COLDWATER LAKE AND RESERVOIR RESEARCH

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TABLE OF CONTENTS

Research Goals…..…………………………………………………………………………………5

Research Priority: Summer Profundal Index Netting for monitoring Lake Trout..........………....…5

Research Priority: Gill lice research……………….……………………….……….…..….........11

Research Priority: Mercury contamination in sport fish………………..……………………........13

Research Priority: Management of Walleye broodstocks…..…………...…………………….......16

Research Priority: Long-term food web dynamics in Horsetooth Reservoir………………..…...36

Research Priority: Triploid Walleye research………………..……...……...…………..………..83

Research Communication and Technical Assistance………..........................…………………....98

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COLDWATER LAKE AND RESERVOIR RESEARCH

Period covered: December 2020 – November 2021.

RESEARCH GOALS: Address questions and problems facing lake and reservoir fisheries managers throughout Colorado. Use field sampling, modeling and experiments to (1) diagnose the primary factors (e.g., harvest, habitat, recruitment, food supply, competition, predation or disease) driving the dynamics or limiting the production of important populations of fish; (2) use this information to identify and evaluate alternative approaches for improving or maintaining fish populations and their fisheries; and (3) develop new standardized sampling tools and reference points that improve the robustness of monitoring data and enable rapid assessment of fishery condition.

RESEARCH PRIORITY: Summer Profundal Index Netting for monitoring Lake Trout *Salvelinus namaycush*.

OBJECTIVES:

Use standard survey methods to estimate the abundance and size structure of Lake Trout in key coldwater reservoirs.

INTRODUCTION:

Lake Trout are top predators, reproduce naturally, and are important sport and food fish for anglers in Colorado's lakes and reservoirs. Monitoring their abundance and size structure is necessary for assessing the appropriateness of harvest regulations, ensuring Lake Trout remain in balance with prey fish populations, and determining whether management goals are achieved. However, estimating the abundance of Lake Trout in large coldwater reservoirs at the frequency needed to inform management using conventional methods such as mark-recapture is impractical.

Summer Profundal Index Netting (SPIN) is a quantitative survey method for rapidly estimating the density of Lake Trout (Sandstrom and Lester 2009). Previous investigations by Colorado Parks and Wildlife concluded that SPIN is a viable alternative to more intensive methods for estimating and tracking trends in the abundance of Lake Trout to help guide management (Lepak 2011; Lepak 2013). Four water bodies have been sampled using SPIN: Taylor Park Reservoir (surveyed in 2013), Lake Granby (2014), Grand Lake (2013, 2016), and Blue Mesa Reservoir (2011, 2014, 2016, 2018, and 2020). Results from the 2021 survey on Blue Mesa Reservoir are reported here.

METHODS:

SPIN uses suites of standardized gill nets (three 1.8 × 64 m nets consisting of eight panels with mesh sizes of 57-, 64-, 70-, 76-, 89-, 102-, 114- and 127-mm stretch measure placed in random order) to capture Lake Trout in a way that allows us to estimate their density directly (i.e., number per ha). These estimates of density are then scaled up to a total abundance based on the area of the lake or reservoir surveyed.

Catch rates of Lake Trout in gill nets fished in Colorado reservoirs are compared to catch rates in the same type of gill nets in other water bodies where independent estimates of Lake Trout density were available. The catch is adjusted for the size-selectivity of the gill nets. Nets are set along the bottom in random orientation. Set locations are selected at random and stratified by depth (2-10 m, 10-20 m, 20-30 m, 30-40 m, 40-60 m, 60-80 m, and >80 m). Sampling is also stratified by different regions within the lake or reservoir if necessary, to account for differences in Lake Trout habitat. Sampling is conducted when surface temperatures exceed 18°C and the nets are set for two hours during daylight. Netting was conducted from 9 to 12 August, 2021 in Blue Mesa Reservoir. The power of this method is the use of data from numerous other systems as a calibration tool to quantify Lake Trout densities in Colorado that can be used to estimate total abundance versus techniques that just provide estimates of relative abundance through time and across systems.

RESULTS & **DISCUSSION**:

Sampling was completed over the course of four days during August 2021, wherein 90 nets were set, capturing a total of 121 Lake Trout ranging in size from 237 mm to 830 mm FL (mean = 425 mm ± 113 mm SD). Lake Trout were most prevalent in 30-40 m depths across Cebolla and Sapinero basins. No Lake Trout were encountered in Iola, likely due to the low reservoir elevations observed in 2021. The depth distribution, size structure, and extent of the catch in 2021 at the corresponding water surface elevation of the reservoir produced a total Lake Trout abundance estimate of 9,775 fish ≥237 mm FL (lower 68% confidence limit = 7,213; upper limit = 12,627). The catch of Lake Trout <250 mm FL was incidental (1.65%). Therefore, these abundance estimates best reflect those of fish ≥250 mm FL as in previous SPIN surveys on Blue Mesa Reservoir (Sandstrom and Lester 2009; Table 1).

We examined whether there has been a disproportionate change in the estimated abundance of Lake Trout ≥363 mm FL or 400 mm TL (predominately age-4 and older) when compared to all fish vulnerable to the gear in Blue Mesa Reservoir over the period of record. In general, this length cutoff encompasses the most piscivorous fraction of the Lake Trout population, and those most vulnerable to anglers and ongoing suppression efforts (Lepak 2011; Pate et al. 2014). Abundance estimates for this separate size group of fish were lower, but exhibited a similar declining temporal trend as those incorporating all sizes of Lake Trout between 2011 and 2016. This indicated that there was not a disproportionate change in the abundance of this secondary size group when compared to all sizes of fish over the 2011-2016 period (Figure 1). However, this pattern changed in 2018 whereby the abundance of Lake Trout ≥400 mm TL continued to decline while the estimated abundance of all fish vulnerable increased (Figure 1). This indicated that there was a pulse of small fish, which were not yet fully vulnerable to capture by anglers or suppression efforts, about to enter the piscivorous size range.

Table 1. Summary data from each SPIN survey conducted to date. Abundance estimates are for all Lake Trout vulnerable to the sampling gear (generally those ≥250 mm FL or 275 mm TL). The acronym LCL stands for lower 68% confidence limit, and UCL stands for upper 68% confidence limit for the abundance estimate. Adjusted CUE is the area-weighted (area of different depth strata and reservoir basins) catch of Lake Trout per gill net set, after also correcting the catch for size-selectivity. Asterisks indicate the presence of *Mysis diluviana*.

Survey year	Lake or reservoir	Number of net sets	Number of lake trout caught	Mean total length (mm)	SD of total length (mm)	Adjusted CUE	Density (fish/ha)	Total area surveyed (ha)	Abundance estimate	LCL	UCL
2011											
	Blue Mesa	81	129	437	110	2.29	11.14	3,059	34,071	27,144	41,929
2013											
	Grand Lake*	36	87	419	107	2.61	12.71	193	2,452	1,974	2,996
	Taylor Park*	36	271	416	94	4.03	19.61	610	11,950	9,871	14,341
2014											
	Blue Mesa	81	211	425	97	1.61	7.85	3,409	26,753	18,383	33,716
	[a]Lake Granby*	71	501	417	79	11.78	57.26	2,780	159,193	135,533	186,844
2016											
	Blue Mesa	83	180	438	114	1.47	7.15	3,409	24,368	16,538	30,948
	Grand Lake*	36	109	436	147	3.34	16.22	193	3,131	2,561	3,783
2018											
	Blue Mesa	95	313	414	98	2.34	11.36	2,629	29,857	23,826	36,702
2020											
	Blue Mesa	90	212	441	92	1.51	7.32	2,247	16,443	12,518	20,842
2021	Blue Mesa	90	121	465	126	1.23	5.97	1,637	9,775	7,213	12,627

[a]Estimates for Lake Granby are subject to change. Food web interactions could make Lake Trout more vulnerable to the sampling gear causing the SPIN method to overestimate their abundance.

Comparing the size structure of Lake Trout captured during the 2018 SPIN survey to previous years confirmed that there was a higher frequency of small fish <400 mm TL (predominately age 2-3; Pate et al. 2014) present in the system, and that these fish would grow into a more piscivorous size range within the next 1-2 years (Figure 2). In addition to being ecologically significant, we detected a statistically significant difference in the size structure of Lake Trout captured during SPIN in 2011, 2014, 2016, and 2018 (Kruskal-Wallis One Way Analysis of Variance on Ranks; $H = 70.76$; $P < 0.001$) (Figure 2). Post-hoc comparisons to determine which years differed from each other indicated that the size structure of Lake Trout captured in 2018 differed from those captured in 2014 and 2016 ($P < 0.005$), largely due to the greater frequency of fish <400 mm TL (Figure 2). Because this pulse of small fish was likely comprised of age 2-3 individuals, it seems reservoir conditions during the 2014-2015 spawning seasons for Lake Trout were favorable.

Given the reduced abundance of piscivorous-sized Lake Trout as estimated from SPIN and corresponding boosts in the abundance of kokanee *Oncorhynchus nerka*, fall suppression netting was not completed in 2018 or 2019. Rather, an incentivized angler harvest tournament for Lake Trout ≤660 mm TL was conducted winter through early summer in 2020 in anticipation for the pulse of small fish observed in 2018 entering the size range fully vulnerable to anglers. Thus, the 2020 SPIN survey was completed after the tournament was concluded and reflects angler harvest that accrued earlier in the year. Tournament anglers turned in 4,055 Lake Trout, 44% (1,791) of which were fish ≥400 mm TL. The smallest fish turned in was 203 mm TL. Relative to 2018, the estimated abundance of all fish vulnerable in 2020 was much reduced, whereas the estimated abundance of piscivorous-sized fish was slightly elevated supporting the notion that small fish observed in 2018 recruited into the piscivorous size range as anticipated (Figure 1). The higher relative frequency of fish ≥400 mm observed in the SPIN catch in 2020 compared to 2018 also supported this notion (Figure 2).

The angler harvest incentive tournament was repeated in 2021 to reinforce results from 2020 and apply continued harvest pressure to the population of small Lake Trout. This year, 178 anglers participated turning in 1,704 heads, which was down from 2020 when 338 anglers participated and turned in 4,055 heads. Anglers generally captured larger fish in 2021 when compared to 2020, and the majority of heads were from fish >400 mm TL. Despite the reduced angler participation, SPIN estimates from 2021 (for all fish vulnerable and for most piscivorous fraction) were lower than those in 2020, and the lowest on record to date (Figure 1), indicating that harvest levels being achieved during the tournaments (combined with natural mortality) are sufficient for keeping small Lake Trout in check. In addition, we did not see a significant difference between the size-structure of Lake Trout captured during SPIN in 2021 versus 2020 ($P > 0.95$), but a highly significant difference ($P < 0.001$) between 2021 and 2018 (when a large pulse of small fish was moving through the system), suggesting that there may not be a large crop of small fish for anglers to catch in 2022 and the tournaments so far have been successful.

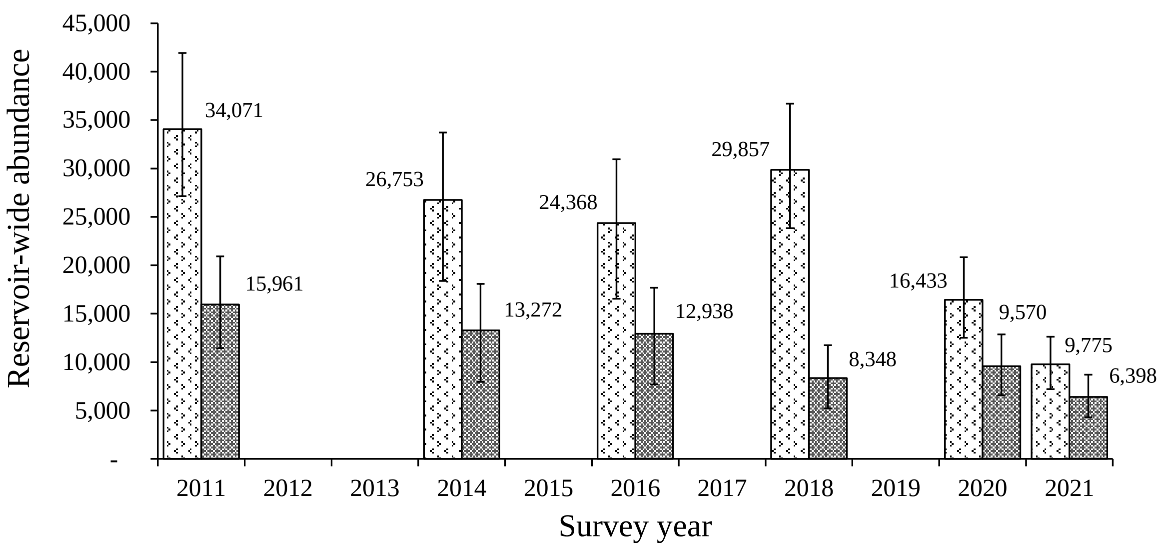

Figure 1. Abundance estimates for all Lake Trout vulnerable to the sampling gear (generally those ≥250 mm FL or 275 mm TL) in Blue Mesa Reservoir (white bars) and just those ≥363 mm FL or 400 mm TL (gray bars) from all SPIN surveys conducted to date. Error bars represent 68% confidence intervals.

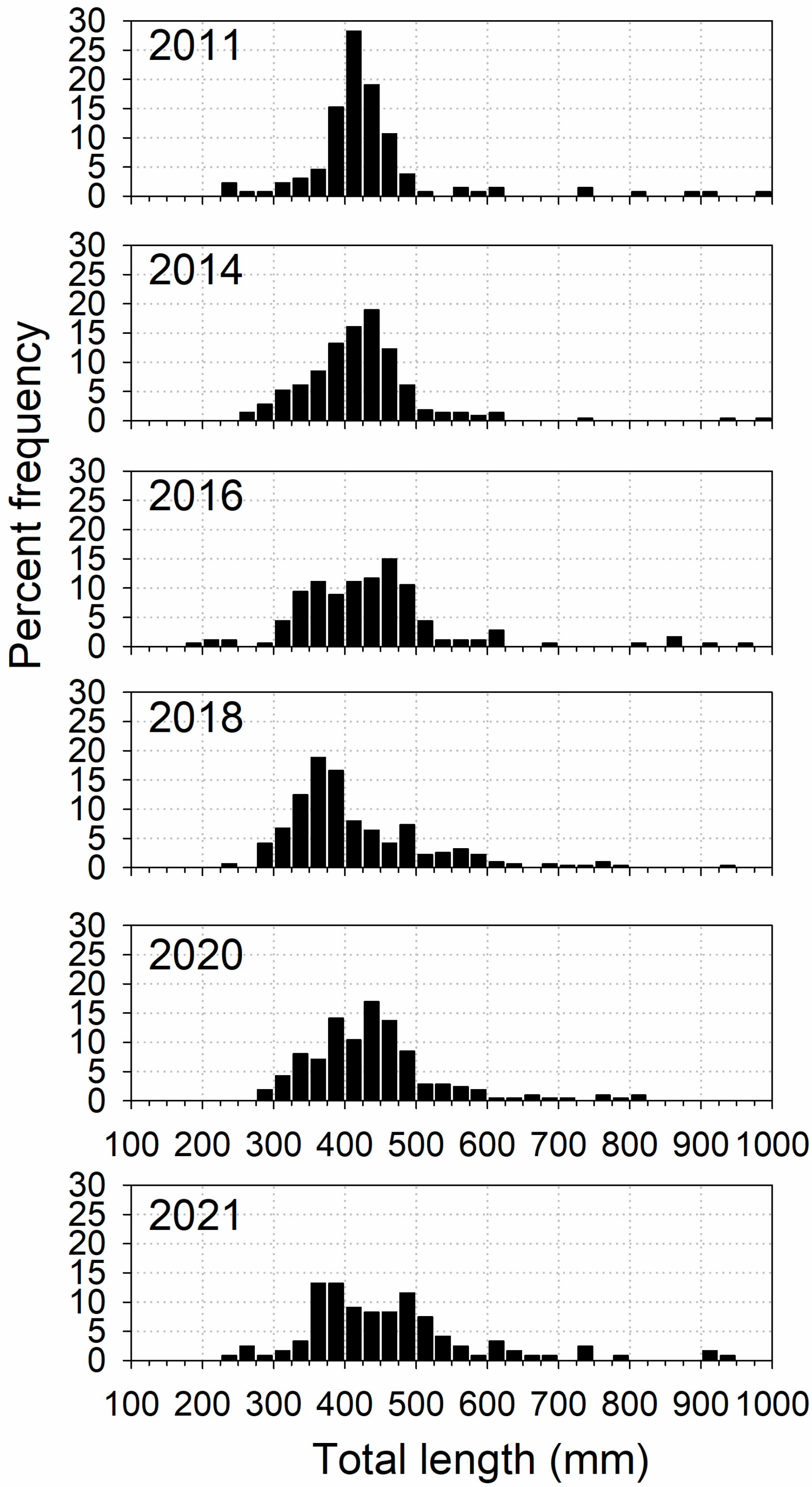

Figure 2. Length-frequency distributions (25 mm size bins) of Lake Trout captured during consecutive SPIN surveys on Blue Mesa Reservoir.

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RESEARCH PRIORITY: **Gill lice research:** Monitoring of gill lice and the distribution, size- and age-structure of kokanee in Blue Mesa Reservoir.

PUBLICATION:

Lepak, J. M., **A. G. Hansen**, M. B. Hooten, D. Brauch, and E. M. Vigil. 2021. Rapid proliferation of the parasitic copepod, *Salmincola californiensis* (Dana), on kokanee salmon, *Oncorhynchus nerka* (Walbaum), in a large Colorado reservoir. Journal of Fish Diseases. Available online: https://onlinelibrary.wiley.com/doi/10.1111/jfd.13539?af=R.

OBJECTIVES:

Characterize parasite-host dynamics between *Salmincola californiensis* and kokanee through intensive seasonal and annual monitoring of immature and mature fish in Blue Mesa Reservoir.

INTRODUCTION:

The 2020 Coldwater Lake and Reservoir Research Annual Report describes the history of interactions between *Salmincola californiensis* and kokanee salmon in Colorado, and specifically in Blue Mesa Reservoir. In 2021, the Lake and Reservoir Research Laboratory focused on reporting findings observed from 2016-2020 in the valuable kokanee salmon population in Blue Mesa Reservoir. The result was the publication listed above in the Journal of Fish Diseases in summer 2021.

Observations showed that the invasion front (and associated exponential population growth) of *Salmincola californiensis* largely drove the patterns identified in Blue Mesa Reservoir. The publication abstract is below, and the primary figure (Figure 1) from the publication is included.

MANUSCRIPT ABSTRACT:

Ecologically and economically valuable Pacific salmon and trout (*Oncorhynchus* spp.) are widespread and susceptible to the ectoparasite *Salmincola californiensis* (Dana). The range of this freshwater copepod has expanded, and in 2015, *S. californiensis* was observed in Blue Mesa Reservoir, Colorado, USA, an important kokanee salmon (*O. nerka*, Walbaum) egg source for sustaining fisheries. Few *S. californiensis* were detected on kokanee salmon in 2016 (<10% prevalence; two adult *S. californiensis* maximum). By 2020, age-3 kokanee salmon had 100% *S. californiensis* prevalence and mean intensity exceeding 50 adult copepods. Year and kokanee salmon age/maturity (older/mature) were consistently identified as significant predictors of *S. californiensis* prevalence/intensity. There was evidence that *S. californiensis* spread rapidly, but their population growth was maximized at the initiation (the first 2-3 years) of the invasion. Gills and heads of kokanee salmon carried the highest *S. californiensis* loads. *S. californiensis* population growth appears to be slowing, but *S. californiensis* expansion occurred concomitant with myriad environmental/biological factors. These factors and inherent variance in *S. californiensis* count data may have obscured patterns that continued monitoring of parasite-host dynamics, when *S. californiensis* abundance is more stable, might reveal. The rapid proliferation

of *S. californiensis* indicates that in 5 years, a system can go from a light infestation to supporting hosts carrying hundreds of parasites, and concern remains about the sustainability of this kokanee salmon population.

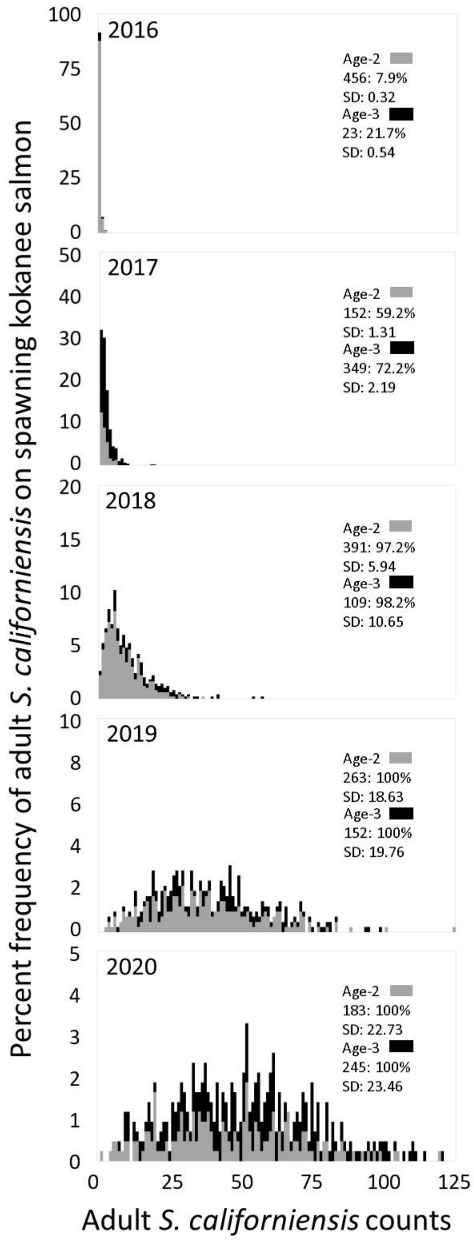


Figure 1. Prevalence and intensity of adult *S. californiensis* on kokanee salmon collected during the Blue Mesa Reservoir spawning run at the Roaring Judy Hatchery from 2016 to 2020. Essentially all kokanee salmon were estimated to be age-2 (light gray bars) or age-3 (black bars). Note the difference in scales on the x-axes for percent frequency of adult *S. californiensis* counts on kokanee salmon. In the upper right of each panel, sample sizes are provided by age class, followed by the prevalence of adult *S. californiensis* infections on kokanee salmon, and the standard deviation (SD) of adult *S. californiensis* counts each year (upper left).

<u>RESEARCH PRIORITY</u>: **Mercury contamination in sport fish:** Revisiting mercury research from 2014 Annual Report—Predictors of mercury contamination in Colorado sport fish: implications for informing TMDL development and the protection of human and ecological health.

MANUSCRIPT SUBMISSION:

Lepak, J. M., B. M. Johnson, M. B. Hooten, B. A. Wolff, and **A. G. Hansen**. Predictors of Sport Fish Mercury Contamination in Heavily Managed Reservoirs: Implications for Human and Ecological Health. Rejected with positive reviews: Science of the Total Environment.

Lepak, J. M., B. M. Johnson, M. B. Hooten, B. A. Wolff, and **A. G. Hansen**. Predictors of Sport Fish Mercury Contamination in Heavily Managed Reservoirs: Implications for Human and Ecological Health. In revision for Environmental Science and Technology.

OBJECTIVES:

To prepare and submit a manuscript that identified factors at the landscape scale that are influencing mercury concentrations in Northern Pike *Esox lucius*, Smallmouth Bass *Micropterus dolomieu*, and Walleye *Sander vitreus*. We also included an evaluation of theoretical changes in mercury deposition and food web structure to compare the magnitude and timing of potential changes in sport fish mercury concentrations from those changes. This manuscript is now in revision for resubmission.

INTRODUCTION:

Fish consumption advisories associated with mercury contamination have been put in place by the Colorado Department of Public Health and the Environment. The Lake and Reservoir Research Laboratory has provided technical advice for setting these advisories for several years, and multiple research projects have been conducted to address this issue from a food web perspective in Colorado. For example, Lepak et al. (2012a), Lepak et al. (2012b), Stacy and Lepak (2012), Johnson et al. (2015), Lepak et al. (2016), and Wolff et al. (2017) all provide Colorado-specific information about mercury contamination in sport fish and how management may influence mercury concentrations. Continuation of this work at the landscape level for more predictive purposes was made possible by compiling data from across the state and applying a machine learning approach to inform what might be driving mercury concentrations in Northern Pike, Smallmouth Bass, and Walleye. We also evaluated the magnitude and timing of potential changes in sport fish mercury concentrations based on different deposition and food web change scenarios. Below are the manuscript highlights, manuscript abstract, as well as the graphical abstract. The first submitted manuscript is also included as Appendix A.

