness in early life stages may exceed our observed incidence among adults. If larval pugheadedness is high, larval mortality may be negatively impacting Clear Lake Hitch reproductive success and recruitment. In addition to the known threats Clear Lake Hitch face from habitat loss, poor water quality, and introduced predators (CBD 2012), teratogeny may be an additional factor impacting the species. Additional investigation is needed to determine the extent of pugheadedness within the population. Future research, including tissue and otolith analyses of pugheaded individuals, may yield more evidence towards a potential cause.

Author Contributions

Conceived and designed the study: FF, MY
Collected the data: FF, MY, JK
Performed the analysis of the data: MY, JK
Authored the manuscript: JK
Provided critical revision of the manuscript: MY, FF

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Unusual feeding observations of the California condor in the wild

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Atypical, non-digestible items consumed by the California condor (*Gymnogyps californianus*) are well documented (Collins et al. 2000; Houston et al. 2007; Mee et al. 2007). Termed 'micro-trash', previous papers have suggested that metal, plastic, and glass artifacts consumed by condors might be mistaken for bone materials consumed as a source of calcium, might be ingested to assist in pellet formation, or may represent exploratory quests for novel food items (Collins et al. 2000; Houston et al. 2007). It is currently unknown if this behavior is an attempt to consume additional calcium, to take what is perceived as needed bone fragments back to developing nestlings, or some other explanation. It is, however, known that micro-trash ingestion was the primary cause of nest failure in the reintroduced condor population from 2001 to 2005 and continues to threaten the reestablishment of a viable breeding population in southern California (Mee et al. 2007). In fact, micro-trash ingestion by condor chicks was found to be the leading cause of death for wild condor nestlings (Rideout et al. 2012). Between 2002 and 2005, of nine nestlings hatched in the wild, six died and two more were removed from the wild for health reasons. Most of this group were found to have consumed substantial quantities of non-digestible items. Micro-trash items have been recovered from birds post-mortem or discovered in and around nest sites, presumably regurgitated by the adult during the feeding of chicks.

Due to these feeding peculiarities, and the lethal outcome that can result, we believe field observations on condor feeding habits, no matter how brief, are noteworthy. Empirical observations of condors feeding in the wild are rare and anecdotal information on birds ingesting non-digestible items can have important management implications.

On 24 May 2018 at 1400, we observed 13 juvenile and adult California condors in a closed turnout on Templin Highway, 0.77 miles east of Interstate 5 in Southern California (N 34 34'17.85, W 118 40'33.38). Condors were observed for 30 minutes and remained at the turnout when biologists left. Several birds were perched on the outer guardrail, while others were standing in the turnout. All condors observed were tagged and appeared in good health.

Tag colors and tag numbers were as follows: Red 56, Red 61, Green 91, Green 32, Green 72, Green 30, Green 40, Green 96, Purple 48, Maroon 7, Blue 74, Yellow 47, and White 87.

During our initial observation, it appeared that the birds were simply resting in the turnout. We then noticed that the group were focused on two full plastic sandbags laying on the ground. Adult and juvenile birds would approach the bags and tug at them, in what appeared to be an attempt to rip open the plastic. Several condors were perched on the guardrail tugging at a third bag that had been placed in a 5-gallon bucket outside the turnout area. During our observation, birds were attempting to tug the bag out of the bucket as well as lift the bucket by the handle. Additionally, these bags appeared to be the focus of competition. Larger, older birds would frequently chase juvenile birds away from the bags during this encounter.

Twice we witnessed 2 different condors tugging on the woven, plastic bags and ingesting the strands of polypropylene material that made up the bag. Once the birds gained access to the contents, they began to actively manipulate the fine sand inside and appeared to ingest small quantities of the material. Eight of the thirteen condors observed stuck their heads inside the open bags to access the contents. Once small openings were torn in the bag, several birds were seen picking at and consuming the sand inside the bag. Digital photos and video were taken to document the observation.

One of the bags was collected when the birds moved away from it. The generic bag was made of woven polypropylene and filled with a fine grain cement sand. Bags of these types are frequently used to stem erosion or assist in channeling flood waters off roadways. These bags are constructed of treated plastic strips that are woven into sack-shaped bags with one open end. A long, thick strip of plastic is attached to the open end and is used to tie the bag closed after filling. The recovered bag measured 65 cm X 35.5 cm and appeared to be the standard sandbag used for various emergency flood events and erosion control.

Publications consistently state that condors are curious and intelligent (Collins et al. 2000; Houston et al. 2007; Mee et al. 2007). Empirical data made by researchers suggests that they frequently find food by investigating their environment, and this behavior does appear to fit what we observed. Interacting with human-made materials is also consistent with previously described condor behavior. However, the actual consumption of the materials in question could also suggest that they mistook the sandbags for a food source, or that they were attempting to access minerals that may have been present in the sand fill used in filling sandbags (Collins et al. 2000; Houston et al. 2007). These suggested interpretations of the observed behavior are purely speculative.

Although there is a body of published work documenting the consumption of micro-trash by condors, we did not find any information that would lead us to a firm conclusion of why this group collectively interacted with and ingested the sand. While many studies speculate on why condors consume non-digestible items (mineral deficiency, pellet formation or exploring novel food items), these inferences do not fit the behavior we observed. Given the described observation and current research information, we can only speculate why condors were observed picking at the sandbags and consuming the sand inside. It is unknown what negative effects, if any, consuming polypropylene strips from the bag or the sand would have on adult condors, or eventually their young if fed to fledglings. However, noting the lethal impacts of micro-trash on young fed non-digestible items by the adults, minimizing any foreign objects from the diet of the adults and chicks would be beneficial.

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INFORMATION FOR AUTHORS

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Front. California condor (*Gymnogyps californianus*). Photo by Jim Bahn (CC BY-ND 2.0)

Back. Barred Sand Bass (*Paralabrax nebulifer*). Photo by Josh More (CC BY-NC-ND 2.0).

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