HIGHLIGHTS:

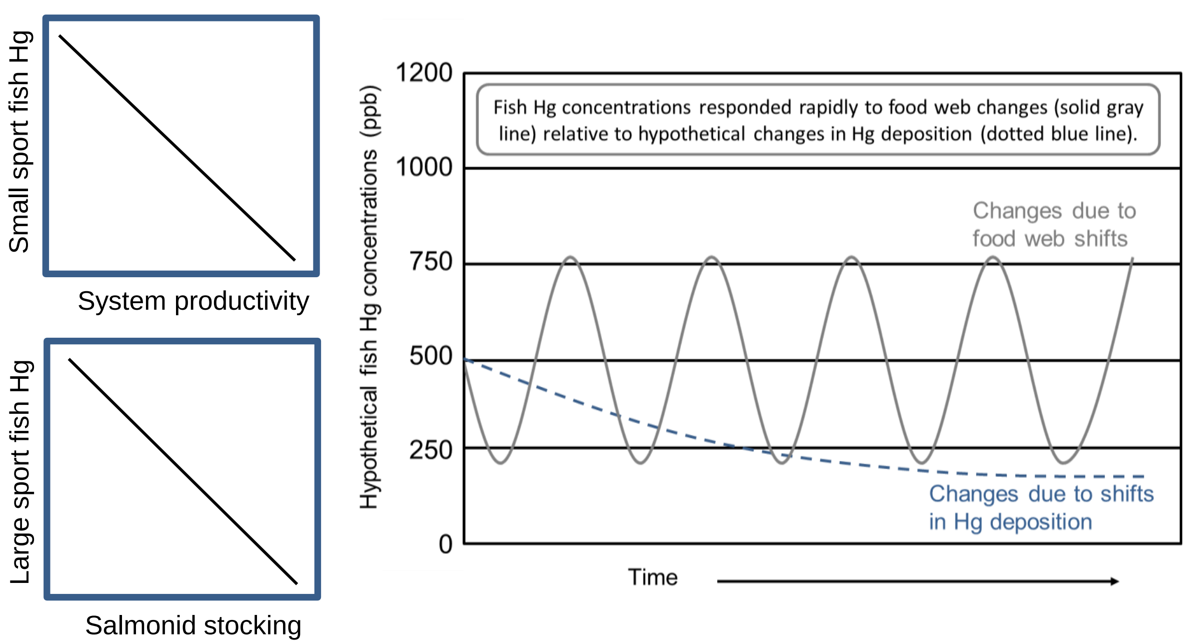
- Mercury concentrations in sport fish were evaluated using a random forest approach

- Potential change in fish Hg from food web and Hg deposition changes were compared
- System productivity and salmonid stocking were important for predicting fish Hg
- Food web shifts can cause significant changes in fish Hg in the short-term
- Long-term global reductions in Hg deposition should reduce fish Hg concentrations

MANUSCRIPT ABSTRACT:

Mercury (Hg) is an important contaminant due to its widespread distribution and tendency to bioaccumulate to harmful levels in the environment. We used a machine learning approach called Random Forest (RF) to evaluate different predictors of Hg concentrations in three species of Colorado (USA) sport fish. We also compared empirical and conceptual rates of change in sport fish Hg concentrations resulting from multiple data sources including empirical observations and theoretical rates of change from different Hg deposition scenarios. Our RF models indicated that the best predictors of large northern pike (*Esox lucius*) Hg concentrations at 864 mm were covariates related to salmonid stocking in each study system, while system-specific metrics related more to productivity and forage base were the best predictors of Hg concentrations of smallmouth bass (*Micropterus dolomieu*) and walleye (*Sander vitreus*) at 381 mm. Our theoretical and empirical comparisons indicated that system-specific food web characteristics (e.g., forage base, stocking history) can be important drivers of rapid and large shifts in some sport fish Hg concentrations compared to what might be expected in response to large-scale changes in Hg deposition over time. Importantly, protecting human and ecological health from Hg contamination requires an understanding of fish Hg concentrations and variability across the landscape and through time, and the RF approach can be applied to predict how sport fish Hg concentrations may change as a result of a variety of factors to help prioritize, focus, and streamline monitoring efforts.

GRAPHICAL ABSTRACT:



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RESEARCH PRIORITY: **Management of Walleye broodstocks**: Assessment of Walleye fry stocked into Chatfield and Cherry Creek Reservoirs as part of a broader study evaluating population dynamics in key brood-waters.

OBJECTIVES:

Evaluate the post-stocking performance of Walleye fry in Chatfield and Cherry Creek Reservoirs to help inform appropriate stocking procedures.

INTRODUCTION:

Walleye are highly sought after sport and food fish east of the continental divide in the state of Colorado. Given generally poor natural-recruitment and high fishing pressure, most Walleye fisheries are sustained through annual stocking of fry in early April and pond-reared 1-2 inch fingerlings later in May or early June. Statewide stocking requests are supported through spring egg-take operations from wild broodstocks in three primary reservoirs: Chatfield, Cherry Creek and Pueblo. Current harvest regulations (18 inch minimum length limit; daily bag limit = 3-5 depending on reservoir; only one >21 inches is allowed) on each of the broodstock reservoirs are restrictive to protect larger females essential for meeting egg collection goals and statewide stocking schedules. However, anglers have expressed interest in relaxing regulations at Pueblo Reservoir to allow harvest of smaller fish given a perceived lack of 'keepers' over the 18 inch minimum length limit. In addition, spring egg-takes at Chatfield Reservoir have been relatively poor in recent years, placing greater burden on Pueblo and Cherry Creek Reservoirs.

Given recent questions from anglers regarding harvest regulations at Pueblo and concerns over poor egg-takes at Chatfield, we initiated a new study to characterize the population demographics of each Walleye broodstock to (1) help inform population models for objectively assessing the appropriateness of different harvest regulations, and (2) make standardized comparisons among populations to help discern potential factors limiting Walleye in Chatfield vs. elsewhere and to identify potential management solutions or areas in need of additional research.

Fall Walleye Index Netting surveys conducted in 2019 indicated that there could be an early growth and survival bottleneck influencing fry stocked into Chatfield Reservoir (see 2020 Annual Report). Therefore, in spring 2021, we repeatedly sampled each reservoir using ichthyoplankton nets to track trends in the density and body size of stocked fry through time in relation to environmental factors and food supply (i.e., zooplankton). Sample processing is still ongoing. Below we provide a manuscript evaluating preservation effects on larval Walleye and Gizzard Shad *Dorosoma cepedianum* body size—needed for accurately estimating growth for the broader study of fry stocking success.

MANUSCRIPT SUBMISSION:

Cristan, **E. C.**, **A. G. Hansen**, and **J. M. Lepak**. *In review*. Effects of ethanol preservation on larval and fingerling Walleye and Gizzard Shad body size. North American Journal of Fisheries Management.

Effects of Ethanol Preservation on Larval and Fingerling Walleye and Gizzard Shad Body Size

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Abstract

Characterizing the dynamics of larval fish can help identify factors contributing to poor or strong recruitment events and inform stocking practices. Central to dynamics early in life is growth, which can influence access to prey during critical periods and survival. However, obtaining accurate body size measurements of larval fish in the field can be challenging, and often means samples must be preserved for later analysis. Accounting for potential preservation effects is needed for accurately characterizing growth and size-dependent predator-prey interactions. Here, we examined the effects of preservation in 95% ethanol on the total lengths (TL; mm) and wet weights (WW; mg) of larval and fingerling Walleye *Sander vitreus* and Gizzard Shad *Dorosoma cepedianum* collected from the field using pushed ichthyoplankton nets. We also estimated the time required for preservation to stabilize. Responses to preservation were similar between species. We observed consistent reductions in WW of ~40-70% depending on fish size, which took ~2 months to stabilize. Conversely, individuals increased or decreased in TL by ~5-10%. Because responses occurred in both directions, the overall effects of preservation on TL were minimal (<2.5% reductions) when averaging across individuals. Measurements of Walleye and Gizzard Shad body size play an important role in understanding their dynamics early in life, and preservation effects should be considered to accurately and precisely collect these metrics.

INTRODUCTION

Accurate and precise morphometric measurements of larval fish are essential for understanding the interplay among feeding and growth (May et al. 2020), survival (Letcher et al. 1996), and recruitment success (Heath and Gallego 1997; Quist et al. 2003; Gostiaux et al. 2021) during early life stages. For example, repeated measures of larval fish density and size in relation to food supply and environmental variables across seasons can help identify the extent and timing of potential ecological bottlenecks contributing to poor year-class strength (McDonnell and Roth 2014). However, collecting accurate and precise measurements of larval fish size can be challenging, especially in the field when poor conditions and time constraints are common (Shields and Carlson 1996; Paradis et al. 2007; Greskiewicz and Fey 2018). The difficulty of accurately measuring larval fish in the field often requires that samples be preserved and transported for later analysis in a laboratory.

Fish are commonly preserved using ethanol, formalin, or freezing, and each preservation method can significantly alter the length and weight of larval fishes (Korwin-Kossakowski 2014). In addition, preservation effects are highly variable among species, preservation types, and concentrations of preservative (Fey 1999). Despite extensive research documenting the effects of different preservation methods on larval fish (Theilacker 1980; Jennings 1991; Johnston and Mathias 1993; Shields and Carlson 1996; Fey 1999; Smith and Walker 2003; Paradis et al. 2007; Korwin-Kossakowski 2014; Greskiewicz and Fey 2018), little is known about the effects of ethanol preservation on larval and fingerling-sized Walleye *Sander vitreus* and Gizzard Shad *Dorosoma cepedianum*. The effects of different preservation methods including formalin, freezing and 70% ethanol on young Walleye have been previously conducted (Glenn and Mathias 1987; Johnston and Mathias 1993), however, the effects of preservation in

95% ethanol have not been evaluated despite being used commonly in studies evaluating potential recruitment bottlenecks for Walleye (e.g., Gostiaux et al. 2021). To our knowledge, evaluations of preservation effects on Gizzard Shad have not been conducted, or have not been reported in an accessible peer-reviewed outlet (i.e., Pitts 1992).

Walleye are a popular sport and food fish throughout North America (Hushak et al. 1986) and often co-occur with Gizzard Shad in Great Plains and Western reservoirs (Wuellner et al. 2008). Understanding interactions between these two species and what influences their growth, survival, and recruitment is important for mangers to better balance predator-prey ratios and maintain or enhance recreational angling opportunities for Walleye, particularly in systems stocked to supplement natural reproduction (Quist et al. 2003). Here, we examine the effects of ethanol preservation on larval and fingerling Walleye and Gizzard Shad. The objectives of this study were to: 1) test whether preservation in 95% ethanol effects the length and weight of larval and fingerling Walleye and Gizzard Shad, 2) calculate percent changes in the lengths and weights of larval and fingerling Walleye and Gizzard Shad post-preservation, 3) develop species-specific models enabling correction for preservation effects if present, and 4) estimate the amount of time (d) it takes for the effects of preservation in 95% ethanol to stabilize.

METHODS

Larval Walleye and Gizzard Shad were collected from Chatfield and Cherry Creek reservoirs in Arapahoe County, Colorado, using an ichthyoplankton net with twin 75 cm diameter rings each equipped with a 1,000 μm mesh conical net constructed with a 4:1 length-to-mouth diameter ratio (Sea-Gear® Corporation). Fish were collected from surface waters after dark every two weeks after the reservoirs were stocked with Walleye fry and post-hatch of

Gizzard Shad throughout April, May, June, and July 2021. All samples were stored in 35-L containers filled with chilled lake water for transport back to the laboratory, and measurements were taken post-mortem less than 12 h after capture. Pre-measurement storage and transport methods followed those of other studies evaluating preservation effects on larval fish collected in the field (Shields and Carlson 1996; Fisher et al. 1998; Paradis et al. 2007) rather than being received from a hatchery or reared directly in the laboratory (e.g., Grezkiewicz and Fey 2018). However, both reservoirs were stocked with larger hatchery pond-reared fingerling Walleye (25-40 mm) in June, and subsamples of these fish from the hatchery were transported live back to the laboratory for immediate measurement and preservation post-euthanasia with Aqui-S®.

Walleye and Gizzard Shad were selected at random for analysis. The total length (TL) of all fish <14 mm were measured to the nearest 0.01 mm using a compound microscope equipped with an Infinity X camera and corresponding Xfinity Analyze software (Teledyne Lumenera). Fish ≥14 mm were measured to the nearest 0.01 mm using digital calipers. Wet weights (WW) of all sizes of fish were measured using an Ohaus Pioneer digital balance with 0.1 mg precision. Individual fish were first blotted on Kimwipes® and placed into a beaker of water on the balance to minimize dehydration. Once initial measurements were complete, individual Walleye and Gizzard Shad were stored in scintillation vials containing 95% ethanol and re-measured every 7 – 10 d until preservation effects reached equilibrium. All individuals damaged during repeated measurements were eliminated, and only intact individuals were considered in analyses.

We assumed some inflation in body size occurred before initial measurements were taken as a result of overnight storage in freshwater and the cessation of osmoregulation following death (Black 1957; Jennings 1991). Therefore, a subsample of Gizzard Shad (20-28 mm TL; 0.05-0.18 g WW; *N* = 14) and Black Crappie *Pomoxis nigromaculatus* (17-30 mm TL; 0.06-0.33 g WW; *N*

= 15) were measured directly after capture in the field, stored in chilled lake water, and re-measured 12 h later to estimate the extent of inflation and correct for this bias. Black Crappie were included to broaden the TL and WW range of fish available and to incorporate a spiny-rayed fish as a surrogate for Walleye which were no longer vulnerable to the ichthyoplankton nets at the time of sampling. Both species exhibited similar levels of inflation and data from each were combined. Inflation in TL and WW averaged 3.77% (SE = 0.45%) and 15.42% (SE = 1.17%), respectively. Inflation was not dependent on body size for either TL or WW (R^2 = 0.054-0.061; P = 0.223-0.235). Thus, we reduced all TL and WW measurements taken prior to preservation by their respective mean percent inflation factor.

To test whether adjusting for inflation effectively removed bias in the initial TL and WW measurements, we compared the effects of preservation on Gizzard Shad requiring adjustment (GSD_a) to Gizzard Shad not requiring adjustment (GSD). The latter group was collected from Chatfield and Cherry Creek reservoirs during daylight and transported live to the laboratory for immediate measurement post-euthanasia. We used ANCOVA models to examine whether the linear relationships between unpreserved and preserved (once stabilized) TLs and WWs differed between the adjusted [10.87-24.45 mm TL and 3.0-104.8 mg WW fish (N = 32 for both)] and unadjusted [9.46-27.83 mm TL and (N = 34) and 1.8-110.0 mg WW (N = 25) fish] Gizzard Shad for overlapping size-ranges of fish. The interaction term was not significant for both TL (P = 0.066) and WW (P = 0.114) at an α level of 0.05, indicating that regression slopes did not differ between groups. Further, marginal means did not differ between groups for both TL [mean TL = 18.04 mm (95% CI = 17.87-18.21) for GSD_a and 18.24 mm (18.07-18.41 mm) for GSD; P = 0.094] and WW [mean WW = 26.9 mg (25.9-27.9 mg) for GSDa and 26.8 mg (25.7-27.9 mg) for

GSD; $P = 0.951$] demonstrating that our adjustments for inflation effectively removed bias from the initial TL and WW measurements.

We used linear regression analyses to test whether preservation in ethanol affected the TLs and WWs of Walleye and Gizzard Shad and to estimate mean time to stabilization after adjusting for inflation. First, we compared relationships between unpreserved and preserved (once stabilized) TLs and WWs to a 1:1 relationship both visually and by asking whether the fitted slope and intercept parameters differed significantly from 1.0 and 0.0, respectively, based on corresponding 95% CIs. These relationships also enabled back-calculation of unpreserved measurements from preserved measurements. For relationships using WWs, we observed a clear reduction in slope at ~60 mg (unpreserved) and ~20 mg (preserved) perhaps due to shifts in body morphology. Therefore, linear relationships were developed for Walleye and Gizzard Shad above and below 60 mg separately, whereas all sizes of fish [both larvae (generally <25 mm) and fingerlings (≥25 mm)] were included in the models for TL. Lastly, because WWs were affected most by preservation in 95% ethanol (see results), we regressed the mean percent change in WW (calculated from one post-preservation measurement date to the next) computed for each collection group (i.e., individuals sampled on the same date) of Walleye and Gizzard Shad as a function of days post-preservation to estimate time to stabilization. Percent changes in WW and days post-preservation were natural log transformed to linearize the data and the estimated x-intercept (i.e., days post-preservation when change in WW was effectively zero) and 95% CI reflected time to stabilization and corresponding uncertainty.

RESULTS

The effects of preservation in 95% ethanol on the TLs of individual fish were variable, and we observed both reductions in TL and increases in TL post-preservation for Walleye (ranged between 5.90% reductions to 9.76% increases) and Gizzard Shad (ranged between 10.47% reductions to 9.70% increases) across body sizes examined. However, preservation effects on TL were minimal when averaging across individuals by fitting the linear regression models to unpreserved and preserved TLs. Although slope and intercept values were significantly different from 1.0 and 0.0 (Table 1), respectively, the relationship between unpreserved and preserved TLs followed a 1:1 line closely for both species despite variability observed at the individual level (Figure 1). For Walleye, a significant slope slightly >1.0 appeared to be driven by fingerling-sized individuals ≥25 mm, which were mostly above the 1:1 line indicating a more consistent reduction in length post-preservation by an average of 2.24% [SE = 0.55%; ranged between 4.95% reductions to 7.05% increases (only one individual)]. Conversely, larval Walleye were more evenly distributed across the 1:1 line and only reduced in length by 0.20% (SE = 0.46%; ranged between 5.90% reductions to 9.76% increases) on average. Such nuances were less apparent for Gizzard Shad, which reduced in length post-preservation by an average of 1.31% (SE = 0.41%) across all sizes of fish.

Unlike TLs, changes in WWs post-preservation in 95% ethanol were notable and in a consistent direction. The WWs of Walleye and Gizzard Shad <60 mg (unpreserved) decreased by an average of 69.61% (SE = 0.82%; range = 56.46-83.45%) and 70.65% (SE = 1.02%; range = 61.47-92.61%), respectively. As a result, the relationships between unpreserved and preserved WWs largely diverged from a 1:1 line and exhibited slope and intercept values significantly greater than 1.0 and 0.0, respectively (Table 1; Figure 2). Reductions in WWs were less extreme for Walleye and Gizzard Shad >60 mg, but relationships between unpreserved and preserved WWs still largely deviated from a 1:1 line (Table 1; Figure 3). Overall, reductions in WWs averaged 41.53% (SE = 1.47%; range = 37.68-47.19%) for Walleye and 55.90% (SE = 1.72%; range = 46.00-65.78%) for Gizzard Shad over the larger weight range.

The effects of preservation in 95% ethanol on the WWs of Walleye and Gizzard Shad stabilized at similar rates. The rate of change in WW was most rapid during the first 15-30 days of preservation, then slowed (Figure 4). The fitted linear regression model between percent changes in WW (i.e., %ΔWW) and days post-preservation [d_{post}; $\ln(\%\Delta WW) = -1.364 \times \ln(d_{post}) + 6.409$; $N = 58$; $R^2 = 0.503$; $P < 0.001$] indicated that Walleye and Gizzard Shad collectively displayed no further reductions in WW at an estimated 52 d_{post} (lower 95% CI = 41 d_{post}; upper 95% CI = 66 d_{post}) on average.

DISCUSSION

Changes in the TLs of individual Walleye and Gizzard Shad larvae and fingerlings post-preservation in 95% ethanol were measurable (~5-10%), but occurred in both directions (reductions and increases observed), resulting in minimal effects when averaging across individuals. Alternatively, we observed consistent, biologically significant reductions in WW ranging from ~40-70% depending on fish size (larger reductions were observed in smaller fish). The magnitudes of reduction were similar between species, and WWs required at least two months to stabilize. Therefore, to appropriately use the size-dependent linear relationships between unpreserved and preserved WWs developed in this study to correct for the effects of preservation in 95% ethanol, we suggest taking post-preservation measurements after at least 66 days (estimated upper 95% CI for time to stabilization) to ensure equilibrium has been reached. Otherwise, our regression models may overcorrect for loss in weight. Because reductions in TL were <2.5% on average across species, using our regression models to estimate unpreserved TLs should be less sensitive to the timing of post-preservation measurements, and we leave it to practitioners to determine whether these minor percent reductions in length on average are biologically relevant. However, results from this study now provide the information needed to account for the effects of a common preservative if necessary.

The effects of preservation observed in our study generally aligned with those reported for several fish species, with some exceptions. Others observed that smaller fish exhibited greater reductions in length and weight post-preservation when compared to larger individuals (Theilacker 1980; Johnston and Mathias 1993; Fey 1999; Korwin-Kossakowski 2014). We observed this same pattern in the WWs of larval and fingerling Walleye and Gizzard Shad, but such patterns were not as pronounced in the TLs of Gizzard Shad and opposite in the TLs of Walleye (fingerling-sized fish exhibited slightly greater reductions in length on average than larvae). In addition, others reported the highest rate and magnitude of change in TL and WW occurred within the first several days or hours of preservation, with limited changes thereafter (Fisher et al. 1998; Fey 1999; Smith and Walker 2003; Paradis et al. 2007). Although our data changed most rapidly during early periods of preservation, we observed that significant changes could still occur weeks after initial preservation, but may depend on the concentration or type of preservative used. To our knowledge, there has been no explicit statistical characterization of how long it takes for preservation effects to stabilize in larval fish, which we provide for 95% ethanol. Similar responses observed from Walleye and Gizzard Shad suggest that time to stabilization in 95% ethanol could be general across species, but requires investigation.

The extent of changes in the TLs and WWs of larval Walleye and Gizzard Shad observed in this study were on the same order as other co-occurring species preserved in ethanol, but differences in methodology can complicate comparisons among studies and species. For example, larval Yellow Perch *Perca flavescens* sampled with a 500-µm mesh push net and stored in 75% ethanol decreased in TL by 9.6% and in WW by 61.1% on average 15 days post-preservation—some additional minor reductions were observed eight months post-preservation (Paradis et al. 2007). Fisher et al. (1998) observed 11.5-14.3% reductions in the TLs of larval Yellow Perch stored in 50-100% ethanol after capture in an ichthyoplankton net (500-µm mesh) similar to that used by Paradis et al. (2007). These responses were relatively insensitive to the concentration of ethanol used and stabilized within 5-10 days, but measurements were not taken after 21 days. Similar to our approach, both studies took initial measurements prior to preservation on fish post-mortem after storage and transport to the laboratory in lake water over a period of hours. However, neither tested nor corrected for potential osmotic inflation/hydration (described in Methods), which may partially explain the greater reductions observed on the TLs of larval Yellow Perch when compared to Walleye in this study, but does not exclude the influence of other potential species-specific or methodological differences (e.g., we used a 1,000-µm mesh net).

Responses to preservation may depend on how experimental fish are collected. Interestingly, Glenn and Mathias (1987) reported consistent 4-11% reductions in the mean TLs of Walleye ranging from 9.6-31.6 mm after only 3 days post-preservation in 70% ethanol. Although responses observed in our study were on a similar magnitude at the individual level after approximately 2 months, changes consistently occurred in both directions, so effects were minimal when averaging across individuals. Greszkiewicz and Fey (2018) observed a 3.4% reduction on average in the lengths of larval Northern Pike *Esox lucius* 90 days post-preservation in 96% alcohol. Consistent between these two studies was that each used fish that were maintained live in laboratory tanks or beach seined live from hatchery ponds prior to preservation rather than captured with a pushed or towed ichthyoplankton net in the field. The latter types of nets can influence the response of larval fish to preservation (Theilacker 1980; Fey 1999). Thus, practitioners should consider the methodological details of preservation studies before application of correction factors. Here, our field methodology reflected those commonly used in studies evaluating recruitment bottlenecks in Walleye (Quist et al. 2003; Gostiaux et al. 2021), and we accounted for potential artifacts arising during pre-measurement storage and transport from the field to laboratory.

Management Implications

Fish that express a prolonged pelagic larval stage such as Walleye can experience high interannual fluctuations in recruitment often stemming from processes influencing mortality during the first few months of life (Cushing 1990; Ludsin et al. 2014). Identifying factors contributing to poor recruitment is needed to determine management strategies that alleviate ecological bottlenecks early in life, but contributing factors may vary system to system requiring system-specific studies. For example, recruitment of Walleye in Lake Erie was strongly positively correlated with the biomass of copepods and weakly correlated with the biomass of cladocerans during the spring larval period (May et al. *in press*). Conversely, metrics of zooplankton abundance and size-structure could not distinguish northern Wisconsin lakes with declining trends in Walleye recruitment from those with sustained natural reproduction (Gostiaux et al. 2021). In systems where Gizzard Shad and Walleye co-occur, Gizzard Shad may reduce the growth and survival of Walleye fry through competition for zooplankton, or enhance growth and survival depending on the timing of Gizzard Shad emergence in relation to ontogenetic shifts to piscivory (Momot et al. 1976; Donovan et al. 1997; Quist et al. 2003).

Characterizing processes such as size-structured predator-prey interactions early in life and dependence on environmental variables requires reliable information on the lengths and weights of individuals, particularly from a bioenergetics perspective (Letcher et al. 1996; McDonnell and Roth 2014). Skewed estimates of Walleye body size and growth could lead managers to misidentify factors driving survival and recruitment. Knowing the timing and causes of recruitment bottlenecks can help structure management strategies to offset recruitment declines and maximize the effectiveness of Walleye fry and fingerling stocking (Gostiaux et al. 2021). Measurements of Walleye and Gizzard Shad body size play an important role in understanding their dynamics early in life, and preservation effects should be considered to accurately and precisely collect these metrics. This is particularly relevant for studies requiring information on the weights (most sensitive to preservation) of individuals through time in addition to lengths (minimally sensitive to preservation in this study).

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TABLES:

TABLE 1. Summary of Walleye and Gizzard Shad (GSD) preserved in 95% ethanol from this study with sample sizes (*N*), mean TLs and WW's, corresponding body size ranges, and results from linear regression analyses relating unpreserved measurements to preserved measurements (once stabilized) which can be used to correct for the effects of preservation.

Species and size-group	*N*	Mean ± SD[a]	Range[a]	Slope	95% CI (Slope)	Intercept	95% CI (Intercept)	R^2
				Total length (mm)				
Larval Walleye <25 mm	64	11.33 ± 1.90	9.21 – 15.33	1.031	1.018 – 1.044	-0.326	-0.572 to -0.079	0.997
Pond-reared fingerling Walleye ≥25 mm	20	32.81 ± 2.97	27.39 – 38.89					
GSD (all sizes)	66	18.14 ± 4.53	9.46 – 27.83	0.967	0.940 – 0.993	0.789	0.304 – 1.274	0.988
				Wet weight (mg)				
Walleye <60 mg	64	10.2 ± 7.6	3.6 – 28.9	2.442	2.341 – 2.543	1.874	1.411 – 2.336	0.974
Walleye >60 mg	20	237.9 ± 53.3	162.8 – 349.9	1.438	1.375 – 1.501	36.220	27.067 – 45.372	0.992
GSD <60 mg	50	18.7 ± 14.7	1.8 – 60.0	2.760	2.657 – 2.863	1.971	1.147 – 2.796	0.984
GSD >60 mg	16	115.2 ± 38.5	67.7 – 180.0	1.466	1.220 – 1.711	35.981	21.424 – 50.539	0.921

[a]Unpreserved measurements (adjusted for inflation if necessary).

FIGURES and FIGURE CAPTIONS:

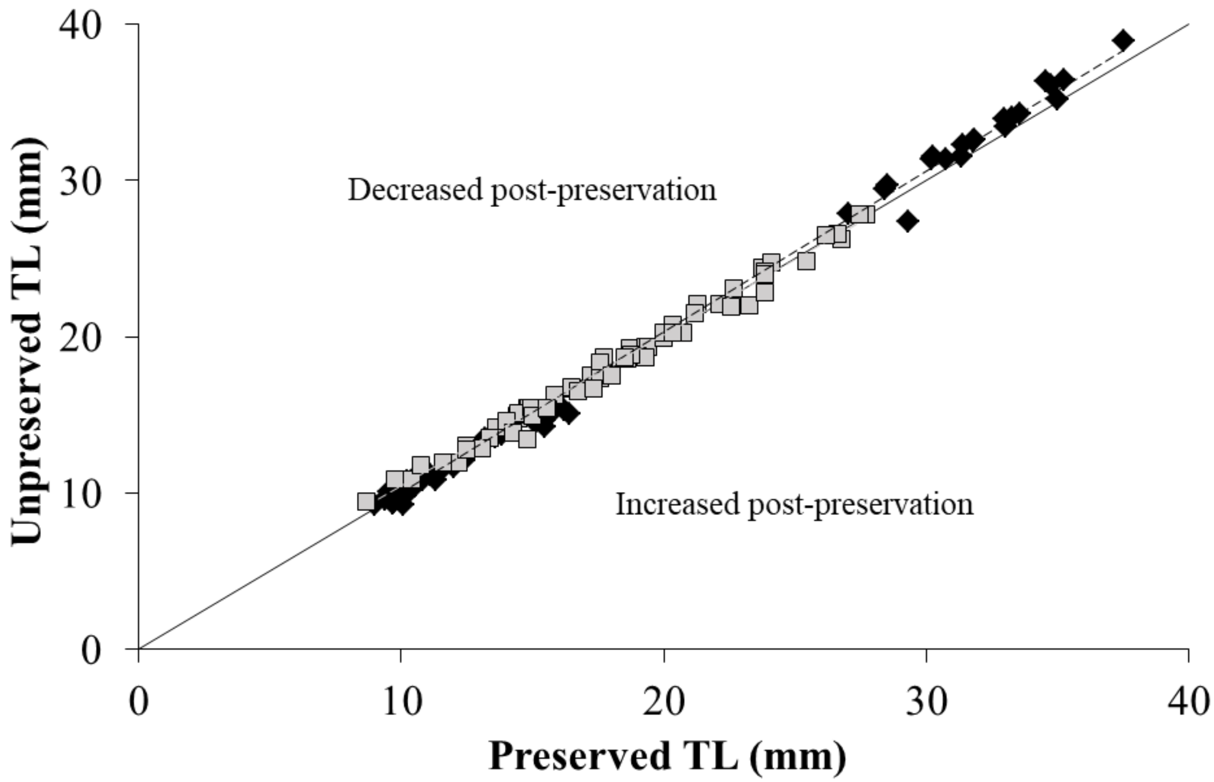

FIGURE 1. Linear relationships between unpreserved TLs and preserved TLs (once stabilized in 95% ethanol) of Walleye (black diamonds and dotted line; y = 1.031x - 0.326; N = 84; R^2 = 0.997) and Gizzard Shad (grey squares and dotted line; y = 0.967x + 0.789; N = 66; R^2 = 0.988) in relation to a 1:1 line (solid black). Individuals falling above the 1:1 line decreased in length post-preservation whereas those falling below the line increased post-preservation.

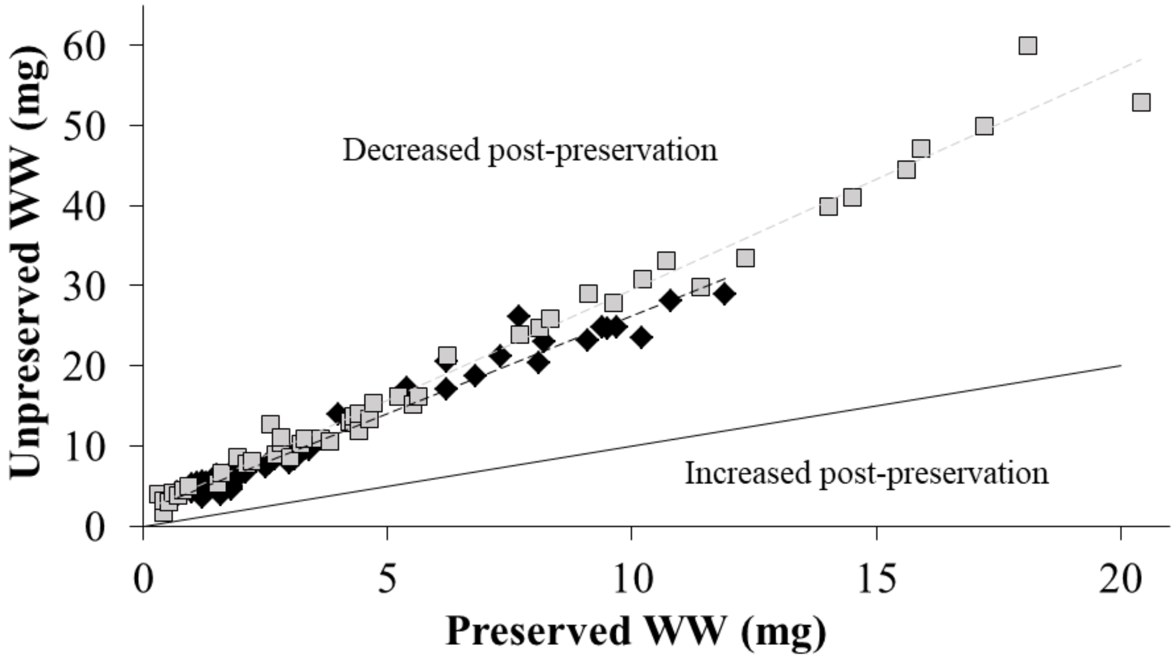

FIGURE 2. Linear relationships between unpreserved WWs and preserved WWs (once stabilized in 95% ethanol) of Walleye (black diamonds and dotted line; y = 2.442x + 1.874; N = 64; R^2 = 0.974) and Gizzard Shad (grey squares and dotted line; y = 2.760x + 1.971; N = 50; R^2 = 0.984) <60 mg (unpreserved) in relation to a 1:1 line (solid line). All individuals decreased in size post-preservation.

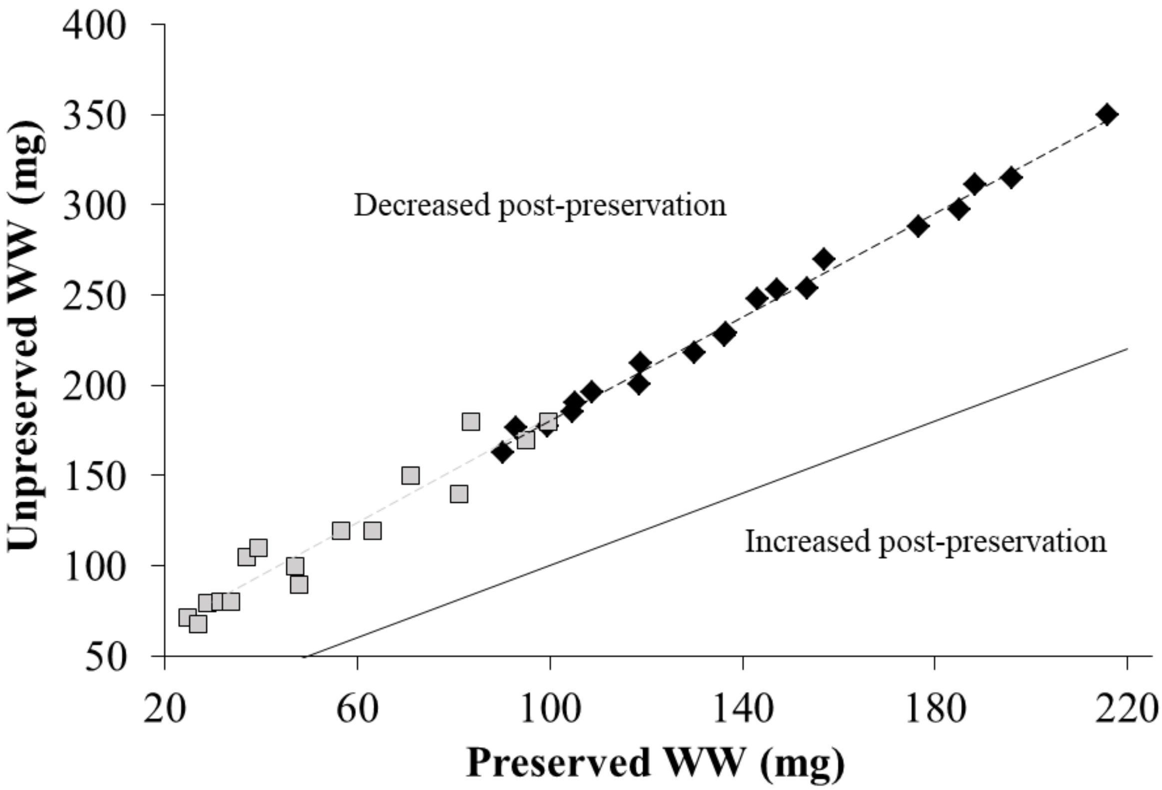

FIGURE 3. Linear relationships between unpreserved WWs and preserved WWs (once stabilized in 95% ethanol) of Walleye (black diamonds and dotted line; y = 1.438x + 36.220; N = 16; R^2 = 0.992) and Gizzard Shad (grey squares and dotted line; y = 1.466x + 35.981; N = 20; R^2 = 0.921) >60 mg (unpreserved) in relation to a 1:1 relationship (solid line). All individuals decreased in size post-preservation.

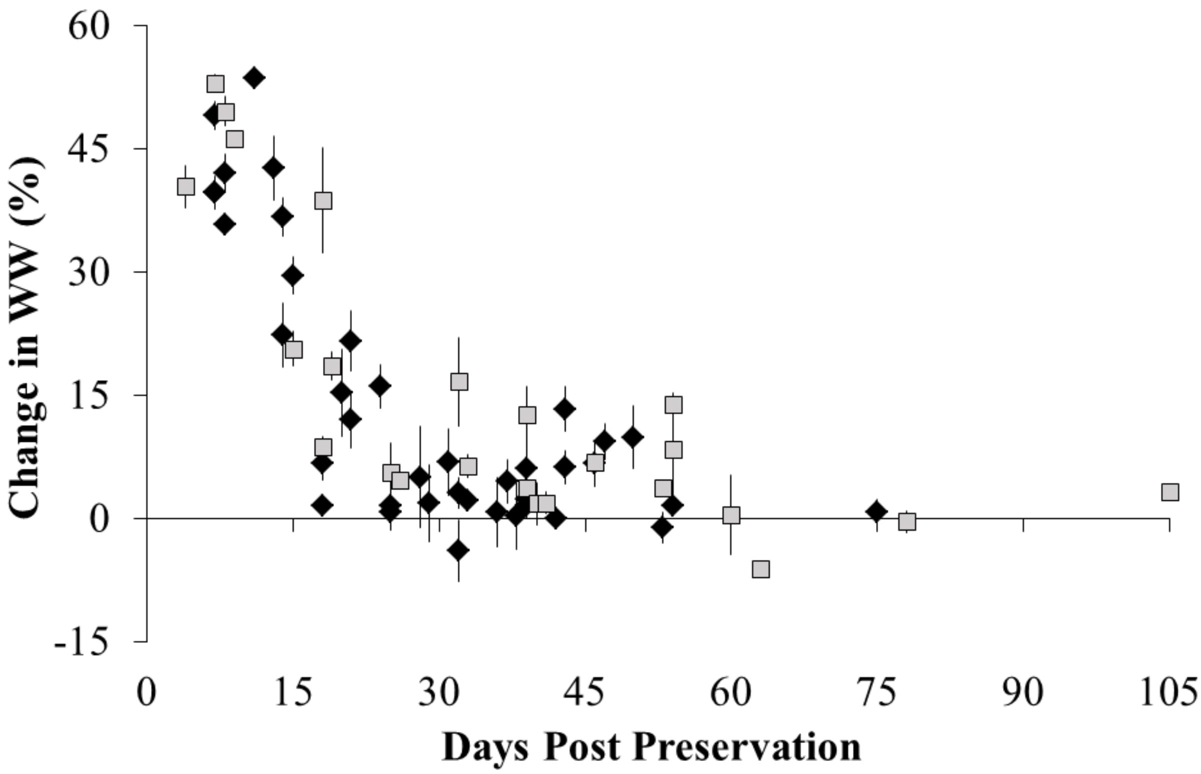

FIGURE 4. Mean percent changes in the WWs of different sample groups of Walleye (black diamonds; N = 39) and Gizzard Shad (grey squares; N = 22) as a function of days post-preservation. Mean percent changes were referenced from one post-preservation measurement date to the next. Error bars represent 1 SE.

RESEARCH PRIORITY: **Long-term food web dynamics in Horsetooth Reservoir:** Integrating 40 years of fisheries and aquatic data from Horsetooth Reservoir (Larimer County, CO) to characterize predator-prey interactions primarily focused on Rainbow Smelt *Osmerus mordax*, Walleye, and *Mysis* shrimp.

MANUSCRIPT SUBMISSION:

Lepak, J. M., **A. G. Hansen**, B. M. Johnson, K. Battige, E. T. Cristan, W. M. Pate, **K. B. Rogers**, **A. J. Treble**, B. A. Wolff, and C. J. Farrell. *In preparation*. Middle-out control by an introduced fish: Rainbow Smelt *Osmerus mordax* as an ecosystem influencer. Canadian Journal of Fisheries and Aquatic Sciences.

OBJECTIVES:

To compile and report information collected from Horsetooth Reservoir to evaluate the response of the system/fishery to the introduction and subsequent population fluctuations of Rainbow Smelt. To prepare and submit a manuscript that describes this data compilation.

INTRODUCTION:

A working draft of the manuscript is written (provided below), but collaborative data analyses must still be completed and included.

MANUSCRIPT ABSTRACT:

Species introductions can have significant and widespread impacts on recipient ecosystems. Rainbow smelt (*Osmerus mordax*) have been introduced into many systems to improve the forage base to enhance sport fisheries. As intended, walleye (*Sander vitreus*) growth in Horsetooth Reservoir (Colorado, USA) increased after rainbow smelt introduction, but walleye recruitment failed for several years, opossum shrimp (*Mysis diluviana*) became absent from predator diets and intermittent surveys, and *Daphnia* densities declined significantly and the dominant species shifted from *Daphnia pulex/pulicaria* to *D. galeata mendotaea*. These patterns were repeated during two different time periods of increasing rainbow smelt abundance in Horsetooth Reservoir, suggesting they have a strong influence on multiple components within the ecosystem. The repetition of biotic responses to rainbow smelt offered the opportunity to evaluate indicators of potential ecosystem regime shifts that restructure predator-prey dynamics across trophic levels. Several indicators (i.e., the decline in *Mysis diluviana* and the change in *Daphnia* density and community composition) were observed that preempted walleye recruitment failure. Indicators like these could inform timely and prompt management decisions to maximize the benefits that influential introduced species (like rainbow smelt) offer, while minimizing or overcoming the barriers they present.

Middle-out control by an introduced fish: rainbow smelt (*Osmerus mordax*) as an ecosystem influencer

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Abstract

Species introductions can have significant and widespread impacts on recipient ecosystems. Rainbow smelt (*Osmerus mordax*) have been introduced into many systems to improve the forage base to enhance sport fisheries. As intended, walleye (*Sander vitreus*) growth in Horsetooth Reservoir (Colorado, USA) increased after rainbow smelt introduction, but walleye recruitment failed for several years, opossum shrimp (*Mysis diluviana*) became absent from predator diets and intermittent surveys, and *Daphnia* densities declined significantly and the dominant species shifted from large-bodied *Daphnia pulex/pulicaria* to smaller-bodied *D. galeata mendotaea*. These patterns were repeated during two different time periods of increasing rainbow smelt abundance in Horsetooth Reservoir, suggesting they have a strong influence on multiple components within the ecosystem. The repetition of biotic responses to rainbow smelt offered the opportunity to evaluate indicators of potential ecosystem regime shifts that restructure predator-prey dynamics across trophic levels. Several indicators (i.e., the decline in *Mysis diluviana* and the change in *Daphnia* density and community composition) were observed that preempted the negative impacts of walleye recruitment failure. Indicators like these could inform timely and prompt management decisions to maximize the benefits that influential introduced species (like rainbow smelt) offer, while minimizing or overcoming the barriers they present.

Introduction

Intentional introductions of aquatic species have been widespread in freshwater systems, occurring at large scales, and worldwide. However, introductions (or assisted colonization) can have a variety of unintended consequences, and negatively influence native and desirable fish species, as well as other taxa, leading to the discouragement of such introductions (Moyle et al. 1986; Ricciardi and Simberloff 2009; Sala et al. 2000). More contemporary species introductions have been approached with increased caution, but they continue to occur, and can have outcomes that are unexpected. We are now in a situation where fisheries management in inland freshwater systems must often include considerations of introduced species and their environmental and economic impacts, even at spatial scales as large as the Great Lakes (Pimentel et al. 2000; Pimentel 2005).

Rainbow smelt (*Osmerus mordax*) are an important forage species in their native range for a variety of sport fish, and they also support sport and recreational fisheries during their spawning migrations. Because of some of their desirable characteristics, rainbow smelt introduction has been used as a management tool to enhance fisheries by providing prey and promoting sport fish growth. Thus, rainbow smelt have been introduced intentionally in many inland systems (Evans and Loftus 1987; Mercado-Silva et al. 2006). These introductions have been linked with increased growth rates in multiple sport fish species including lake trout (*Salvelinus namaycush*), landlocked Atlantic salmon (*Salmo salar*), walleye (*Sander vitreus*), and others (Havey 1973; Evans and Loftus 1987; Jones et al. 1994). Rainbow smelt now inhabit multiple inland lakes, and have spread from northeastern North America throughout the Great

Lakes region (including all five Great Lakes) and the West (USGS 2021 NAS database; Mercado-Silva et al. 2006).

Although rainbow smelt introductions have been associated with increased growth of multiple sport fish species as intended, they have also been associated with undesired food web and fishery changes (Mercado-Silva et al. 2007). For example, rainbow smelt appear to compete with, and consume several coregonine species in their native range, sometimes resulting in recruitment failure (Evans and Waring 1987; Hrabik et al. 1998; Loftus and Hulsman 1986). Further, declines in walleye recruitment/abundance has been associated with increasing smelt abundance in several instances (Schneider and Leach 1977; Johnson and Goettl 1999; Mercado-Silva et al. 2007). Competition with young walleye for prey resources (i.e., zooplankton, dipterans or other invertebrates) may contribute to these observations, as definitive evidence of predation of walleye by rainbow smelt is lacking (Lawson and Carpenter 2014; Mercado-Silva et al. 2007; Johnson and Goettl 1999). However, rainbow smelt are known to be highly piscivorous (cannibalistic) in some cases (e.g., Evans and Loftus 1987; Jones et al. 1994), and there is some evidence of rainbow smelt consuming the eggs of predators like lake trout (*Salvelinus namaycush*) and burbot (*Lota lota*) in other cases (Evans and Loftus 1987). Rainbow smelt might also influence top predators because they contain thiaminase that can interfere with their predators' reproduction. However, common walleye prey species like alewife (*Alosa pseudoharengus*) and gizzard shad (*Dorosoma cepedianum*) have even higher thiaminase concentrations, and walleye reproduction does not appear to be influenced (Honeyfield et al. 2007; Lepak et al. 2013).

The influence that rainbow smelt exert on themselves and taxa that are trophically above and below them make them a fundamental component of many food webs as they can serve roles as planktivores, piscivores, and sport fish prey at different ontogenetic stages of their life cycle. For example, Galbraithe (1967) found that when rainbow smelt and fathead minnows (*Pimephales promelas*) were established in Sporley Lake (Michigan, USA), *Daphnia pulex* were extirpated and replaced by *D. galeata mendotae* and *D. retrocurva*, and similar findings were observed by Reif and Tappa (1966) in Harvey's Lake (Pennsylvania, USA) in response to rainbow smelt introduction. There is also evidence that rainbow smelt may be capable of controlling populations of other "preferred" invertebrate prey species like opossum shrimp (*Mysis diluviana*) in some cases (Johnson and Goettl 1999). However, this type of information is generally limited, and was suggested as a potential information gap to fill in the past (Evans and Loftus 1987). Thus, rainbow smelt exhibit strong interactions across multiple trophic levels and taxa, and often act as important ecosystem influencers from the "middle out" (DeVries and Stein 1992).

Rainbow smelt were introduced into Horsetooth Reservoir (Larimer County, CO, USA) to enhance walleye and smallmouth bass (*Micropterus dolomieu*) growth (Goettl and Jones 1984). Initial intentions of promoting increased walleye growth were realized, although smallmouth bass growth did not respond, likely due to limited habitat overlap with rainbow smelt (Jones et al. 1994, Johnson and Goettl 1999). While walleye growth responded positively to rainbow smelt introduction, negative consequences (including walleye recruitment failure) began to arise following a significant increase in rainbow smelt abundance in the late 1980's. The rainbow smelt population declined following that time, and then increased once again beginning around 2010, providing an iterative opportunity to evaluate multi-trophic level responses to rainbow smelt as an ecosystem influencer. Here, we use a Bayesian approach to characterize community-level interactions between rainbow smelt and other biota including walleye, *Mysis diluviana*, and *Daphnia*. We couple information from historic datasets to identify triggers/thresholds with change-point regression to determine indicators that may be diagnostic of time periods with undesirable outcomes (e.g., walleye recruitment failure). Using these results we can inform/prepare fisheries managers for conditions resulting from introductions of rainbow smelt and changes in their abundance.

Methods

Site description

Horsetooth Reservoir was completed in 1949 to store water for agricultural and domestic use. When full (capacity of ~200 million m^3), Horsetooth Reservoir is 755 ha at an elevation of 1655 meters. The reservoir is relatively long (~11 km long) and thin (~1 km wide) with a mean depth of 25 m and a maximum depth of 70 m. Horsetooth Reservoir has three relatively distinct, but comparable basins that are similar due to reservoir morphometry (Figure 1). Soon after completion, and through 2000, Horsetooth Reservoir was managed as a two-tiered fishery with a coldwater component consisting primarily of stocked, catchable-sized salmonids (on the order of 8,000 kg annually), and a coolwater component consisting largely of naturally reproducing walleye and smallmouth bass.

Mysis diluviana (opossum shrimp) were introduced into Horsetooth Reservoir to enhance the forage base for salmonids. Direct plants of *Mysis diluviana* occurred in Horsetooth Reservoir, and the reservoir is also downstream from other reservoirs that received *Mysis diluviana* from

1971-1974 (Nesler 1986), and these systems supply water to Horsetooth Reservoir contemporarily. Planting *Mysis diluviana* was done based on increased kokanee salmon (*Oncorhynchus nerka*) growth observed in Kootenay Lake (British Columbia) in the early 1970's (Nesler and Bergersen 1991) as a result of a *Mysis diluviana* introduction. Later it was found that increased kokanee salmon growth observed in Kootenay Lake was a unique situation, and *Mysis diluviana* often have limited spatial overlap with salmonids, but compete for zooplankton resources (Beattie and Clancey 1991; Northcote 1991). In 1981, *Mysis diluviana* were confirmed in Horsetooth Reservoir with a benthic trawl (Nesler 1986), and they represented the majority of some fish diets through 1987, but were not observed in the reservoir during sampling, or in fish stomachs from 1988 to 2003 (Jones 1985a, Johnson and Goettl 1999, Silver et al. 2021). The absence of *Mysis diluviana* in Horsetooth Reservoir during sampling has been linked with high densities of rainbow smelt (Johnson and Goettl 1999), and a brief resurgence in *Mysis diluviana* was observed following 2003.

Rainbow smelt were introduced into Horsetooth Reservoir in 1983. Their population grew following the introduction, and increased walleye growth was observed (as intended) through 1988 (Jones et al. 1994). Walleye had been reproducing and recruiting naturally in Horsetooth Reservoir in the presence of rainbow smelt. However, by 1990, walleye recruitment had declined significantly. In response, ~5, and then ~6 million walleye fry were stocked in 1992 and 1993, respectively. These efforts appeared unsuccessful, and approximately ~50,000, ~50,000, ~73,000, and ~88,000 walleye fingerlings were stocked in 1994 through 1997, respectively. Walleye recruits were sampled in larger numbers again following these stocking events, and walleye stocking did not occur for 22 years in Horsetooth Reservoir (1998 to 2020). In 2021, 3.6 million walleye fry were stocked in an attempt to offset walleye recruitment failure.

Horsetooth Reservoir fluctuates significantly each year (on the order to 10-30 m), but at the end of the year 2000, the reservoir was drawn down significantly for maintenance to ~4% (~8 million m^3) of capacity by early 2001. There were indications that the Horsetooth Reservoir rainbow smelt population had begun to decline in the years leading up to the drawdown (Johnson et al. 1999). In the early spring of 2001, 2002 and 2003, Horsetooth Reservoir was at ~10% capacity or less, limiting rainbow smelt access to known spawning grounds. The rainbow smelt population appears to have been reduced significantly following the drawdown through 2010. Following 2010, the rainbow smelt population increased again, and changes were observed in walleye recruitment and growth, *Mysis diluviana* density, and zooplankton density and community structure.

Following the drawdown in 2000, Horsetooth Reservoir has received ~500-600 kg of catchable salmonids annually aside from a large influx in 2012 (~11,000 kg). This was due to a salvage operation related to poor water quality associated with a wildfire upstream of the Colorado Parks and Wildlife Watson Hatchery production facility (Larimer County, CO, USA). Another large influx (~9,500 kg) of stocked rainbow trout occurred in 2021 to focus more effort on the salmonid tier of the two-tiered fishery.

Walleye recruitment and abundance indices

Routine gill netting (late April through May) surveys occur intermittently in Horsetooth Reservoir (see Jones et al. 1994 for gear and methodology). Catch per unit effort during gill netting surveys (number of walleye caught per overnight net set) was calculated annually when sampling was conducted for two size classes of walleye; 150-300 mm (indicative of age-1 and age-2 walleye recruits), and > 450 mm (indicative of large walleye with elevated growth being present during the survey), to characterize walleye population dynamics. Colorado Parks and Wildlife walleye spawning operations in 1992-1995 provided Chapman's modified Petersen mark-recapture estimates of the abundance of walleye ≥ 350 mm (see Goettl and Johnson 1998). Walleye were marked during the spawning operation, and then recaptured during routine gill netting. In addition, in 2020, Colorado Parks and Wildlife conducted fall walleye index netting (FWIN; Ontario Ministry of Natural Resources) to estimate the number of walleye ≥ 350 mm (see Hansen 2020).

Walleye growth, diet and stable isotope analyses

Routine gill netting (late April through May) and boat electrofishing (July-August) surveys occur intermittently in Horsetooth Reservoir (see Jones et al. 1994 for gear and methodology). Individual walleye for analyses were retained opportunistically from these sampling events to determine growth, diet, and muscle carbon and nitrogen stable isotope signatures (a time-integrated indicator of diet; see Post et al. 2002; Johnson et al. 2015).

Walleye length at age-3 in 1991 and prior was estimated using ages and back-calculated lengths interpreted from walleye scales collected in 1983 (n = 204), 1984 (n = 210), 1985 (n = 94), 1986 (n = 93), 1988 (n = 68), 1989 (n = 97), 1990 (n = 138), 1991 (n = 94), and 1992 (n = 187). These methods and results are described in Goettl and Jones (1984, 1986), Jones (1985b), Goettl and Thomas (1987), and Goettl (1990, 1991, 1992, 1993). Walleye lengths at age-3 from 2001 and later were estimated using ages and back-calculated lengths interpreted from sectioned walleye otoliths (see Farrell et al. 2021 for methods) collected in 2012 (40), 2013 (119), 2019

(131), and 2021 (75). Means across walleye lengths at age-3 (from both scale and otolith interpretation) were taken by cohort, but only when two or more individuals were represented. For each cohort, mean length at age-3 was evaluated when an individual was age-3 (i.e., not by year class) to indicate length at age-3 (i.e., data presented for 1980 represent fish born in 1977). Standard error was calculated for data from 2001 to 2016, but only cohort means were available for earlier data so error was not available, and these were weighted by sample size to develop cohort means.

Walleye diets from 1983 to 1996 (no diets were analyzed in 1993) were quantified using stomach content analysis of fish collected during routine surveys. Stomach contents were removed and analyzed under a dissecting microscope, identified to the lowest feasible taxon, and quantified as mean percent volume by prey item (Jones 1985a). After 1996, diets were quantified using walleye carbon and nitrogen stable isotope signatures (as well as their prey signatures) combined with Bayesian mixing models. An example of this approach, and a description of 2008 diet quantification for Horsetooth Reservoir walleye can be found in Johnson et al. (2015). A similar approach was taken in 2013 and 2017-2019, where potential walleye prey carbon and nitrogen stable isotope signatures were measured, as well as walleye themselves, as indicators of which prey were being consumed. These data were then coupled with a stable isotope mixing model in R (SIMMR 0.4.5) recently developed (Parnell 2021). Trophic fractionation and error for $\delta^{13}C$ (0.4 and 1.3, respectively) and $\delta^{15}N$ (3.4 and 1.0) were set to values established by Post et al. (2002). Values for burn-in (1,000) and the number of iterations (10,000) remained at default settings, and models converged in all cases such that Gelman diagnostics never exceeded 1.01, and additional iterations were unnecessary.

In 2013, 30 walleye were collected for analyses. Prey species were targeted for collection that were abundant, of edible size, and common in walleye diets in the past. These prey items were collected opportunistically in Horsetooth Reservoir using beach seines, various nets, minnow trapping, and boat electrofishing. These prey species included rainbow smelt (n = 4), crayfish (n = 6), hatchery-reared rainbow trout (n = 9), dipterans (n = 5 composite monthly samples of multiple individuals collected from May to October), large zooplankton (n = 3 composite samples of multiple individuals representing bulk zooplankton collected with a 500 micron mesh net in June, July, and September), gizzard shad (n = 3), and yellow perch (n = 3). These prey items were collapsed into categories including rainbow smelt, crayfish, salmonidae (rainbow trout), other invertebrates (dipterans and zooplankton), and other fish (gizzard shad and yellow perch).

Stable isotope signatures were measured using a Thermo Delta V isotope ratio mass spectrometer (IRMS) interfaced to a NC2500 elemental analyzer at the Cornell Isotope Laboratory (Ithaca, New York, USA). In-house standards (mink tissue) were analyzed every 10 samples to ensure precision and accuracy. The overall standard deviation of these samples was 0.16‰ for $\delta^{13}C$ and 0.14‰ for $\delta^{15}N$. A methionine standard was used to quantify the ability of the instrument to measure across a gradient of amplitude intensities. Values for $\delta^{15}N$ obtained from these samples between 100mV and 17,000mV had 0.30‰ of error, and 0.79‰ of error for $\delta^{13}C$ between 200mV and 20,000mV. Isotope corrections were performed using a two-point normalization (linear regression) of all $\delta^{13}C$ and $\delta^{15}N$ data using two additional in-house standards (corn and trout). Values for $\delta^{13}C$ and $\delta^{15}N$ were corrected using primary references of Vienna Pee Dee Belemnite, and atmospheric air. To avoid potential bias from differing lipid concentrations among samples and species, corrections for lipid content from Post et al. (2007) were applied to δ^{13}C values:

$$\delta^{13}C_{normailzed} = \delta^{13}C_{measured} - 3.32 + 0.99 \text{ x } C{:}N,$$

where C:N is the carbon-to-nitrogen ratio.

In 2017, 2018, and 2019, 42, 14, and 42 walleye were collected for analysis, respectively. During this time period, prey species were targeted for collection that were abundant, of edible size, and common in walleye diets in the past. Cursory diet analyses indicated that walleye were focused largely on consuming rainbow smelt. Thus, rainbow smelt were targeted with fine mesh gill nets all three years, and other prey species were collected opportunistically from 2017-2019 with netting, trapping, and boat electrofishing efforts. Prey items collected for analyses included rainbow smelt (n = 213 across all three years), crayfish (n = 6), large zooplankton (n = 6 composite samples of multiple individuals representing bulk zooplankton collected monthly with a 500 micron mesh net from June through November 2019 with signatures averaged across samples from each of the three reservoir basins), and gizzard shad (n = 36 from 2017 and 2018). These categories were collapsed to rainbow smelt, crayfish, other invertebrates (zooplankton), and other fish (gizzard shad). Because samples from 2017-2019 were collected during a relatively short time window, and signatures for rainbow smelt stayed relatively consistent during this time, prey items from all three years were used for mixture modeling of walleye diets.

Stable isotope signatures were measured and corrected as described above. However, these isotopic runs took place at a different time with unique quality assurance and quality control information. During this run (2107-2019 samples), in-house standards (deer tissue) were analyzed every 10 samples to ensure precision and accuracy. The overall standard deviation of these samples was 0.05‰ for $\delta^{13}C$ and 0.05‰ for $\delta^{15}N$. A methionine standard was used to quantify the ability of the instrument to measure across a gradient of amplitude intensities. Values for $\delta^{15}N$ obtained from these samples between 100mV and 15,000mV had 0.21‰ of error, and 0.37‰ of error for $\delta^{13}C$ between 200mV and 10,000mV.

Rainbow smelt abundance and indices

The most consistent rainbow smelt sampling gears used to characterize the Horsetooth Reservoir population were hydroacoustic and trawl surveys. Hydroacoustic surveys (1993-1996) were conducted with a BioSonics 420 kHz, dual-beam scientific echosounder and analyzed using echo integration with in situ data to determine mean rainbow smelt target strength (-50 dB) as described in Johnson and Goettl (1999). Subsequent hydroacoustic surveys (1998 to 2013) were conducted similarly, but with a Hydroacoustic Technology Incorporated Model 243, 200 kHz split beam digital echosounder using a target tracking algorithm (due to lower rainbow smelt abundance). Individual fish targets were identified as those with a minimum of 5 echoes corresponding to individuals longer than 5 cm but less than 20 cm (based on suspended fine mesh gill net data, Lepak unpublished data) based on the target strength-to-length relationship for rainbow smelt developed by Rudstam et al. (2003). Rainbow smelt were largely observed near the thermocline or in the epilimnion during sampling (based on hydroacoustics and vertical gill netting), and targets below 30 m (generally fish larger than 20 cm) were removed from analyses. Finally, from 2017-2021, surveys were conducted with the same equipment as 1998-2013, but used echo integration (versus target tracking) to enumerate rainbow smelt from data collected following zig-zag transects (versus longitudinal transects) down the center of the reservoir's north-south axis (see Figure 1 for reservoir map). For these surveys, in situ target strengths were used to determine rainbow smelt density similar to Johnson and Goettl (1999).

Trawl surveys were conducted using a net with a 6 x 6 m opening at the mouth that was deployed in a stepped-oblique approach from the surface to a depth of 18 m (Kirn and LaBar 1991). Trawl surveys were used to estimate rainbow smelt density in Horsetooth Reservoir, and the connection between these surveys and hydroacoustic surveys are described in Johnson and Goettl (1999). In addition to the two methods described above, we also used intermittent, routine gill netting (late April through May) and boat electrofishing (July-August) survey data (see Jones et al. 1994 for gear and methodology) to develop an annual index of rainbow smelt encountered during the use of these disparate gear types over time. The index was represented by the total rainbow smelt catch across these surveys divided by the number of sampling events (standard spring gill net surveys and summer boat electrofishing surveys) that occurred in a given year.

Rainbow smelt diet and stable isotope analyses

Rainbow smelt were collected using fine and large mesh gill nets, trawls, boat electrofishing, and beach seines throughout the study period. Rainbow smelt diets were quantified using stomach content analysis of fish collected in 1984 (Jones 1985a), 1987 (Thomas 1989), 1989 to 1992 (Goettl 1990, 1991, 1992, 1993), 1994 (Goettl and Johnson 1995) and 1995 (Goettl and Johnson 1996). Rainbow smelt ≥ 100 mm were commonly designated as their own size category in historic diet analyses given interest in their potential to consume *Mysis diluviana*, walleye larvae, and rainbow smelt (cannibalism). When available (1984, 1987, 1989, and 1991) data from rainbow smelt ≥ 100 mm were used. If multiple size classes were included in analyses (e.g., rainbow smelt from 100 to 125 mm and from 125 mm to 150 mm), diet composition was weighted by sample sizes within each respective size class designated over 100 mm, respectively. Other years (1990, 1992, 1994, and 1995) during this period were represented by diets from all size classes of rainbow smelt. Stomach contents were removed and analyzed under a dissecting microscope, identified to the lowest feasible taxon, and quantified as mean percent volume by prey item (Jones 1985a).

In 2017, 2018, and 2019, large (≥ 130 mm) rainbow smelt (n = 20, 9 and 73, respectively) diets were quantified using the same approach (stable carbon and nitrogen isotope measurements of predator and prey coupled with a Bayesian mixing model; SIMMR 0.4.5) that was described above for walleye over the same time period. We noted a change in isotope signatures (indicating a diet shift) when rainbow smelt reached 130 mm, so we separated rainbow smelt into predators (≥ 130 mm) and prey (≤ 130 mm). Other items that were measured and included in the stable isotope mixing models were zooplankton (as described in the walleye diet analysis), and dipterans. Dipterans were not collected in 2017-2019, but were found regularly in rainbow smelt diets. Thus, we included data from dipterans collected in 2013 (described in the walleye diet analysis) despite disparate collection time periods. Therefore, stable isotope analysis quality assurance and quality control results listed above from both the 2013 and 2017-2019 analyses are applicable to these data. In addition to stable isotope analyses, in 2019, we collected rainbow smelt (n = 500) using fine mesh gill nets from May to November. All of the stomach contents from these fish were inspected for the presence of *Mysis diluviana* and larval/juvenile walleye. In all cases (volumetric diet composition and composition established with stable carbon and nitrogen isotope analyses) rainbow smelt prey categories were collapsed into: *Mysis diluviana*, rainbow smelt, zooplankton (e.g., daphnids, copepods), and other invertebrate prey (e.g., dipterans, amphipods).

Mysis diluviana abundance and indices

Mysis diluviana sampling was conducted almost exclusively using a 1 m diameter, 500 µm mesh net towed vertically through the water column in representative depth strata at 10 locations in Horsetooth Reservoir by either Colorado Parks and Wildlife or Colorado State University personnel (Silver et al. 2021). With these data, a reservoir-wide density estimate of *Mysis diluviana* could be determined. In 1981, a benthic trawl was used to confirm the presence of *Mysis diluviana* and develop a conservative density estimate (Nesler 1986). Additionally, observations of *Mysis diluviana* in fish diets were noted intermittently during the study period, and used to inform presence-absence.

Daphnia peak abundance and dominant species indices

Zooplankton sampling took place with a metered Clarke-Bumpus sampler (predominantly conducted April to September) from 1987-1996 [see Johnson and Goettl (1999)]. From 1987 to 1993, the upper 0–5 m were sampled, while in 1994 to 1996, samples were taken from 0-10 m. More limited (less temporal coverage) zooplankton collections and analyses using a 153 µm Wisconsin net from 2002-2018, and 2020 were analogous to those in 1987-1996 (with the exception of the gear used), and represent a compilation of data collected from Colorado State University personnel (0-10 m sampled), and data available through the Bureau of

Reclamation and the Northern Colorado Water Conservancy District (a combination of data collected from 0-5 and 0-10 m). Zooplankton collections and analyses from 2019 and 2021 were conducted in 0-10 m with a 153 μm Wisconsin net by Colorado Parks and Wildlife personnel. Horsetooth Reservoir has three distinct basins, and zooplankton (number/L) were collected and enumerated from each basin (and averaged) when feasible throughout the study period. Since some methodology and sampling depths changed over time, a single datum (maximum) was used to represent each year. Thus, the sampling occasion from each year with the highest estimated abundance of *Daphnia* was used as an index of the zooplankton community. The number of *Daphnia* L^{-1} and the dominant species (*Daphnia pulex/pulicaria* or *Daphnia galeata mendotae*) were determined based on these data.

Trend analysis

There are clear and biologically significant relationships between rainbow smelt abundance and walleye recruitment, walleye population size structure, walleye growth, predator diets, *Mysis diluviana* presence/absence, *Daphnia* density, and zooplankton community structure. We have compiled these disparate data sets and will collaborate with external personnel to identify triggers/thresholds (e.g., zooplankton density and species composition) with change-point regression to determine indicators that may be diagnostic of time periods with undesirable outcomes (e.g., walleye recruitment failure). Given the data available coupled with a Bayesian approach and using an observed versus predicted framework, we should also be able to determine the accuracy and precision of our predictions.

Results

Walleye recruitment and abundance indices

Based on standardized gill net catches, walleye 150 mm to 300 mm (generally represented by age-0 to age-3 individuals) were rarely encountered following periods of relatively high rainbow smelt abundance. Catch rates of this size class of walleye were generally below 0.5 fish per net during these periods indicating poor or negligible walleye recruitment (Figure 2). There was no direct evidence that this decline in walleye recruitment was due to predation on larval walleye by rainbow smelt, but resource competition could explain some of our observations. Concomitant with walleye fingerling stocking to supplement natural reproduction (Figure 2), an apparent decline in rainbow smelt abundance, and following a drawdown of Horsetooth Reservoir (2001-2003), catch rates of walleye 150 mm to 300 mm generally exceeded 0.5 fish per net, and approached or exceeded 2.0 fish per net until 2012, when the rainbow smelt population appeared to rebound (Figure 2). Walleye fry stocking (3.6 million) took place in 2021, and there have been recent observations of small walleye during boat electrofishing surveys. Based on previous patterns, these data may be an indication that increased walleye recruitment might be observed in the immediate future.

Large walleye (≥ 451 mm) capture rates (fish/net) in standardized gill net sampling showed increases following periods of increasing rainbow smelt abundance (Figure 3). This was expected, as walleye growth rates have shown positive responses to rainbow smelt consumption in Horsetooth Reservoir in the past, and this likely increased the amount of large fish available for capture (Jones et al. 1994; Johnson and Goettl 1999). This pattern of increasing catches in large walleye was observed in the 1990’s when rainbow smelt were abundant (Johnson and

Goettl 1999), and then again beginning around 2010. Catch rates have declined in recent years, analogous to previous observations prior to rainbow smelt resurgence (Figure 3). We note that large walleye catchability using standardized gill nets may be relatively high, and those fish may have been more susceptible to surveys post-2010, artificially increasing the observed catch rates of this size class of walleye. However, an increase in large walleye catch rates was apparent, and rigorously evaluating walleye catchability at length was outside the scope of this study.

Mark-recapture estimates of walleye (≥ 350 mm) abundance in 1992 through 1995 produced estimates that were comparable to the estimate using the fall walleye index netting approach (Hansen 2020). Estimates (and lower and upper 95% confidence intervals) were 8,280 (6,685 and 10,242) in 1992, 5,298 (4,410 and 6,364) in 1993, 6,511 (5,152 and 8,222) in 1994, and 6,025 (4,580 and 7,915) in 1995 (Goettl and Johnson 1996). During the 2020 fall walleye index netting survey, walleye (≥ 350 mm) abundance was estimated to be 7,795 (5,010 and 10,066), and these 95% confidence intervals overlapped with those estimated during 1992-1995. These similarities suggest that large walleye catchability in gill nets may be elevated, and catch rates of individuals exceeding 450 mm observed during standardized gill net surveys may be artificially high post-2010 (Figure 3).

Walleye growth, diet and stable isotope analyses

Walleye growth responded positively to increases in rainbow smelt abundance in the late 1980's to the mid-1990's (Figure 4). The response in walleye growth (an increase in length by 50% at age-3; Jones et al. 1994) after the initial introduction of rainbow smelt appeared to remain relatively stable in the years following. However, at times this was difficult to determine because poor walleye recruitment created large gaps when age-3 walleye were not available for sampling (e.g., periods following 1989 and 2013). These data gaps are potentially as informative as the years where we have data available, because they indicate significant declines in walleye recruitment and year class failure coinciding with elevated rainbow smelt abundance.

In 1984, only the stomach of one 255-mm walleye contained rainbow smelt (Jones 1985a). Rainbow smelt in walleye diets increased quickly thereafter, and were a significant component of walleye diets from 1988 to 1996, when intensive diet analyses were discontinued. (Jones et al. 1994; Johnson and Goettl 1999). Based on conversations with Colorado Parks and Wildlife personnel, walleye were largely consuming crayfish during periods when rainbow smelt were less abundant (K. Kehmeier pers. comm.; Johnson et al. 2015). This information in concert with stable carbon and nitrogen isotope signatures in 2008, 2013, and 2017-2019, indicated that walleye in Horsetooth Reservoir were consuming a mix of prey sources in the reservoir in 2008, but transitioned to primarily a rainbow smelt diet by 2017 (Figure 5 and Figure 6). In general, walleye collected in 2017-2019 had stable carbon and nitrogen isotope signatures that were similar, and walleye collected in 2013 had similar signatures to each other, but disparate from the 2017-2019 fish (Figure 6), indicating a more mixed diet in 2013. Rainbow trout sometimes represented a significant component of walleye diets, especially when salmonid stocking was more prevalent in the 1980's, and rainbow smelt numbers were relatively low (Figure 5). Thus, rainbow trout were included in the 2013 walleye diet mixture for this reason and because of the pulse (11,000 kg) of stocking in 2012 (see study site description). This prey item was not collected or included in 2017-2019 as stocking was relatively minimal during this time period (~500 kg). Zooplankton were sampled consistently in 2019 and were included as a potential prey item for walleye in 2017, 2018, and 2019, but were generally 5% or less of walleye diets using the mixing model approach (Figure 5 and Figure 6). These data provide relatively strong evidence that walleye consume rainbow smelt when they are available.

Rainbow smelt abundance and indices

Hydroacoustic and trawl surveys showed analogous patterns of increasing rainbow smelt estimates following their introduction in 1983 through the mid-1990's (Figure 7). Rainbow smelt estimates/indicators peaked at this time, but began to decline based on trawl and particularly hydroacoustic data until 2000. In 2001, 2002 and 2003, Horsetooth Reservoir was drawn down significantly (at ~10% capacity or less) during the early spring, limiting access to known rainbow smelt spawning grounds (K. Kehmeier, pers. comm.). After this time, rainbow smelt were not observed until 2010, when a single rainbow smelt was captured during a gill net survey. Based on hydroacoustic data (routine trawl surveys were discontinued), rainbow smelt abundance increased analogous to what was observed following their introduction in 1983. Rainbow smelt encountered during routine spring and summer surveys also indicated that there were two pulses of increased rainbow smelt abundance from 1983 to 2021 (Figure 8).

Rainbow smelt diet and stable isotope analyses

When rainbow smelt were first introduced into Horsetooth Reservoir, *Mysis diluviana* made up the majority of the diets from individuals collected. *Mysis diluviana* disappeared from rainbow smelt diets rather quickly, and the last observation from rainbow smelt stomachs was 1988 (Johnson and Goettl 1999). *Mysis diluviana* were replaced by prey items like *Daphnia* and dipterans, and cannibalism was first observed in 1987, and increased in the 1990's until rainbow smelt numbers began to decline (Figure 9). Only zooplankton were observed in rainbow smelt diets in 1994 and 1995 (Goettl and Johnson 1995; Goettl and Johnson 1996), but in 2017-2019, results indicated that rainbow smelt (≥ 130 mm) were getting large portions of their energy from cannibalism (Figure 9). These findings were corroborated by 500 rainbow smelt stomachs from fish collected across seasons in 2019 from May to November. No *Mysis diluviana* or larval walleye were observed in these stomachs, and the large majority of biomass was rainbow smelt.

Mysis diluviana abundance and indices

Mysis diluviana were introduced into Horsetooth Reservoir directly, and their presence was confirmed in 1981 using a benthic trawl, but this was not a highly rigorous, or quantitative method (Nesler 1986). The presence of *Mysis diluviana* was also apparent in fish diets (e.g., rainbow smelt ≥ 100 mm) until 1988 (Jones 1985a; Thomas 1989). Quantitative sampling for *Mysis diluviana* began in 1999, but very few were captured until after the drawdown of Horsetooth Reservoir and refilling in 2004. For the following 10 years, *Mysis diluviana* appeared more frequently during sampling, and quantitative surveys indicated that their abundance had increased relative to what was observed around 2000 (Figure 10). These fluctuations in *Mysis diluviana* population and occurrence coincide strongly with changes in rainbow smelt abundance and indices, suggesting that rainbow smelt are responsible for the changes in *Mysis diluviana*.

Daphnia peak abundance and dominant species indices

Peak abundance of *Daphnia* as measured in this study did not exceed 10 individuals L^{-1} from 1989 to 1994. Similarly, when *Daphnia* density was below 10 individuals L^{-1}, *Daphnia galeata mendotae* was the dominant species present. The same pattern was observed (less than 10 *Daphnia* L^{-1} dominated by *Daphnia galeata mendotae*) in Horsetooth Reservoir from 2012 to 2020 (Figure 11). These time periods correspond to relatively high rainbow smelt (an effective zooplanktivore) abundance estimates. *Daphnia pulicaria/pulex* are generally considered a more desirable prey item for fish species because they tend to be larger than *Daphnia galeata mendotae*. Thus, it appears that when rainbow smelt abundance is high, the zooplankton community in Horsetooth Reservoir responds accordingly as described currently (2012 to 2020), and previously from 1989 to 1994 (Johnson and Goettl 1999). In contrast, when rainbow smelt abundance appears to be relatively low, the zooplankton community shifts, and higher densities of *Daphnia* ($\geq$ 10 individuals L^{-1}) generally dominated by *Daphnia pulicaria/pulex* are observed during sampling (Figure 11).

Trend analysis

The results from this analysis are pending, though there are clear relationships within these data that are driven by the abundance of rainbow smelt, and we will pursue this quantitatively.

Discussion

Rainbow smelt have exhibited a strong middle-out influence in Horsetooth Reservoir since their introduction in 1983. Significant responses by organisms at trophic levels above and below rainbow smelt were noted at the onset of rainbow smelt population introduction and increase, and reciprocal observations were made when the population subsequently declined (Jones et al. 1994; Johnson and Goettl 1999). We observed the influence of increasing rainbow smelt abundance on Horsetooth Reservoir biota during two separate time periods. This provided an opportunity to evaluate biotic responses iteratively, and data showed that walleye, *Mysis diluviana,* and *Daphnia* expressed consistent, analogous responses to high rainbow smelt abundance, suggesting that rainbow smelt interact strongly with all these species in Horsetooth Reservoir, and act as ecosystem influencers.

Rainbow smelt have been associated with walleye recruitment failure in the past (Evans and Loftus 1987; Mercado-Silva et al. 2017). This undesirable outcome also occurred in Horsetooth Reservoir. Walleye recruitment essentially ceased during periods of high rainbow smelt abundance, which we note is also associated with periods of low *Daphnia* density, and a species composition dominated by *Daphnia galeata mendotae* versus more desirable (as prey) *Daphnia pulex/pulicaria*. Similar to others (e.g., Pothoven et al. 2009), our work did not confirm any predation on larval or juvenile walleye by rainbow smelt, though this mechanism has been posited as a reason for observed declines in walleye recruitment (Mercado-Silva et al. 2007). Thus, our results suggest that competition for zooplankton or other resources may be the strongest interaction that rainbow smelt are having with small walleye versus a direct predator-prey interaction, but we cannot rule out the possibility of rainbow smelt predation on larval walleye. Whatever the mechanism, we noted in these data (especially back-calculated length at age-3) that repeated walleye year class failures followed periods of high rainbow smelt abundance. However, based on our results (and others), rainbow smelt also appear to provide a forage base that can support large, faster growing walleye compared to populations without this prey resource (Evans and Loftus 1987; Jones et al. 1994; Johnson et al. 1999).

Intraspecific interactions between rainbow smelt include competition for prey resources, and also direct predation of small rainbow smelt by larger individuals (cannibalism). Rainbow smelt cannibalism has been observed widely (e.g., Evans and Loftus 1987; Jones et al. 1994), and may contribute to overall rainbow smelt population control (Hendersen and Nepszy 1989; He and LaBar 1994). It was evident that, especially at smaller sizes, rainbow smelt were effective planktivores in Horsetooth Reservoir, and likely compete strongly with other planktivorous species/stages of fish. A distinct enrichment was noted in rainbow smelt carbon stable isotope signatures at 130 mm, indicating a shift to cannibalism within the study system. Thus, this size class of rainbow smelt is largely functioning as a rainbow smelt predator (and competitor with larger walleye), creating recruitment pressure on rainbow smelt themselves.

Mysis diluviana responded strongly to rainbow smelt introduction in Horsetooth Reservoir. After their introduction in the early 1970's, *Mysis diluviana* were observed in a benthic trawl in 1981 (Nesler 1986), and were a dominant prey item in rainbow smelt stomachs during analyses in 1984 and 1987 (Jones 1985a; Thomas 1989), but after 1988, *Mysis diluviana* were not observed in fish diets or during surveys in Horsetooth Reservoir until 2003. Rainbow smelt are *Daphnia* consumers and can compete for zooplankton resources with *Mysis diluviana*, but based on diet information, rainbow smelt also consume *Mysis diluviana* directly. Thus, *Mysis diluviana* are likely experiencing pressure from rainbow smelt through resource competition as well as predation. These factors in combination appear to have reduced the *Mysis diluviana* population within Horsetooth Reservoir below our ability to detect them when rainbow smelt abundance is high. There are sources of *Mysis diluviana* upstream from Horsetooth Reservoir, and they are able to re-establish if extirpated, but to our knowledge, an effect this strong has not been observed in an established invasive *Mysis* spp. population (e.g., Pothoven et al. 2009; Bruel et al. 2021).

The zooplankton community in Horsetooth Reservoir appeared to be restructured in the presence of high rainbow smelt abundance. In particular, the peak density of *Daphnia* in surface waters dropped below 10 individuals L^{-1} and the dominant species shifted from *Daphnia pulex/pulicaria* to *Daphnia galeata mendotae*, and this shift was essentially complete (100% change in species composition). These findings are analogous to those of others where entire zooplankton communities and their densities were restructured, and species even experienced extirpation (e.g., Reif and Tappa 1966; Galbraithe 1967). The type of influence observed in Horsetooth Reservoir has direct impacts on zooplankton communities, and may also be creating conditions where other organisms like larval walleye and *Mysis diluviana* do not have access to the prey resources they require, resulting in a negative influence on their recruitment and populations (Cushing 1990; May et al. 2021; Silver et al. 2021).

Rainbow smelt exhibited significant influence on multiple food web components in Horsetooth Reservoir during two disparate increases in abundance. The responses by walleye, *Mysis diluviana,* and *Daphnia* were analogous during these two different time periods, corroborating that increases in rainbow smelt abundance were responsible for these observations. Rainbow smelt were introduced in Horsetooth Reservoir with the intent of increasing walleye growth, and that was achieved. However, unintended consequences (e.g., Moyle et al. 1986) occurred, and like others (e.g., Evans and Loftus 1987; Mercado-Silva et al. 2007), we observed walleye recruitment failure (twice). In addition, we observed several indicators (i.e., the decline in *Mysis diluviana* and the change in *Daphnia* density and community composition) that preempted the full negative impacts felt from walleye recruitment failure. This information could be applied by managers to inform timely and prompt decisions in response to changes in relatively easily measurable invertebrate indicators. We acknowledge the conditions in Horsetooth Reservoir are unique, but managing in the presence of introduced species is becoming more common in systems around the world (Ricciardi and Simberloff 2009; Sala et al. 2000), and heavily managed reservoirs in western North America, like Horsetooth Reservoir, are no exception. Thus, understanding how (and when) to maximize the benefits that influential introduced species (like rainbow smelt) offer, while minimizing or overcoming the barriers they present, will improve the efficacy of fisheries management in the future.

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Figure captions and figures:

Figure 1. Horsetooth Reservoir bathymetric map. Contours appear in 10 m intervals.

Figure 2. Catch per unit effort of walleye (150-300 mm) captured during routine Colorado Parks and Wildlife spring gill netting surveys. Standard errors are provided, and incidents of walleye stocking are noted along the x-axis (text and time periods correspond in color to the type of walleye stocked: fry or fingerling). Gray boxes indicate periods of relatively high rainbow smelt abundance.

Figure 3. Catch per unit effort of walleye (≥ 451 mm) captured during routine Colorado Parks and Wildlife spring gill netting surveys. Standard errors are provided, and gray boxes indicate periods of relatively high rainbow smelt abundance.

Figure 4. Walleye length at age-3. Growth in 1991 and prior was estimated using ages interpreted from scales collected from walleye in 1983-1986, and 1988 to 1992. Walleye sample sizes (earlier to later for this time period) were 39, 64, 140, 114, 79, 49, 68, 55, 62, 53, 172, 233, 33, and 24, respectively. Growth from 2001 on was estimated using ages interpreted from sectioned walleye otoliths collected in 2012, 2013, 2019 and 2021. Walleye sample sizes (earlier to later for this time period) were 4, 3, 2, 7, 22, 22, 18, 19, 50, 35, 32, 46, 41, 20, and 2, respectively. Note that there were no data for the 2014 year class. Means across walleye lengths at age-3 were taken by cohort, but only when two or more individuals were represented. For each cohort, mean length at age-3 was plotted three years later to indicate size at age-3 (i.e., data from

1980 represent fish spawned in 1977). Standard error could be calculated for data from 2001 to 2016, but only cohort means were available for data from 1980 to 1993. Gray boxes indicate periods of relatively high rainbow smelt abundance.

Figure 5. Horsetooth Reservoir walleye diets. Walleye diets between 1983 and 1996 were quantified using stomach content analysis of fish collected during routine surveys. Walleye diets between 2008 and 2019 were quantified using walleye carbon and nitrogen stable isotope signatures (as well as their prey signatures) combined with Bayesian mixing models. Walleye sample sizes for years with data (earlier to later) were 99, 90, 129, 99, 255, 115, 233, 160, 195, 163, 21, 2, 40, 30, 30, 42, 14, and 42, respectively. Prey item categories (coded by color) are provided above the figure. The presence of prey items or the inclusion of prey items (in the case of Bayesian mixing model results), varied by time period and the species represented by “Other invertebrates”, and “Other fish” are described in the Methods. Gray boxes indicate periods of relatively high rainbow smelt abundance.

Figure 6. Horsetooth Reservoir predator and prey carbon and nitrogen stable isotope signatures. Predator and prey mean isotopic signatures (and standard deviations) are provided, and the year the data were collected (text on figure) and points correspond in color to the species information provided in the legend. Large rainbow smelt data are portrayed with larger sized points noted in the legend.

Figure 7. Estimates of rainbow smelt abundance (hydroacoustics) and density (trawl). Hydroacoustic data (black squares) correspond to the y-axis on the left, and trawl data (white

squares) correspond to the y-axis on the right (note the exponential scale). Standard deviations for trawl estimates are provided. Gray boxes indicate periods of relatively high rainbow smelt abundance.

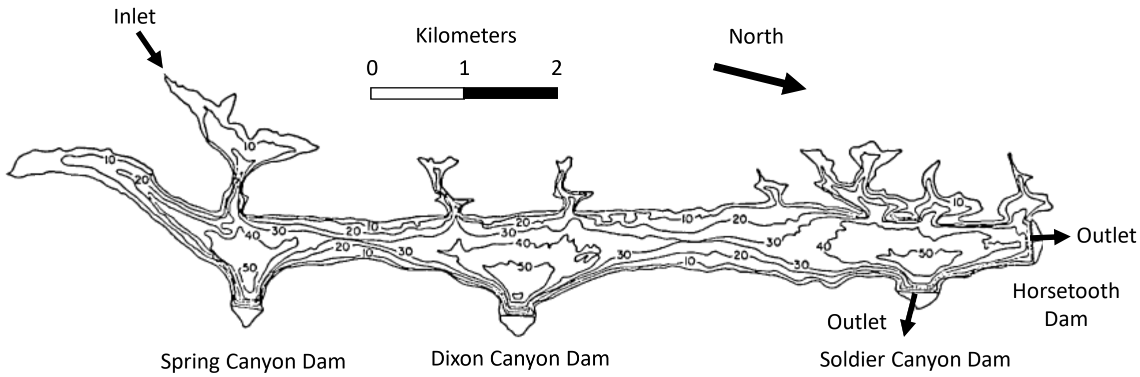
Figure 8. Rainbow smelt encountered during routine Colorado Parks and Wildlife gill netting (late April through May) and boat electrofishing (July-August) surveys. Annual indices are represented by the summation of rainbow smelt encountered per survey, divided by the number of total surveys that occurred in a given year. One individual rainbow smelt (181 mm) was captured during a routine gill net survey in 2010 and this is noted. Gray boxes indicate periods of relatively high rainbow smelt abundance.

Figure 9. Horsetooth Reservoir rainbow smelt diets. Rainbow smelt diets between 1984 and 1995 were quantified using stomach content analysis of fish collected during routine and targeted surveys. Fish greater than 100 mm were separated when possible, but when no size classes were designated (1990, 1992, 1994, and 1995), all diets were included. Rainbow smelt diets between 2017 and 2019 were quantified using rainbow smelt carbon and nitrogen stable isotope signatures (as well as their prey signatures) combined with Bayesian mixing models. These rainbow smelt were all ≥ 130 mm. Rainbow smelt sample sizes for years with data (earlier to later) were 5, 88, 90, 94, 99, 212, 435, 131, 20, 9, and 73. Prey item categories (coded by color) are provided above the figure. Prey items represented by "Other invertebrate prey," were primarily dipterans and amphipods. Gray boxes indicate periods of relatively high rainbow smelt abundance.

Figure 10. Estimates of *Mysis diliuviana* density (m^{-2}). Standard deviations of estimates are provided. We note that *Mysis diluviana* were collected in 1981 using a benthic trawl (Nesler 1986), and the presence of *Mysis diluviana* was confirmed in rainbow smelt diets until 1988 (Jones 1985a; Thomas 1989). Gray boxes indicate periods of relatively high rainbow smelt abundance.

Figure 11. Peak zooplankton abundance and dominant *Daphnia* species measured in Horsetooth Reservoir. Samples dominated by *Daphnia pulex/pulicaria* are denoted with black squares, and samples dominated by *Daphnia galeata mendotae* are denoted with white squares. Gray boxes indicate periods of relatively high rainbow smelt abundance.

Figure 1.



Inlet
Kilometers
0
1
2
North
Outlet
Horsetooth
Dam
Outlet
Spring Canyon Dam
Dixon Canyon Dam
Soldier Canyon Dam

Figure 2.

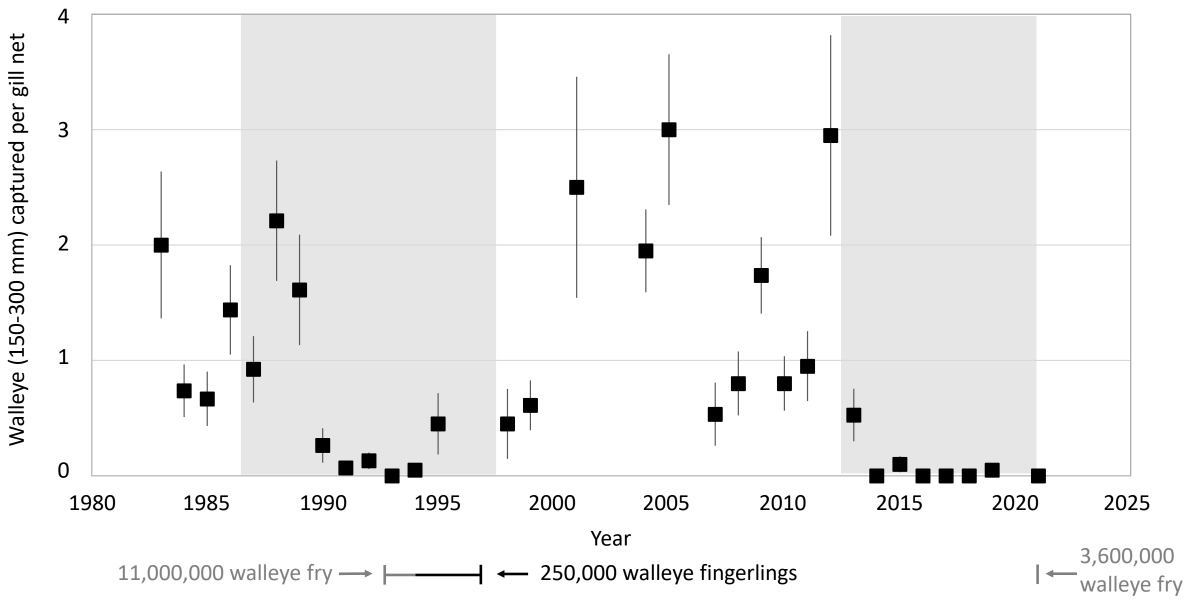
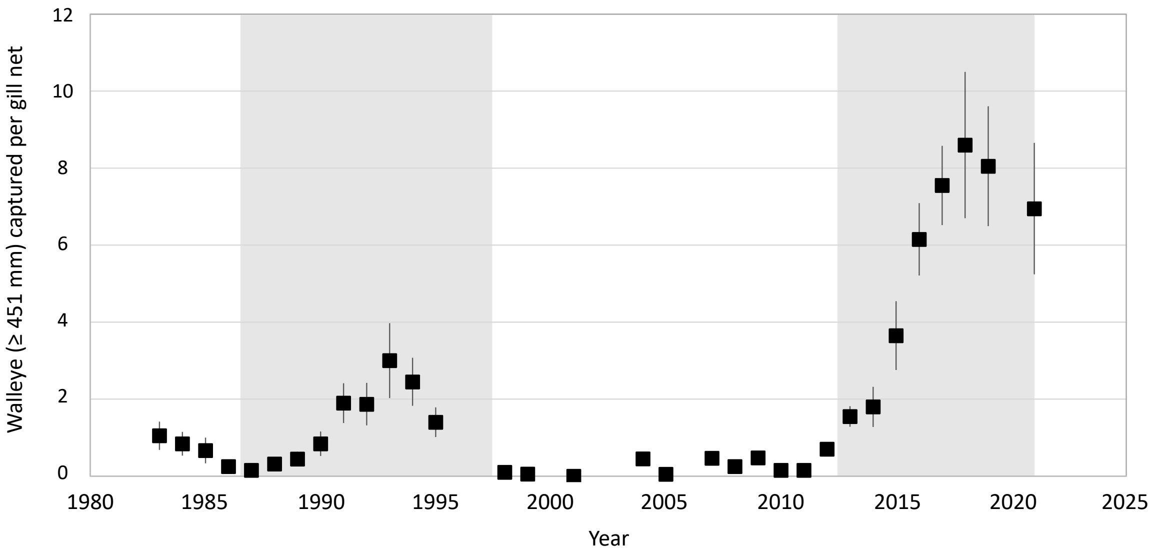

Walleye (150-300 mm) captured per gill net
0
1
2
3
4
1980
1985
1990
1995
2000
2005
2010
2015
2020
2025
Year
11,000,000 walleye fry
250,000 walleye fingerlings
3,600,000
walleye fry

Figure 3.



Walleye (≥ 451 mm) captured per gill net
0
2
4
6
8
10
12
1980
1985
1990
1995
2000
2005
2010
2015
2020
2025
Year

Figure 4.

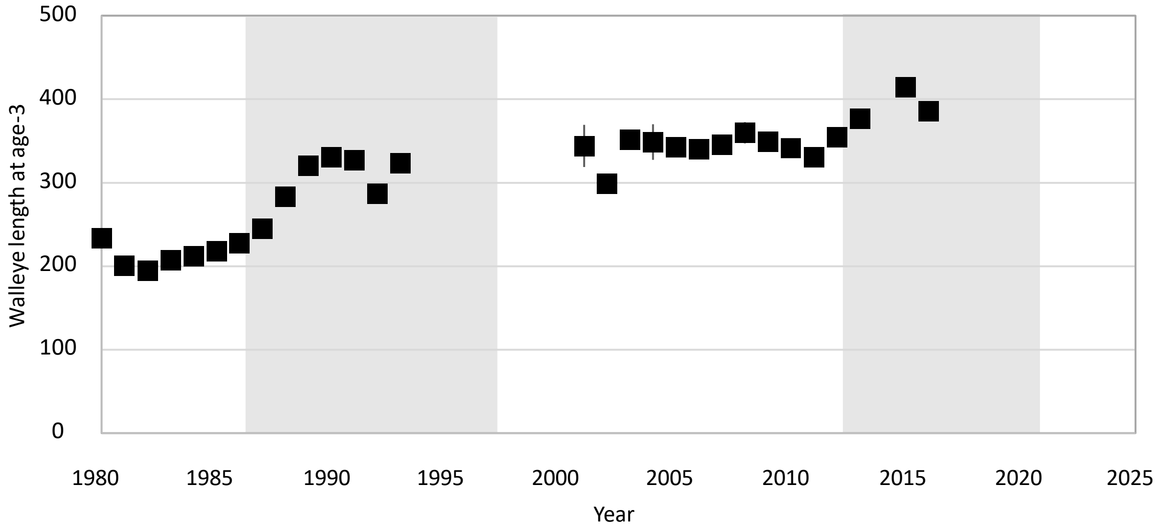
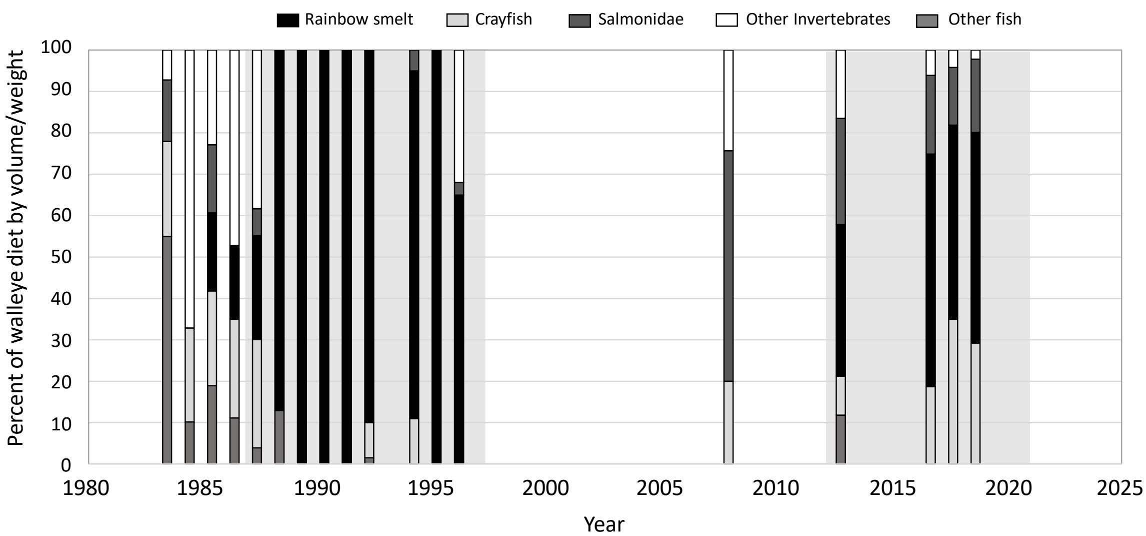

Walleye length at age-3
500
400
300
200
100
0
1980
1985
1990
1995
2000
2005
2010
2015
2020
2025
Year

Figure 5.



Rainbow smelt
Crayfish
Salmonidae
Other Invertebrates
Other fish
Percent of walleye diet by volume/weight
100
90
80
70
60
50
40
30
20
10
0
1980
1985
1990
1995
2000
2005
2010
2015
2020
2025
Year

Figure 6.

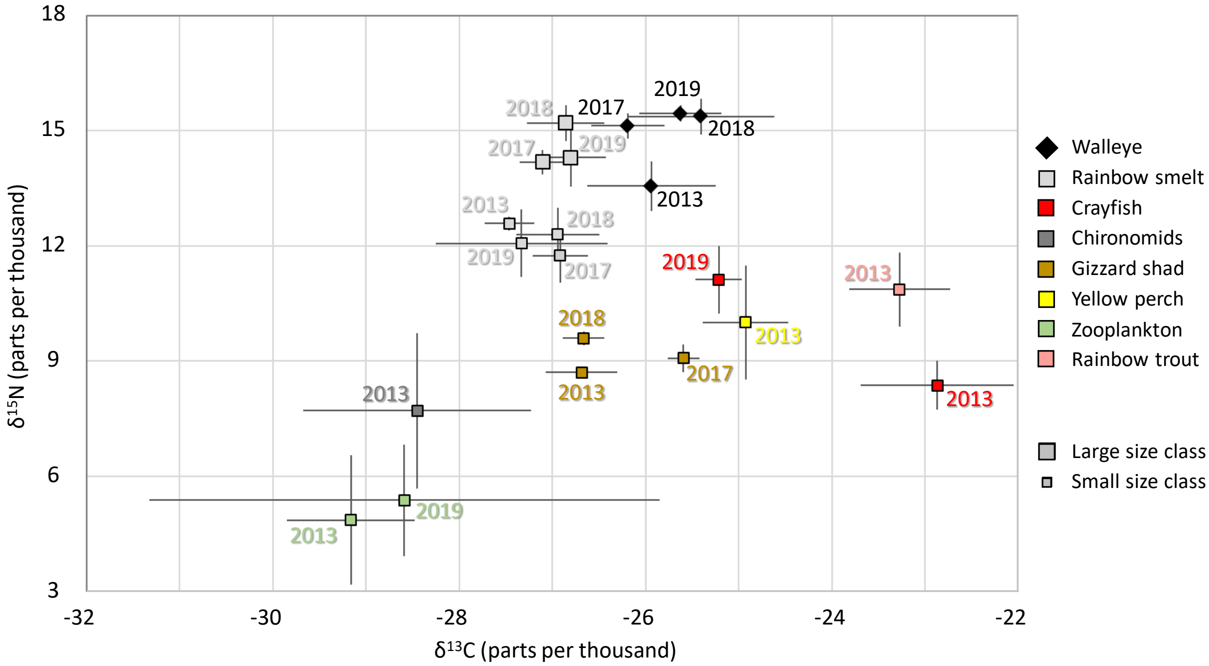

18
15
12
9
6
3
-32
-30
-28
-26
-24
-22
δ15N (parts per thousand)
δ13C (parts per thousand)
2018
2017
2019
2018
2017
2019
2013
2013
2018
2019
2017
2019
2013
2018
2013
2017
2013
2013
2013
2019
2013
2019
Walleye
Rainbow smelt
Crayfish
Chironomids
Gizzard shad
Yellow perch
Zooplankton
Rainbow trout
Large size class
Small size class

Figure 7.

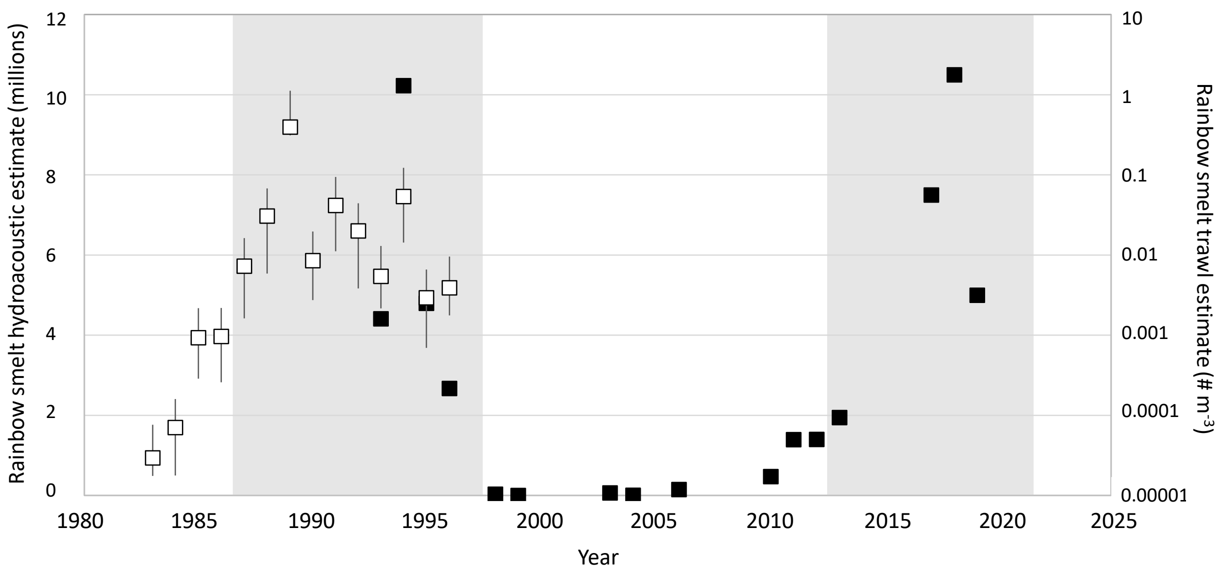
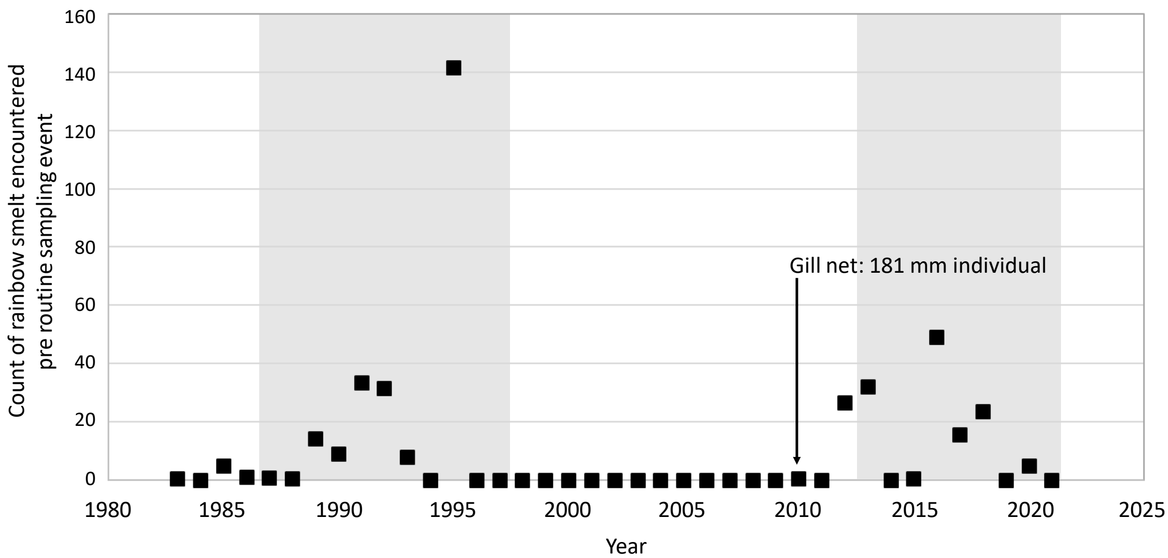
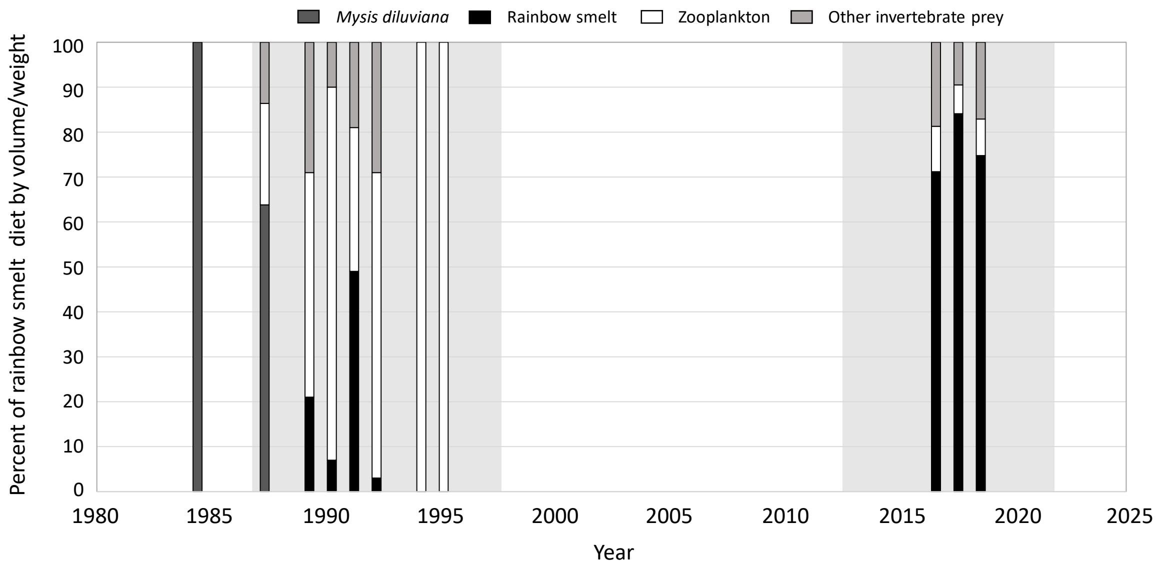

Rainbow smelt hydroacoustic estimate (millions)
Rainbow smelt trawl estimate (# m-3)
Year
12
10
8
6
4
2
0
10
1
0.1
0.01
0.001
0.0001
0.00001
1980
1985
1990
1995
2000
2005
2010
2015
2020
2025

Figure 8.



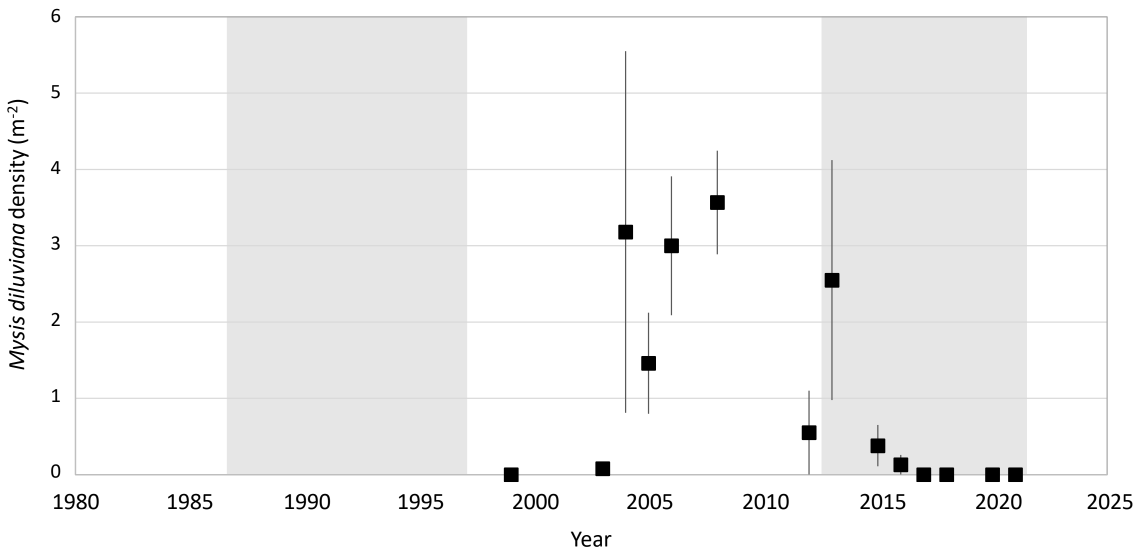
Count of rainbow smelt encountered pre routine sampling event
160
140
120
100
80
60
40
20
0
Gill net: 181 mm individual
1980
1985
1990
1995
2000
2005
2010
2015
2020
2025
Year

Figure 9.



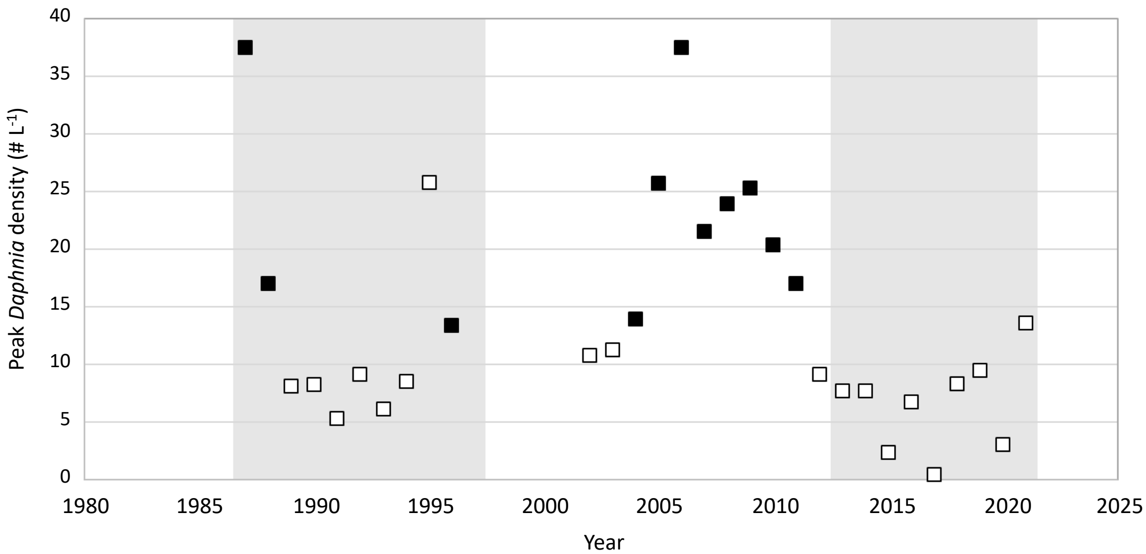
Mysis diluviana
Rainbow smelt
Zooplankton
Other invertebrate prey
Percent of rainbow smelt diet by volume/weight
100
90
80
70
60
50
40
30
20
10
0
1980
1985
1990
1995
2000
2005
2010
2015
2020
2025
Year

Figure 10.



6
5
4
3
2
1
0
Mysis diluviana density (m-2)
1980
1985
1990
1995
2000
2005
2010
2015
2020
2025
Year

Figure 11.


40
35
30
25
20
15
10
5
0
Peak *Daphnia* density (# L^{-1})
1980
1985
1990
1995
2000
2005
2010
2015
2020
2025
Year

<u>RESEARCH PRIORITY</u>: **Triploid Walleye research:** Recent advancements from ongoing Colorado State University PhD project on triploid Walleye ecology in collaboration with CPW. Here we report results from two publications (one *in press,* and one *in review*).

OBJECTIVES:

Evaluate the post-stocking performance [trophic ecology, growth, survival, reproductive characteristics (e.g., gonadal development), and population dynamics] of triploid versus diploid Walleye to help inform management and appropriate stocking procedures on the Western Slope of Colorado.

PUBLICATION:

Farrell, C. J., B. M. Johnson, **A. G. Hansen**, C. A. Myrick. *In press*. Induced triploidy reduces mercury bioaccumulation in a piscivorous fish. Canadian Journal of Fisheries and Aquatic Sciences. Available online: https://doi.org/10.1139/cjfas-2021-0037.

BRIEF SUMMARY:

Walleye *Sander vitreus* (but see Bruner 2021) are highly sought-after sport and food fish in the state of Colorado. Because the upper Colorado River basin (UCRB) contains critical habitat for four endemic species of native fish, reservoir fisheries management in the UCRB emphasizes a suite of measures to reconcile nonnative sport fishing with the recovery of native fishes endemic to the Colorado River and its tributaries. This includes stocking triploid Walleye to diversify recreational angling opportunities in sensitive locations, deter illegal fish stocking, and possibly interfere with diploid reproduction in unwanted populations (Fetherman et al. 2015; Johnson et al. 2009).

Induced triploidy is commonly used in aquaculture operations (Piferrer et al. 2009), for biocontrol of aquatic vegetation (Allen and Wattendorf 1987), and increasingly as a stocking option for recreational fisheries (Cassinelli et al. 2019; Koch et al. 2018; Teuscher et al. 2003), primarily because triploid fish are reproductively sterile (Benfey 1999). Sterility confers several potential advantages for the use of triploids as a stocking option in recreational fisheries, most notably for reproductive containment and the potential for increased growth rates (Piferrer et al. 2009). Differential energetic requirements associated with spawning could modify consumption dynamics and the bioaccumulation of contaminants between diploid and triploid fish — an overlooked element that may have important implications for the use of triploids in recreational fisheries.

Several studies have investigated how MeHg bioaccumulation affects reproduction (Crump and Trudeau 2009), but the effects of reproductive investment (i.e., the cumulative energetic investment into gamete production over a fish’s lifetime) on MeHg bioaccumulation are not well understood. Spawning is energetically costly for both males and females and requires fish to meet this energetic demand by consuming prey (Diana 1983; Trudel et al. 2000), and since food consumption is the primary pathway of Hg uptake in predators (Hall et al. 1997), the energetic costs associated with spawning should at least partially regulate MeHg bioaccumulation (Nicoletto and Hendricks 1988). Therefore, we would expect a positive correlation between MeHg concentrations (i.e., [MeHg]) and reproductive investment. Previous studies noted that reproductive investment was correlated with higher [MeHg] in sharks (Coelho et al. 2010; Pethybridge et al. 2010) and ray-finned fishes (Nicoletto and Hendricks 1988; Son et al. 2014). However, the effects of reproductive investment could not be isolated from age and maturity status.

Given that the development of ovaries is typically more energetically costly than the development of testes (McBride et al. 2015), one would expect that reproductive females would consume more food, and therefore bioaccumulate more MeHg than reproductive males. However, several studies demonstrated that reproductive males have similar or higher [MeHg] relative to reproductive females of the same age (Bastos et al. 2016; Gewurtz et al. 2011; Madenjian et al. 2016). Differences in resource use (Lepak et al. 2012), activity levels (Henderson et al. 2003), and standard metabolic, Hg elimination, or growth rates (Madenjian et al. 2014, 2016; Trudel and Rasmussen 2006) between males and females complicate studies using between sex comparisons to examine the effects of reproductive investment on [MeHg]. No previous study has effectively accounted for age or other potential sex-dependent variables to discern the relative importance of reproductive investment in governing the bioaccumulation of Hg. Differential gonad development arising from ploidy manipulation gives us the ability to isolate the effects of reproductive investment on Hg bioaccumulation and overcome previous limitations.

We compared mercury bioaccumulation in triploid and diploid Walleye in Narraguinnep Reservoir, Colorado, and made several hypotheses that sex- and ploidy-specific differences in the allocation of energy towards reproductive development would affect mercury bioaccumulation. We tested our hypotheses with linear regression and a bioenergetics model informed by field data. We found diploid Walleye had 28%–31% higher mercury concentrations on average than triploids, but there were no differences between sexes of the same ploidy (Figure 1). Triploids of mature age exhibited minimal gonadal development when compared to diploids (Figure 2). After accounting for reproductive investment, the bioenergetics model accounted for most of the observed difference in average mercury concentration between ploidies for females (Figure 3). Conversely, the energetic cost of producing testes was low, and gonadal development could not explain observed patterns for males. Costs associated with elevated swimming activity and metabolism by diploid males relative to other groups could explain the difference but requires further investigation. The use of triploid fish in stocking programs could prove useful for reducing mercury in fish destined for human consumption.

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FIGURES:

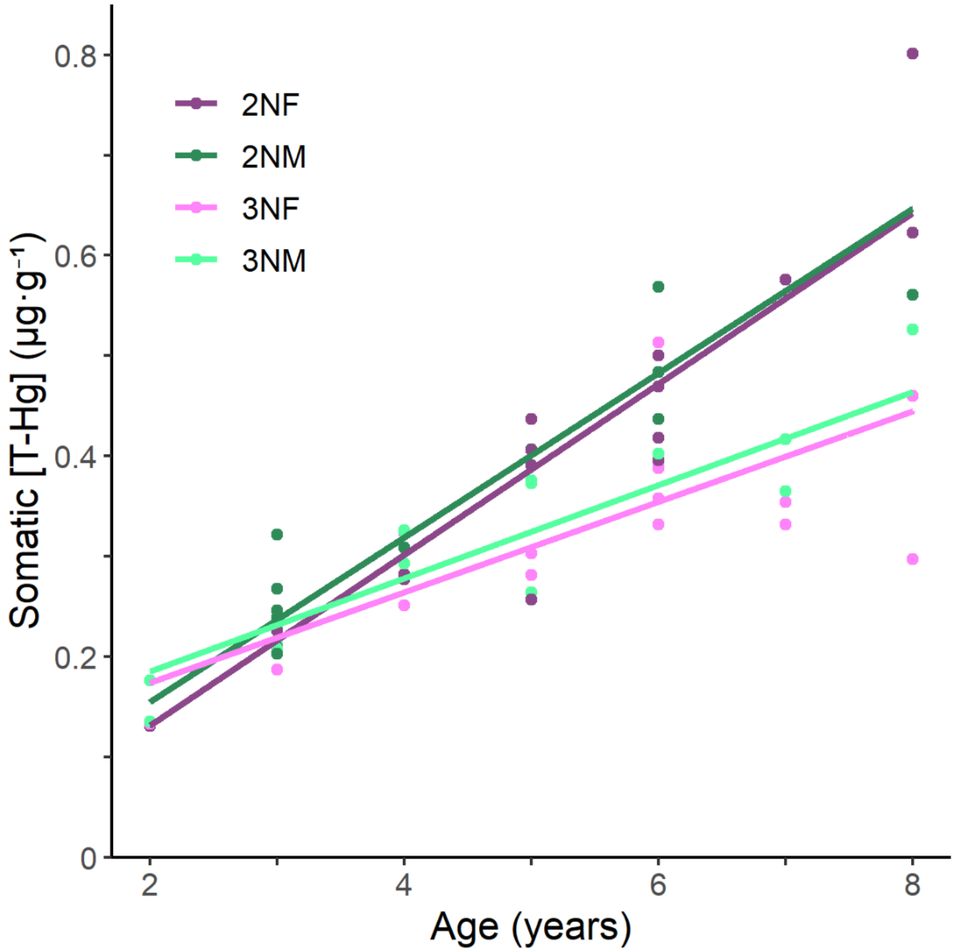

Figure 1. Observed somatic mercury concentrations [T-Hg] by age for Walleye from Narraguinnep Reservoir (points). Lines represent model-averaged estimates of regression coefficients for retained models (cumulative $\omega_i \leq 0.95$). 2N = diploid, 3N = triploid, F = female, M = male.

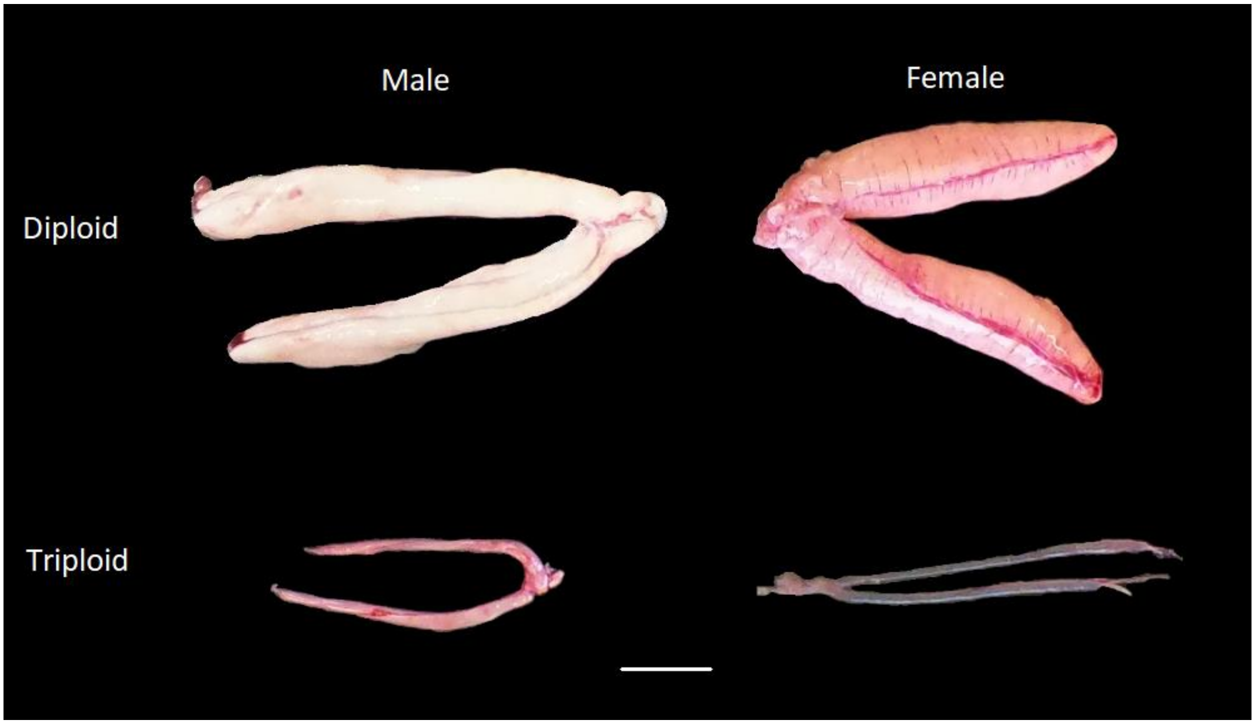

Figure 2. Example gonads from diploid and triploid individuals of reproductive age for diploid fish (3+ for males, 5+ for females). Gonads are from similarly aged and sized individuals within each sex.

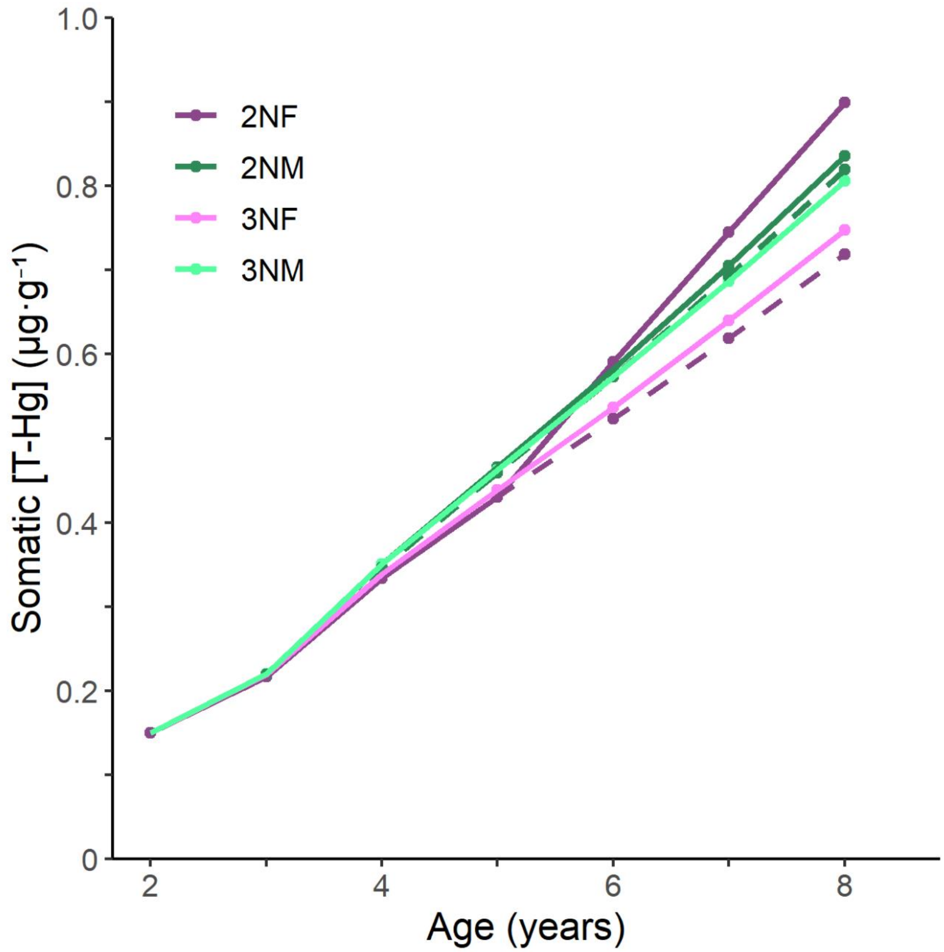

Figure 3. Somatic total mercury concentrations ([T-Hg]; µg·g^{-1}) by age for Walleye estimated by the bioenergetics model. 2N = diploid, 3N = triploid, F = female, M = male. For diploid Walleye, solid lines represent spawning fish (full gonadal development observed in the field), while dashed lines represent hypothetical non-spawning fish (no gonadal development).

MANUSCRIPT SUBMISSION:

Farrell, C. J., B. M. Johnson, **A. G. Hansen**, C. A. Myrick, E. C. Anderson, T. A. Delomas, A. D. Schreier, and J. P. Van Eenennaam. *In review*. Cytological and molecular approaches for ploidy determination: results from a wild Walleye population. North American Journal of Fisheries Management.

INTRODUCTION:

Artificially induced triploidy is used in aquaculture worldwide to increase growth efficiency, improve flesh quality, and prevent unwanted reproductive development of cultured organisms (Piferrer et al. 2009; Zhou and Gui 2017). Interest in stocking triploid fish is rising among fisheries managers in situations where natural reproduction is undesirable (Budy et al. 2012; Farrell et al. *in press*), because inducing triploidy is the most practical, efficient, and effective method for large-scale production of sterile fish (Maxime 2008). As perfect induction of triploidy is rarely achieved (Fetherman et al. 2015), pre-stocking ploidy determinations are necessary to ensure batches of fish with low induction rates are not released onto the landscape. Likewise, since ploidy is rarely visually identifiable (Maxime 2008), those interested in the effects of triploidy on life-history traits and population dynamics need to determine ploidy post-stocking.

Flow cytometry is a cytological method for determining ploidy and is considered the "gold standard" method among practitioners (Fiske et al. 2019; Hubálek and Flajšhans 2020), as it has been deemed the most accurate technique for determining ploidy in fish (Maxime 2008). Flow cytometry quantifies the relative nuclear DNA content of individual cells, typically erythrocytes in fishes, by measuring the fluorescence emitted by stained nuclear DNA (Allen 1983). Coulter counter analysis is another common cytological method for determining ploidy, which measures nuclear volume of erythrocytes as a proxy for nuclear DNA content (Wattendorf 1986) and is nearly as accurate as flow cytometry (Fiske et al. 2019). There are other ploidy determination methods (e.g., karyotyping or blood smears), but most are time consuming and may not be suitable when processing several samples (Fiske et al. 2019; Maxime 2008).

Molecular-based methods of ploidy determination are relatively new and offer an alternative approach that can overcome the difficulties associated with cytological methods, while also providing additional genetic information about stock structure. Collection and storage of tissue samples for molecular-based ploidy determination is relatively simple. Epithelial tissue from a fin is a preferred source of DNA for extraction (Wandeler et al. 2007), and can easily be collected from a fish non-lethally (Pratt and Fox 2002) using a clean pair of scissors. Properly stored fin clip samples have a shelf life measured in years to centuries rather than weeks. Fish size is less of a concern when using molecular-based methods for ploidy determination. For example, Hou et al. (2021) successfully extracted DNA from ichthyoplankton <4 mm total length (TL). While cytological approaches require fish in relatively good condition, tissue samples used for genetics-based approaches can be used on specimens that have been dead for decades (Wandeler et al. 2007), effectively eliminating the concern of obtaining high quality samples from fish postmortem.

In this study, we compared paired Coulter counter- and genetics-based ploidy determinations for individual fish to test the agreement of these two methods, and assess the utility of molecular-based methods for determining ploidy in a percid. Samples (paired blood and fin tissue) for ploidy determination were collected from a population of diploid and triploid Walleye in a southwestern Colorado reservoir within the upper Colorado River basin.

METHODS:

Narraguinnep Reservoir is a 215-ha irrigation supply reservoir in southwest Colorado, USA. In 2008, Colorado Parks and Wildlife began stocking triploid Walleye into Narraguinnep Reservoir, where an existing diploid Walleye population had existed since as early as 1972, resulting in a mixed population of diploid and triploid Walleye (Farrell et al. *in press*). Fish were collected using gillnets configured for fall walleye index netting (FWIN; Morgan 2002) during spring 2019 and 2020. Gillnets were set overnight. Water temperatures during both sampling periods were less than 10°C, and limited netting mortality occurred. Fish condition was noted prior to processing and fish were euthanized if necessary. Sampling procedures were approved by the Institutional Animal Care and Use Committee (Protocol # 18-7822A) at Colorado State University.

Blood samples were collected with syringes via cardiac puncture immediately following euthanasia (Duman et al. 2019), stored in tubes coated with lithium heparin, and stored at 4°C until Coulter counter ploidy analysis could be performed by the Genomic Variation Laboratory at the University of California Davis, following the methods described by Fiske et al. (2019).

A 3-cm^2 fin clip was collected for genetic analysis from the lower lobe of the caudal fin using scissors and forceps that were sanitized with 70% ethanol for genetic analysis. Fin clips were dried at ambient temperature and stored in Whatman paper until genetic analyses could be completed at the Idaho Fish and Game Department's Eagle Fish Genetics Laboratory. We genotyped 231 samples to use as a training set to establish critical log-likelihood ratios (LLRs) used for determining ploidy from genetics data following the method of Delomas (2019). We then used 118 samples, where Coulter counter-based ploidy determinations were withheld from the genetics laboratory, as a test set to evaluate agreement between the two methods.

All statistical analyses were performed using R 4.0.3 (R Development Core Team 2020). We used Cohen's kappa to assess agreement between the two ploidy determination methods (Cohen 1960). Cohen's kappa adjusts for chance agreement (van Stralen et al. 2012), and values ≤0.2 indicate poor agreement and ≥0.9 indicate excellent agreement (Byrt 1996; Landis and Koch 1977). Cohen's kappa values were calculated using the irr package in R (Gamer et al. 2019). Figures were prepared using the package ggplot2 (Wickham 2016).

RESULTS & DISCUSSION:

The mean modal nuclear volumes of erythrocytes estimated by a Coulter counter for fish in both the training and test sets was 12.4 μm^3 (SD = 0.733 μm^3) for diploids and 17.6 μm^3 (SD = 0.952 μm^3) for triploids (Figure 1). Mean erythrocyte volume for triploid Walleye was approximately 1.42x larger than their diploid conspecifics. Two Walleyes had nuclear volumes of 24.3 and 24.8 μm^3 (Figure 1). Because the nuclear volume of erythrocytes for these fish was approximately 1.40x greater than measured in triploids, they were deemed tetraploid as nuclear volume increases by 50% per unit increase in ploidy level (Benfey 1999).

Calculated LLRs from genetics data had distinct distributions for triploid and diploid Walleye. To determine critical values for assigning ploidy, the LLRs of the training samples were manually assessed. The critical value for diploidy was set at -5, as this value excluded all triploid training samples, and aligned with the critical value found to perform well for similar panels in salmonids (Delomas 2019). The critical value for triploidy was set at 200, as this value excluded all diploid training samples. Fin tissue samples were categorized as either triploid (LLR ≥200), diploid (LLR ≤ -5), or ambiguous (-5 < LLR < 200). Mean LLRs were 1722 (SD = 1039) for diploids and 2270 (SD = 1504) for triploids (Figure 2).

After applying the critical LLR values established from the training set to the test set, we found 98.3% agreement between the molecular and cytological ploidy determinations. These methods disagreed for only two individuals (Table 1). These two individuals were deemed tetraploid via the Coulter counter method, but triploid via genetics. Considering the method CPW uses to produce triploid Walleye (Fetherman et al. 2015), it is unlikely tetraploids exist in this population, and the Coulter readings for the two putative tetraploids could be an artifact of blood coagulation. Cohen's kappa for the comparison of these two methods was 0.965 (95% CI = 0.917 – 1.00), which indicated excellent agreement (Byrt 1996; Landis and Kock 1977). There were no disagreements between the methods for which a triploid was called diploid or where a diploid was called triploid.

Our study further supports that molecular techniques work extremely well for ploidy determination and that collecting samples is simpler and more practical than for those needed for cytological approaches. Tissue samples used to extract DNA for genetics analysis, such as fin clips, are relatively simple to collect, less stressful for the organism (Pratt and Fox 2002), and easily preserved—allowing samples to be archived for future study or retrospective analysis of samples collected before this methodology existed. Obtaining blood samples of satisfactory quantity and quality for cytological ploidy determination requires fish to be large enough and in good condition (i.e., prior to postmortem blood clotting). For example, individual ploidy determination by traditional approaches is difficult on very small individuals (e.g., larvae), yet Walleye are often stocked as larvae (Ellison and Franzin 1992; Logsdon et al. 2004).

Some standardized gillnetting protocols like FWIN require 24h sets (Morgan 2002), far too long to ensure high quality blood samples required for cytological approaches. However, the availability of molecular-based approaches for determining ploidy enables the use of standardized approaches such as FWIN for monitoring and studying actively managed mixed diploid-triploid populations. Furthermore, researchers and managers can also obtain more information for a similar price (\$10 USD·sample^{-1}) using molecular methods to determine ploidy than by using cytological methods. In addition, genetic data obtained from molecular approaches for ploidy determination can be used for other analyses including stock identification, parentage, and estimation of effective population size (Begg et al. 1999; Hauser and Carvalho 2008; Steele et al. 2013). Moreover, tissues collected for DNA extraction can be used for other, non-genetic analyses. For example, fin clips have been used in mark-recapture studies (VanDeValk et al. 2007), for contaminant biomonitoring (Cerveny et al. 2016; Heltsley et al. 2005), and for stable isotope analysis (Sanderson et al. 2009).

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TABLES AND FIGURES:

Table 1. Confusion matrix comparing the ploidy assignments by cytological (Coulter counter) and molecular (genetics) approaches in the test set of samples (N = 118).

Cytological Ploidy Calls	Molecular Ploidy Calls		
	2N	3N	4N
2N	73	0	0
3N	0	43	0
4N	0	2	0

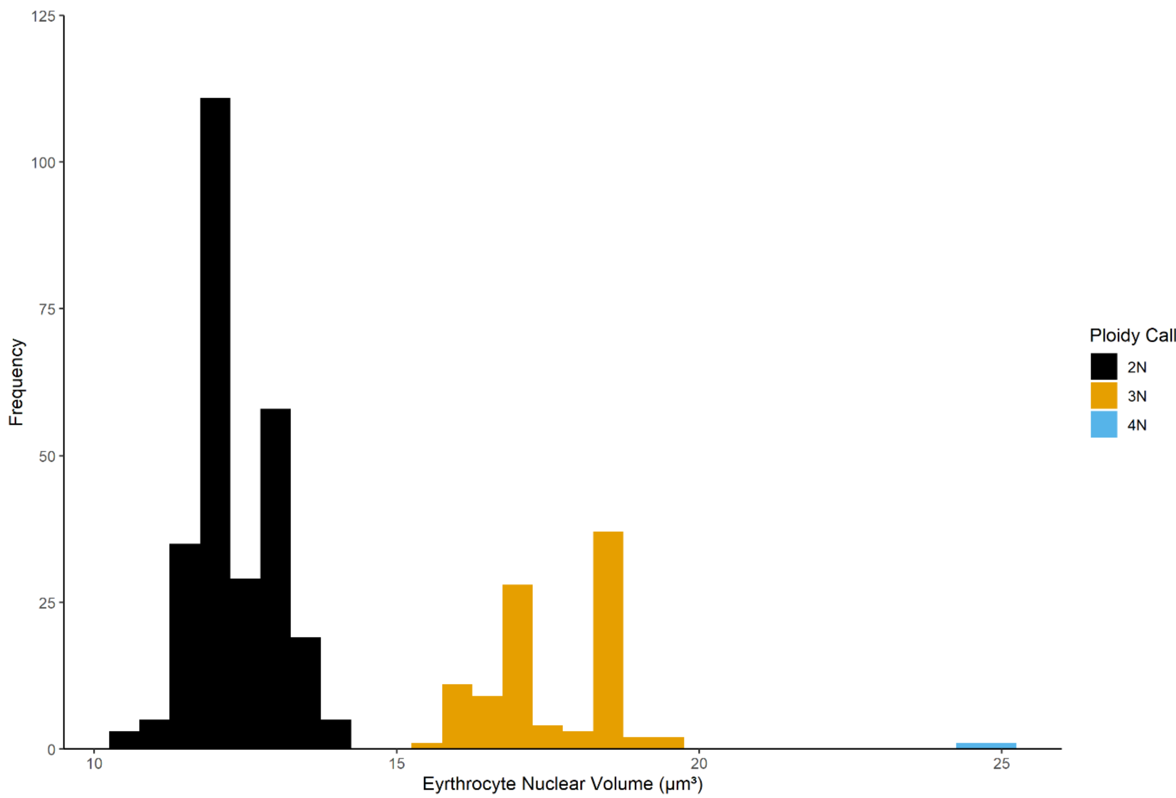

Figure 1. Distribution of modal nuclear volume of Walleye erythrocytes measured via a Coulter counter. 2N = diploid, 3N = triploid, 4N = tetraploid.

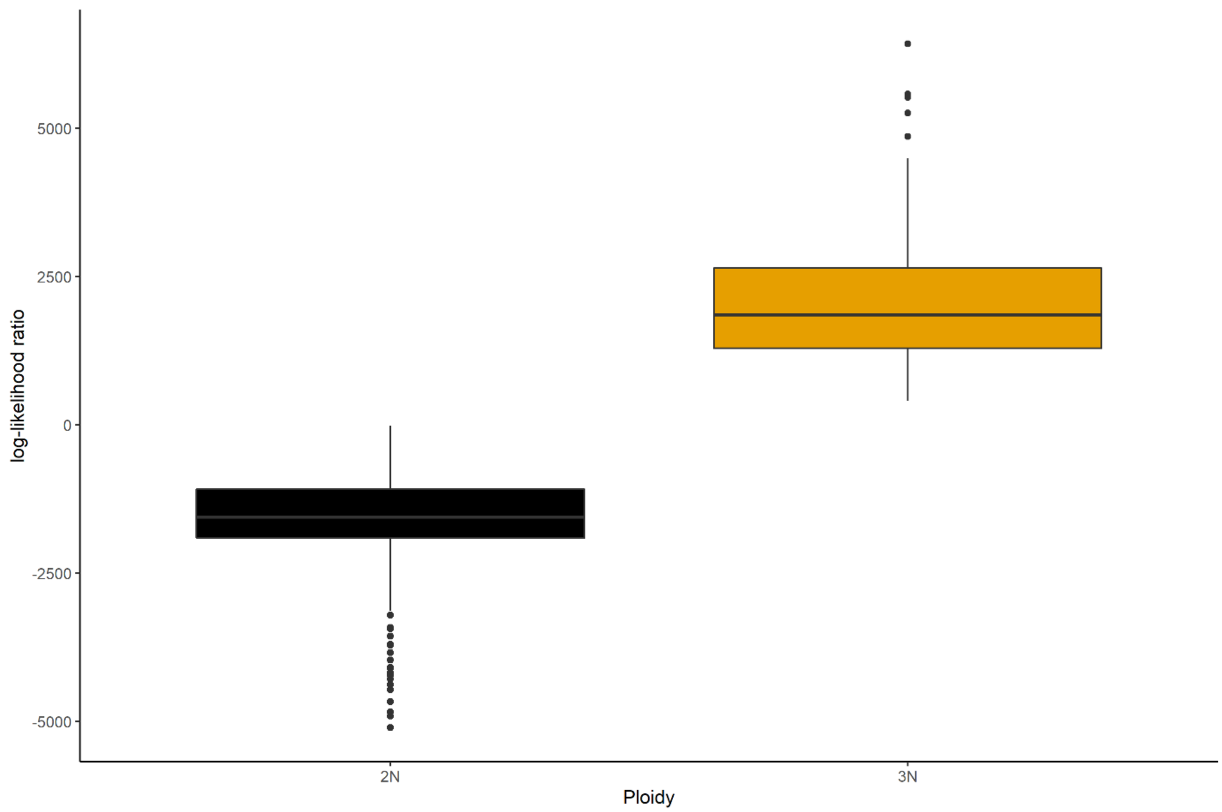

Figure 2. Log-likelihood ratios (LLR) estimated for diploid and triploid Walleye from genetics-based ploidy determinations. 2N = diploid, 3N = triploid.

RESEARCH COMMUNICATION AND TECHNICAL ASSISTANCE

Reporting period: December 2020 – November 2021.

Peer-reviewed Publications:

- Rohan, S. K., D. A. Beauchamp, T. E. Essington, and **A. G. Hansen**. 2021. Merging empirical and mechanistic approaches to modeling aquatic visual foraging using a generalizable visual reaction distance model. Ecological Modelling 457:109688.

- Farrell, C. J., B. M. Johnson, **A. G. Hansen**, and C. A. Myrick. *In press*. Induced triploidy reduces mercury bioaccumulation in a piscivorous fish. Canadian Journal of Fisheries and Aquatic Sciences.

- Lepak, J. M., **A. G. Hansen**, M. B. Hooten, D. Brauch, and E. M. Vigil. *In press*. Rapid proliferation of the parasitic copepod, *Salmincola californiensis* (Dana), on kokanee salmon, *Oncorhynchus nerka* (Walbaum), in a large Colorado reservoir. Journal of Fish Diseases.

- Guo, C., Wei Li, Shiqi Li, Lin Li, T. Zhang, B. J. Hicks, J. Liu, and **A. G. Hansen**. *In press*. Manipulation of fish community structure effectively restores submerged macrophytes in a shallow subtropical lake. Environmental Pollution.

- **Hansen, A. G.**, J. A. Gardner, K. A. Connelly, M. Polacek, and D. A. Beauchamp. *In press.* Resource use among top-level piscivores in a temperate reservoir: implications for a threatened coldwater specialist. Ecology of Freshwater Fish.

External Presentations:

- **Hansen, A. G.**, and D. Brauch. Population dynamics of Lake Trout in Blue Mesa Reservoir, Colorado: new insights from linking a simple population model to long-term survey data. American Fisheries Society, Western Division Virtual Meeting hosted by Utah Chapter. Symposium: *Advancements in the Ecology and Management of Nonnative Lake Trout*. May 14th, 2021.

Other Research Communication & Technical Assistance:

- Silver, D. B., B. M. Johnson, W. M. Pate, **A. G. Hansen**, and K. Christianson. 2021. History and outcomes of opossum shrimp (*Mysis diluviana*) introductions in Colorado. Colorado Parks and Wildlife, Technical Publication Number 58.

- Associate Editor for North American Journal of Fisheries Management. Completed my 2.5 year commitment in August 2021.

- Anonymous peer reviewer for: Lake and Reservoir Management (2 manuscripts); North

American Journal of Fisheries Management (2 manuscripts); Transactions of the American Fisheries society (1 manuscript).

- CPW Fact Sheets: ‘Managing Mercury in Sport Fish.’

- Research on long-term population dynamics of Lake Trout in Blue Mesa Reservoir highlighted in a story written by Erik Cristan (CPW Aquatic Research Technician for Lake and Reservoir Research Laboratory spring 2020-present). Title: *Angler Incentives: lucrative Lake Trout in Blue Mesa Reservoir*. Published in Colorado Outdoors Magazine, Sept./Oct. 2021 issue.

- Organized a virtual symposium at Western AFS in May 2021: *Advancements in the Ecology and Management of Nonnative Lake Trout.*

- The Lake and Reservoir Research Laboratory provided technical assistance to the Colorado Department of Public Health and Environment by participating as invited scientists in their Technical Advisory Committee that develops fish consumption advisories for Colorado. The group has concerns about emerging contaminants including per- and poly-fluoroalkyl substances (PFAS). Dr. Jesse Lepak, employed with the Lake and Reservoir Research Laboratory, contributed expertise on fish species and their behavior/habitat preference. These characteristics have direct implications on their potential contaminant exposure and uptake in the environment. These meetings took place on 23 June 2021 and 25 October 2021.

APPENDIX A

Science of the Total Environment

Predictors of Sport Fish Mercury Contamination in Heavily Managed Reservoirs: Implications for Human and Ecological Health

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Keywords:	Random forest; northern pike; smallmouth bass; walleye; Hg prediction
Corresponding Author:	Jesse Michael Lepak, Ph.D. New York Sea Grant Oswego, NY United States
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	Adam G. Hansen
Abstract:	Mercury (Hg) is an important contaminant due to its widespread distribution and tendency to bioaccumulate to harmful levels in the environment. We used a machine learning approach called Random Forest (RF) to evaluate different predictors of Hg concentrations in three species of Colorado (USA) sport fish. We also compared empirical and conceptual rates of change in sport fish Hg concentrations resulting from multiple data sources including empirical observations and theoretical rates of change from different Hg deposition scenarios. Our RF models indicated that the best predictors of large northern pike (Esox lucius) Hg concentrations at 864 mm were covariates related to salmonid stocking in each study system, while system-specific metrics related more to productivity and forage base were the best predictors of Hg concentrations of smallmouth bass (Micropterus dolomieu), and walleye (Sander vitreus) at 381 mm. Our theoretical and empirical comparisons indicated that system-specific food web characteristics (e.g., forage base, stocking history) can be important drivers of rapid and large shifts in some sport fish Hg concentrations compared to what might be expected in response to large-scale changes in Hg deposition over time. Importantly, protecting human and ecological health from Hg contamination requires an understanding of fish Hg concentrations and variability across the landscape and through time, and this random forest approach can be applied to predict how sport fish Hg concentrations may change as a result of a variety of factors to help prioritize, focus, and streamline monitoring efforts to effectively and efficiently.
Suggested Reviewers:	Feiyue Wang University of Manitoba Manitoba Centre for Earth Observation Science feiyue.wang@umanitoba.ca Conducts research related tot he topic.
	David Ward USGS FSC: US Geological Survey Flagstaff Science Campus dlward@usgs.gov Related research topics.
	Che-Jen (Jerry) Lin Lamar University Jerry.Lin@lamar.edu Related Research.
	Roxanne Karimi Stony Brook University School of Marine and Atmospheric Sciences Roxanne.Karimi@stonybrook.edu Related research topics.

	Lyatt Jaegle University of Washington jaegle@atmos.washington.edu Related research topics.
	Thomas Suchanek University of California Davis thsuchanek@ucdavis.edu Related research topics.
Opposed Reviewers:	

August 16th, 2021

A letter to the Editors; Science of the Total Environment,

Dear Editor(s),

Please consider the submitted manuscript entitled "Predictors of sport fish mercury contamination in heavily managed reservoirs: implications for human and ecological health" for publication as a Research Paper in Science of the Total Environment (STOTEN). As the title states, the manuscript evaluates a set of predictors of sport fish mercury (Hg) concentrations in heavily managed reservoirs. We used a valuable machine learning approach called Random Forest (RF) to evaluate different predictors of Hg concentrations in three species of Colorado (USA) sport fish. We also compared empirical and conceptual rates of change in sport fish Hg concentrations resulting from multiple data sources including empirical observations and theoretical rates of change from different Hg deposition scenarios. This is a continuation of several of the authors' work that has been published previously in STOTEN, and contributes to what is known about Hg contamination and cycling in the environment. This manuscript provides a basis for identifying areas of concern for further investigation, and informing the design of comprehensive monitoring and additional research efforts.

This manuscript fits well with the Aims and Scope of STOTEN because the work focuses on the widespread problem of mercury contamination in the environment which is a topic commonly addressed by manuscripts in STOTEN. The overall goal of the manuscript is to provide information to assess and ultimately minimize health risks associated with mercury exposure in humans and wildlife. Thus, it relates to several of STOTEN's preferred subject areas including but not limited to; 1) atmospheric biogeochemistry, 2) ecotoxicology and risk assessment, 3) wildlife and contaminant, 4) novel contaminant (bio)monitoring and risk assessment approaches, and 5) trace metals and organics in biogeochemical cycles.

Author contributions include: Lepak (conceptualization, investigation, writing original draft, funding acquisition), Johnson (conceptualization, review and editing, funding acquisition, resources), Wolff (Data curation, project administration, review and editing), Hooten (conceptualization, formal analysis, review and editing), Hansen (resources, review and editing). Author preference is for the publication to be in color, but resource limitation may preclude the inclusion of color depending on the cost. The Graphical Abstract and Figure 7 have color components that help clarify what is presented, but only those portions of the manuscript. Permissions for data usage were obtained from multiple institutions, so we will not be making raw data available publicly. However, contemporary fish Hg concentration data for Colorado are available from the Colorado Department of Public Health and Environment (https://coepht.colorado.gov/environmental-data/fish-consumption).

We hereby confirm that the enclosed work has not been published or accepted for publication, and is not under consideration for publication, in another journal or book. Your consideration of this manuscript is greatly appreciated. Please contact me if any additional information is required (phone: 607-351-8310, or Email: Salvelinus205@gmail.com). I look forward to hearing from you.

Sincerely,

Jesse M. Lepak

Predictors of Sport Fish Mercury Contamination in Heavily Managed Reservoirs: Implications for Human and Ecological Health

Jesse M. Lepak[a*], Brett M. Johnson[b], Mevin B. Hooten[c], Brian A. Wolff[b], and Adam G. Hansen[a]

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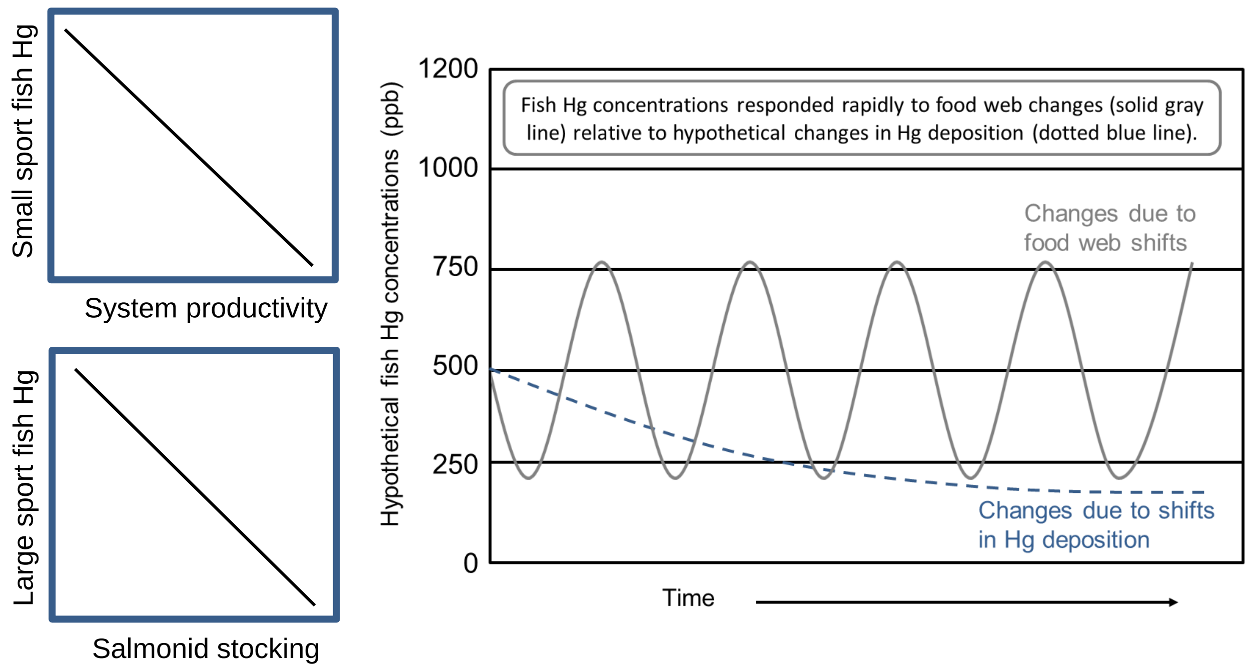
Graphical abstract



Highlights (for review : 3 to 5 bullet points (maximum 85 characters including spaces per bullet point)

Highlights

- Mercury concentrations in sport fish were evaluated using a random forest approach
- Potential change in fish Hg from food web and Hg deposition changes were compared
- System productivity and salmonid stocking were important for predicting fish Hg
- Food web shifts can cause significant changes in fish Hg in the short-term
- Long-term global reductions in Hg deposition should reduce fish Hg concentrations

1. Introduction

Mercury (Hg) is an important contaminant due to its widespread distribution and tendency to bioaccumulate in organisms to levels that impact the health of humans, other organisms, and ecosystems worldwide (Driscoll et al., 2007; Mergler et al., 2007; Wright et al., 2018). Mercury enters the landscape through a variety of natural and anthropogenic pathways including volcanoes, forest fires, erosion, fossil fuel burning, waste incineration, mining operations, and cement production (Pirrone and Mason, 2009). Mercury is a neurotoxin that can adversely affect humans and wildlife in a variety of different ways, potentially impacting survival, growth, behavior and reproduction (Mergler et al., 2007; Scheuhammer et al., 2007). Despite health concerns related to Hg contamination in fish, documented benefits of fish consumption may outweigh the risks (Knuth et al., 2003; Institute of Medicine, 2007; Mergler et al., 2007), and healthy fish consumption has been encouraged (e.g., Niederdeppe et al., 2019).

Sport fish Hg concentrations are generally a preferred indicator of Hg contamination in freshwater systems because consumption of Hg contaminated sport fish is the major method of transfer of aquatic Hg to humans (Driscoll et al., 2007; Mergler et al., 2007). Fish Hg concentrations can be inherently variable due to a variety of factors, including the amount of Hg being contributed to a particular system. There are indications that in some areas, atmospheric Hg has been declining modestly over the past decade due primarily to reductions in anthropogenic emissions of Hg, while in other areas, increases have been observed (Zhang et al., 2016; Lyman et al., 2020). However, changes in contributions of Hg to the environment do not necessarily translate directly to changes in fish Hg concentrations because of complex biogeochemical interactions (e.g., Wang et al., 2019; Brigham et al., 2021). Thus, Hg deposition information alone cannot be used to infer fish Hg concentrations across the landscape. In addition, changing land use, weather patterns, and atmospheric conditions (e.g., Obrist et al. 2018) are also likely to contribute to variability in fish Hg concentrations. These sources of uncertainty can be problematic, and monitoring programs have been developed to capture inherent variability in fish Hg concentrations to protect human health. But, extensive, large-scale monitoring can be cost prohibitive, and many predictors of Hg concentrations in fish can be correlated, and interact in unforeseen ways. These issues make predictive model development (to identify priority areas or species of concern for testing) challenging. Thus, an approach incorporating information about mechanisms influencing fish Hg concentrations and multiple data sources (including correlated predictors), could help overcome some of the obstacles to characterizing dynamic fish Hg concentrations and sources of uncertainty.

Investigators have studied a variety of factors associated with Hg contaminated sport fish in eastern North America, the Great Lakes region, and western North America (e.g., Sorensen et al., 1990; Driscoll et al., 1994; Eagles-Smith et al., 2016), and several patterns have emerged on the larger landscape. For example, when deposited, Hg can be taken up quickly by water and the organisms within it (Harris et al., 2007). Because of this direct water-atmosphere interface, larger systems (higher surface area) receive more overall Hg deposition relative to smaller systems. In addition, systems in close proximity (<100 km) to "large" sources of Hg (similar to those found in the eastern and central United States; Lin et al., 2012) can experience increased deposition from those sources, and Wentz et al. (2014) found that Hg inputs to stream fishes were highest in urban versus rural areas. Thus, it follows that systems located near Hg sources (i.e., population centers or urban areas) could experience higher levels of Hg inputs and exposure.

It is well-established across regions that fish species, size, and trophic position are important factors influencing sport fish Hg concentrations (Bodaly et al., 1993; Power et al., 2002;

Johnston et al., 2003). Specifically, large, piscivorous sport fish with elevated trophic positions tend to have higher Hg concentrations than small, omnivorous or planktivorous fish feeding at lower trophic levels. Therefore, changes in food web structure (especially sport fish forage base) can alter sport fish Hg concentrations by changing fish trophic position, growth, and diet (Eagles-Smith et al., 2008; Lepak et al., 2012b, Johnson et al., 2015). Reservoir food web structure and species interactions are often influenced by fisheries management practices (e.g., fish stocking). Thus, when one is interested in understanding system-specific Hg concentrations, fisheries management practices must be considered because they can influence fish species composition, individual size, forage base, and subsequently piscivore Hg concentrations.

Nutrient inputs influence the productivity of reservoirs and the resulting changes in productivity have the potential to alter Hg concentrations in sport fish. Two mechanisms associated with increased productivity can reduce Hg concentrations in organisms. The first, known as “bloom dilution” occurs when high nutrient availability stimulates population growth of algae and subsequently zooplankton resulting in a higher amount of biomass available to accumulate a given amount of Hg (Pickhardt et al., 2002; Chen and Folt, 2005). The second process is known as “growth dilution” and can occur in organisms at multiple trophic levels. For example, it has been shown that zooplankton growth increases while Hg concentrations decrease when algae with low carbon:nitrogen:phosphorus ratios (and therefore higher quality) are available (Karimi et al., 2007). When high-quality diet items are available to organisms, they typically display high rates of somatic growth paired with lower consumption rates. This reduces both Hg concentration in prey and overall intake by predators. In the case of fish, it has been shown through experimental nutrient additions and observational studies that fish from systems with higher nutrient inputs have relatively low Hg concentrations (Cleckner et al., 1998; Kidd et al., 1999; Essington and Houser, 2003). Thus, increased nutrient inputs to reservoirs can reduce Hg bioaccumulation in sport fish within them.

Although nutrient inputs have the potential to lower Hg concentrations in aquatic organisms, they can also increase bioavailability of Hg and subsequently Hg bioaccumulation. Mercury is methylated (becoming bioavailable) under anoxic conditions by bacteria as a byproduct of their energy sequestration pathway (Compeau and Bartha, 1985; Fleming et al., 2006). Abundant nutrients can stimulate primary production, some of which decays, creating anoxic conditions conducive to Hg methylation (Bodaly et al., 1984). For example, Lienesch et al. (2005) found that increased nutrient inputs intended to enhance sport fish populations resulted in increased hypoxia during the summer and winter. Hypoxic conditions have been associated with high concentrations of Hg in water, zooplankton, and fish (Driscoll et al., 1994; Slotton et al., 1995). These examples indicate that the relationship between nutrients and Hg concentrations in biota is complex.

Water temperature (i.e., growing season) has the potential to increase and decrease Hg in organisms through a variety of mechanisms. For example, temperature can influence growth dilution, community composition, and Hg methylation rates. At small scales, temperature is usually similar across systems. However, where elevation differences are large (e.g., those from eastern to western Colorado, USA), or large differences in latitude exist, the length of the growing season can be different based on system elevation or latitude. All else being equal, fish that grow faster have the potential to be "diluted" in Hg relative to others growing more slowly (Ward et al., 2010). However, increased water temperatures or longer growing seasons can also enhance microbial activity. Microbial Hg methylation rates have been shown to increase up to a temperature of approximately 35 °C before experiencing a decline (Callister and Winfrey, 1986).

Further, Lin et al. (2012) found that Hg deposition is higher in warmer seasons due to stronger Hg^0 oxidation. Thus, elevation and latitude could have positive and/or negative influences on sport fish Hg concentrations related to temperature.

Water level fluctuation has been associated with elevated Hg concentrations in sport fish including walleye (*Sander vitreus*) (Selch et al., 2007), and yellow perch (*Perca flavescens*) in South Dakota systems varying by over 100% in surface area (Sorensen et al., 2005). These authors suggest that fish Hg levels are influenced by water level fluctuation through the rewetting and perturbation of dry soils which are relatively rich in sulfate, stimulating population growth of sulfate-reducing bacteria and Hg methylation rates. Analogous patterns of elevated Hg concentrations in biota (zooplankton and fish) in systems experiencing water level fluctuation were also found by St. Louis et al. (2004). Others have found that reservoirs have fish and other biota that can be particularly high in Hg concentrations in general (Tremblay and Lucotte, 1997; French et al., 1998; Bodaly and Fudge, 1999). In the arid portion of western North America, artificial reservoirs are the predominant lacustrine systems on the landscape. Most reservoirs serve multiple functions including recreational, municipal, and agricultural use. As such, reservoirs tend to be highly managed with respect to their biotic and abiotic characteristics, and this can influence Hg dynamics (Willacker et al., 2016).

In this paper, we describe an approach to evaluate predictors of Hg concentrations in three different sport fish species in Colorado, USA. We use data compiled from sources throughout Colorado to evaluate the relative importance of various predictors of sport fish Hg concentrations related to the factors described above. We used available system-specific predictors as indicators of urbanization, food web structure, productivity, and Hg methylation processes. Further, we used our observations combined with those of others to compare rates of sport fish Hg change that might be expected in response to a variety of factors primarily focusing on empirical changes in food webs and hypothetical changes in Hg emissions. These comparisons provide a basis to inform decisions about the magnitude and relevant spatial and temporal scales for developing appropriate fish consumption advice.

2. Materials and methods

2.1. Data collection and compilation

Sport fish Hg concentrations (2004 to 2014) available from Colorado Department of Public Health and Environment (CDPHE) were used to develop response variables for three fish species; northern pike (*Esox lucius*), smallmouth bass (*Micropterus dolomieu*), and walleye. For each species and each system where they were sampled, simple linear regression was used to estimate fish Hg concentrations at lengths that were related to the most commonly applied length-based harvest regulations in Colorado (864 mm for northern pike and 381 mm for smallmouth bass and walleye). To develop the regression analyses and determine Hg at length, data from individual fish were used, as well as data based on composite samples from multiple fish combined. Composite samples were represented by a single datum as the mean length of the group of fish tested and the resulting Hg concentration. Measurements of Hg that fell below detection were censored by assigning a value equal to half the detection limit (Rao et al., 1991). However, regression analyses were never based solely on data that fell below the Hg concentration detection limit of 0.3 ppm for these data because it was felt the assigned value (0.15 ppm) could mask a relatively large amount of variability (potential empirical range from 0 to 0.3). We selected a regression approach to maximize the amount of data that could be included in these analyses and to represent a size that is informative to the health of anglers and their families that harvest sport fish in Colorado.

Predictors of the response variables (fish Hg concentrations at length) were selected based on previous research, and data availability. Thus, we had *a priori* hypotheses associated with each predictor variable. For each system, predictors included a group of 17 indices developed from a compilation of data from 2000 to 2014. These covariates included: 1) system surface area (Area), 2) shortest linear distance to Colorado Interstate 25 (I-25), 3) population density of the county containing the system (Pop dens), 4) whether or not rainbow trout (*Oncorhynchus mykiss*) were stocked from 2003-2013 (RBT), 5) the total mass of salmonids stocked from 2003-2013 (Salm mass), 6) the mean annual stocking density (g/ha) of rainbow trout from 2003-2013 (RBT mass/area), 7) presence or absence of bluegill sunfish (BGL, *Lepomis macrochirus*), 8) presence or absence of cyprinids (CYP), 9) presence or absence of gizzard shad (GSD, *Dorosoma cepedianum*), 10) presence or absence of green sunfish (SNF, *Lepomis cyanellus*), 11) presence or absence of yellow perch (YPE), 12) index of chlorophyll a (Chl a), 13) index of secchi depth (Secc), 14) index of total phosphorous (TP), 15) system elevation (Elev), 16) system latitude (Lat), and 17) index of water level fluctuation (H_2O ↑↓). For clarity, a tabular representation of these covariates, their hypothesized underlying mechanisms, directionality of the effect expected from each covariate, the data sources used to represent the covariates, and the relevant time frame of the data can be found in Table 1.

Data compiled for these analyses were collected intermittently by different agencies and personnel from 2000-2014 depending on a variety of factors including research or monitoring objectives, resource availability, and sampling conditions. We used these data to develop system-specific indices to characterize covariates and evaluate their relative importance for predicting empirical sport fish Hg concentrations. When possible, we limited data use from 2003-2013 to develop the most relevant covariates for the 2004-2014 Hg concentration data for Colorado sport fish. We did this to ensure that sport fish Hg concentrations were temporally reflective of the system-specific conditions characterized by the indices/covariates. However, contemporaneous data were not always available, and supplemental data from 2000-2002 and 2014 were necessary to characterize some systems. Thus, our indices/covariates were developed to inform the relative importance of past drivers of sport fish Hg concentrations, and design future monitoring efforts, but not to develop fish consumption advice directly.

Data from multiple system-specific surveys and sampling events were available from CPW from 2003-2013 to inform a subset of covariates including, Area, I-25, RBT, Salm mass, RBT mass/area, BGL, CYP, GSD, SNF, YPE, Elev, Lat, and H_2O ↑↓. The presence or absence of forage fish species were assessed as present (captured during any CPW survey from 2003-2013), or absent (not captured during any CPW survey from 2003-2013). A qualitative index (low, medium and high taken from the CPW database) was used to indicate water level fluctuation (H_2O ↑↓) from 2003-2013. Water quality data (chlorophyll a, secchi depth, and total phosphorous) were collected by multiple agencies including the United States Geological Survey, the Bureau of Reclamation, and CDPHE. These data were made available by CDPHE (R. Anthony and K. Richardson, pers. comm.). Data collected during summer (May-September inclusive) 2000-2014 were used to calculate mean, system-specific indices to characterize these water quality covariates. Nutrient data (Chl a, and TP concentrations) below detection limits were censored by assigning a value equal to half the detection limit (Rao et al., 1991). A more tractable list of predictors, abbreviations, mechanisms (as described in more detail in the

Introduction) and expected directionalities of drivers of Hg concentrations in sport fish are provided in Table 1.

2.2. Data analyses

Using these data, we applied a Bayesian nonlinear machine learning technique called random forest (RF; Cutler et al., 2007) to statistically evaluate indices/covariates as predictors of sport fish Hg concentrations within the dataset and determine the relative importance and effectiveness of the predictors for characterizing sport fish Hg concentrations. Given the complexities, and potential correlations within the dataset, the RF approach is ideal because it is based on a form of cross-validation, is entirely nonlinear, accounts for obscure interactions, and simultaneously evaluates multicollinear variables (Breiman, 2001; Cutler et al., 2007). Where other approaches (e.g., linear models) prevent the inclusion of multicollinear variables, resulting in the potential exclusion of valuable predictive data, the RF approach can incorporate all readily available data for prediction. For example, total phosphorus and chlorophyll a are often highly correlated, and with most approaches, one variable or the other would need to be excluded from any single predictive model. Using the RF approach allowed us to incorporate all variables of interest despite any multicollinearity.

The RF approach falls under a much larger class of machine learning methods where prediction is of primary interest. These methods rely on “bagging,” referring to the method of fitting regression trees to numerous bootstrapped versions of the training data (i.e., observations sampled with replacement from the larger data set). The resulting trees are then averaged to yield a predictor with low bias and variance. To perform our RF analyses, we used the ‘randomForest’ package (Liaw and Wiener, 2002; package 4.6-6) in R (R Development Core Team, 2011). In the

RF implementation described here, 2,000 regression trees were used to calculate accuracies and error rates for each observation using out-of-bag predictions (i.e., predicting data that were withheld from each tree). The predictions of data that were not used to evaluate fit can be considered as a form of cross-validation. Variable importance can then be assessed by comparing the increase in; 1) mean squared prediction error, and 2) node purity associated with each individual covariate. We refer the interested reader to Gini (1912), Liaw and Wiener (2002), and Cutler et al. (2007) for more information on these variable importance metrics.

For each fish species, a full model (all predictors) was used to determine predictive power and variable importance. However, we were aware that many of the predictors available in this dataset would not be available to others. To determine how well this approach performed when only the best predictors were used, we removed most predictors from the full models and repeated the RF statistical analyses on a condensed model. Predictors were retained if they were found to be important as measured by both the increase in mean square prediction error and node purity. These selections were made somewhat arbitrarily for demonstration purposes, however, *a priori* hypotheses, and support from other research were considered along with the predictor importance metrics when full models were reduced to the most important 3 or 4 variables. In general, we expected that the best predictors of Hg concentrations in larger (864 mm) northern pike would be those related to stocked salmonids as forage while the best predictors of Hg concentrations in 381 mm smallmouth bass and walleye would be more dependent on system productivity metrics.

2.3. Prediction application

The food web structure in Elkhead Reservoir (Colorado, USA) changed significantly when the stocking of salmonids (up to 3,400 kg of ~ 250 mm rainbow trout stocked in a single year; 2010, with a mean of about 1,500 kg of salmonids stocked each year) was discontinued in the fall of 2011. Based on previous whole-system experimental work (Lepak et al., 2012b) we expected northern pike Hg concentrations in this reservoir to increase from this management action. For comparison, northern pike Hg concentrations were available from 2005 and 2007, prior to this management shift. Due to limited data, 2005 and 2007 samples compiled and categorized as the 2005 sampling date to estimate the most conservative rate of change. We used the full and condensed models developed for northern pike to predict what 864 mm northern pike Hg concentrations would be under these new conditions. The output also provided a value for comparison to empirical northern pike Hg concentration data collected in 2013 following the cessation of stocking, recognizing that the food web structure may or may not have been in equilibrium following this shift.

2.4. Conceptual and empirical rates of change in sport fish Hg concentrations

We developed several conceptual scenarios (hypothetical changes in Hg deposition/emissions) to represent how rapidly fish Hg concentrations might respond to changes in Hg deposition. We used a trend analysis of volume-weighted wet Hg deposition in the United States (Zhang and Jaeglé, 2013) to develop five Hg depositional scenarios with respect to their potential influence on sport fish Hg concentrations in the West for comparison with the food web scenarios described above. All wet deposition scenarios began with a hypothetical fish having a Hg concentration of 0.3 ppm, selected because this concentration is a threshold that prompts a fish consumption advisory in Colorado. Fish Hg concentrations were assumed to respond directly (same proportional change) in the time step (year) immediately following any hypothetical change in Hg deposition/emissions projections. We acknowledge that this is an oversimplification of Hg dynamics, and that sources and existing pools of Hg are ubiquitous and may persist despite localized regulatory efforts. However, Brigham et al. (2014) saw a direct and proportional decline in yellow perch Hg concentrations relative to wet deposition. Thus, for simplicity and comparison, we selected to assume that fish Hg concentrations would respond proportionally and rapidly to hypothetical changes in Hg deposition.

Based on Zhang and Jaeglé (2013), the first scenario was equivalent to an increase in East Asian emissions by 32% to match current estimates of trends in that region. The second was designed to match conditions as measured by the Mercury Deposition Network at Buffalo Pass, Colorado (2.66% increase per year) and the West in general (2.60% increase per year) in 2014. The third scenario represented Hg deposition resulting from a 45% decrease in domestic emissions of Hg. The fourth was a combination of a 45% decrease in domestic emissions of Hg and a 12% reduction in background atmospheric Hg levels as has been detected in some areas of the globe. Finally, the last scenario represented a 12% reduction in background atmospheric Hg levels. These scenarios were projected for a 20-year time span.

Similar to the scenarios for changes in wet Hg deposition, we used Lin et al. (2012) to develop two scenarios comparing total Hg deposition (wet and dry) and the potential for the maximum available control technology (MACT) regulations proposed by EPA to help control Hg bioaccumulation in fish. The first scenario is likely the case throughout most of Colorado where approximately 90% of Hg deposited is from outside the state, region and/or country and so 10% of Hg can be regulated from within Colorado (Lin et al., 2012). The goal of MACT is to reduce Hg in emissions by 91%, so 10% of Hg deposited was reduced by 91% in this case. The second scenario was selected to represent a case where a system was near a “large” Hg source. Lin et al. (2012) stated that, near these types of sources, 75% of Hg deposited can arise directly from those sources. Thus, 75% of this Hg could be reduced by 91% using MACT, and these conditions were selected as the last scenario developed.

For comparison to hypothetical changes in Hg deposition/emissions, we referred to observations from (Lepak et al., 2012b) where northern pike Hg concentrations were experimentally manipulated (reduced) by providing high-quality (relatively high energy density and low Hg concentration) forage in the form of hatchery-raised rainbow trout to mimic management strategies and potential changes in food web structure. Replicated ponds were used as controls in this study, and northern pike Hg concentrations from the ponds were considered as well. We present data from Lepak et al. (2012b) in three ways: 1) the maximum change in Hg concentration observed in an individual northern pike from the whole-lake manipulation, 2) the mean change in Hg concentrations of northern pike as a result of the manipulation at the whole-lake scale, and 3) the mean change in Hg concentrations of northern pike in the pond treatments analogous to the whole-lake manipulation. For an additional comparison, we considered northern pike Hg concentrations in Elkhead Reservoir (described in the *prediction application* section above) from before (2005 and 2007 data) and after (2013 data) the cessation of salmonid stocking in fall 2011.

3. Results

3.1. Random forest modeling results

There were thirteen systems included in the 864 mm northern pike RF analysis in addition to predicting the Hg concentration in northern pike from Elkhead Reservoir experiencing no stocking. Northern pike data (n = 108) were sparse (n < 3) in four systems, but data from other species supported the information provided by these samples. The full model explained approximately 25% of the variability in the data with all three salmonid stocking metrics being the most important predictors of northern pike Hg concentrations (Figure 1). To reduce the number of predictive variables in the model, only the top three predictors related to stocking were included in a second model. This more parsimonious, condensed model (Northern pike Hg concentration as a function of RBT, Salm mass, and RBT mass/area) explained approximately 50% of the variability in the data with a consistent bias where predicted values were higher than those observed at relatively low concentrations and lower than those observed at relatively high concentrations (Figure 2).

There were fifteen systems included in the smallmouth bass RF analysis. Smallmouth bass data (n = 128) were sparse (n < 3) in four systems, but data from other species supported the information provided by these samples. The full model explained approximately 55% of the variability in the data with metrics related to growing season, productivity, and forage base being the best predictors (Figure 3). Although I-25 appeared as the fourth most important predictor in both variable importance indicators, the relationship was contrary to expectations; sport fish Hg concentrations went up with increasing distance from Colorado Interstate 25. Thus, this predictor was removed from further analyses and the top three predictors (Elev, GSD, and Chl a) were used in the condensed model. The more parsimonious, condensed model (Smallmouth bass Hg concentration as a function of Elev, GSD, and Chl a) explained approximately 70% of the variability in the data with a consistent bias where predicted values were higher than those observed at relatively low concentrations and lower than those observed at relatively high concentrations (Figure 4).

There were 26 systems included in the walleye RF analysis. Walleye data (n = 543) were sparse (n < 3) in one system, but data were available from a sample near (388 mm) the desired 381 mm for this analysis. The full model explained approximately 10% of the variability in the data with system productivity and growing season metrics being the best predictors (Figure 5). Thus, the top four predictors (Chl a, Elev, Secc, and TP) were used in the condensed model. The more parsimonious, condensed model (Walleye Hg concentration as a function of Chl a, Elev, Secc, and TP) explained approximately 17.5% of the variability in the data with a consistent bias where predicted values were higher than those observed at relatively low concentrations and lower than those observed at relatively high concentrations (Figure 6).

3.2. Prediction application

The linear regression-based empirical value for Hg concentration of 864 mm northern pike in Elkhead Reservoir based on 2005-2007 data (during salmonid stocking) was 0.02 ppm. When the full model was applied to predict Hg concentration of 864 mm northern pike in Elkhead Reservoir without stocking (setting RBT, Salm mass, and RBT mass/area to zero in the model), the result was 0.55 ppm. The result from the condensed model (including RBT, Salm mass, and RBT mass/area as predictors) when the same was done (stocking set to zero) was 0.93 ppm. The observed 2013 Hg concentration of 864 mm northern pike in Elkhead Reservoir was 0.57 ppm.

3.3. Conceptual and empirical rates of change in sport fish Hg concentrations

Three of the five volume-weighted wet Hg deposition scenarios developed from Zhang and Jaeglé (2013) showed increasing trends in fish responding to hypothetical changes in Hg deposition while the other two (both including a 12% decline in background atmospheric Hg) showed slight declines in fish Hg concentrations (Figure 7). Scenarios mimicking conditions in 2014, increasing East Asian emissions, and a 45% reduction in domestic emissions all resulted in increasing Hg concentrations in theoretical sport fish through time (Zhang and Jaeglé, 2013). The MACT scenario that is likely applicable to most areas in Colorado (91% reduction in the 10% of the Hg deposited that can be regulated; Lin et al., 2012) showed a slight decline in fish Hg concentrations by approximately 9% or from 0.3 ppm to 0.27 ppm. The largest reduction in fish Hg concentrations from a simulated change in wet and dry Hg deposition was due to the MACT scenario. This was driven by the reduction in emissions from a hypothetical large local Hg source (91% reduction in the 75% of the Hg sources contributing to deposition that can be regulated) with a single time step reduction by 68% or from 0.3 ppm to 0.1 ppm (Figure 7). This scenario is likely less applicable to Colorado because "large" Hg sources (as described by Lin et al., 2012) are uncommon in Colorado and are relatively small when compared to more industrial areas like the Midwest and Northeast.

The comparison between responses in sport fish Hg concentrations to potential changes in Hg deposition/emission and food web changes indicated that alterations in food web structure could result in relatively rapid shifts in sport fish Hg concentrations (Figure 7). The most rapid shifts were empirical observations during whole-system experimental manipulations (Lepak et al., 2012b) where northern pike Hg concentrations were reduced from approximately 10 to 50% over the course of about 50 days. We also note that empirical northern pike Hg concentrations (at 864 mm) in Elkhead Reservoir increased significantly (over an order of magnitude) between 2005 and 2013 in concomitant with a biologically significant food web shift (the cessation of salmonid stocking) during this time period in 2011 (Figure 7). We note that, since salmonid stocking was discontinued in 2011, the rate of northern pike Hg concentration changes may have been more rapid than what was captured by the data from 2005 and 2007 compared to those from 2013.

4. Discussion

4.1. Random forest modeling

Our expectations based on previous work in Colorado reservoirs (Lepak et al., 2012a, b; Stacy and Lepak, 2012; Johnson et al., 2015) regarding the factors important for predicting sport fish Hg concentrations were supported by the RF analysis. The most important predictors (based on the RF variable importance indicators) of 864 mm northern pike Hg concentrations in Colorado were related to salmonid stocking, presumably because of biomass dilution from the consumption of stocked fish that are relatively high in energy and low in Hg content (Lepak et al., 2012a, 2012b; Stacy and Lepak, 2012). We expected that the most important predictors of 381 mm smallmouth bass and walleye Hg concentrations would be related to biomass dilution as well, but driven by system productivity rather than fish stocking. This is likely because 381 mm sport fish are not generally capable of consuming large (> 200 mm) stocked salmonids that represent the majority of the biomass of fish stocked in Colorado. Thus, indicators related to system productivity and growing season (i.e., Elev, Chl a, Secc, TP) were found to be relatively important predictors of smallmouth bass and walleye Hg concentrations compared to stocking indices.

Four variables that were included as predictors of sport fish Hg concentrations were found to be relatively unimportant in the RF analysis. The indicators of proximity to emission sources and deposition potential (Area, I-25, Pop dens) were relatively unimportant as was the indicator of water level fluctuation hypothesized to potentially be positively correlated to Hg methylation

(Sorensen et al., 2005; Selch et al., 2007). These predictors might be important in other situations or regions, but they were not among the best predictors of sport fish Hg concentrations in the context of this study and the other predictors described above. Instead, we found that productivity (internal and external sources) in the form of nutrients and forage were better predictors of Hg concentrations in sport fish in this set of systems relative to indicators of Hg deposition and methylation within our dataset. A review of Hg contamination of US streams by Wentz et al. (2014) indicated that length-normalized, median largemouth bass (*Micropterus salmoides*) Hg concentrations were highest in streams draining watersheds that were undeveloped, while they were lowest in streams draining urban, followed by agricultural areas. The authors suggested that, even though Hg inputs were highest in urban areas, largemouth bass Hg concentrations were relatively low because conditions conducive to Hg methylation (e.g., dissolved inorganic carbon inputs and proportion of wetlands in the watershed) are less common in urban areas (Wentz et al., 2014). This observation could explain some of the nature of our findings based on data collected in Colorado.

In Colorado, system elevation is related to several other factors that could both potentially increase and decrease fish Hg concentrations. For example, in general, as elevation increases agricultural land-uses decline along with nutrient inputs, precipitation (linked to Hg deposition) increases, growing season declines, and in-lake forage species (e.g., gizzard shad with relatively high energy and low Hg content) are less abundant, which could all lead to elevated sport fish Hg concentrations. However, with increasing elevation, food sources tend to have relatively low trophic positions, food chains tend to be relatively short, and there are generally fewer local sources of Hg emissions, which could all lead to lower sport fish Hg concentrations. In this case, factors related to elevation that led to increased fish Hg concentrations appeared to have a stronger influence than those leading to decreased fish Hg concentrations. The precise mechanism or combination of mechanisms causing this pattern are unknown, but the other three most valuable predictors of fish Hg concentrations were indicators of system productivity.

An RF approach analogous to what is described here is appropriate to inform and streamline monitoring programs, but not to obtain estimates as an alternative to direct testing. This technique could be used to identify areas where sport fish Hg concentration shifts may be occurring. Appropriate systems and species could then be prioritized for Hg testing so consumption advisories could be updated accordingly to maximize the benefits of fish consumption and better protect anglers and their families from potential health risks. For example, in response to the management actions and subsequent food web shifts observed in Elkhead Reservoir, CDPHE has adjusted fish consumption advice and monitoring efforts there to be more protective of human health.

4.2. Prediction application

The performance of full and condensed RF models was encouraging. Although there was a range of variability described by the full and condensed models, model results were reasonable despite low sample size, and bias appeared systematic (predicted values were consistently higher than those observed at relatively low concentrations and lower than those observed at relatively high concentrations). The application of the full model to Elkhead Reservoir to predict northern pike Hg concentrations following 2011 (when salmonid stocking was discontinued), resulted in a value within 5% of empirical observations from 2013, while the application of the reduced (three stocking variables) model overestimated northern pike Hg concentrations.

Many systems in western North America are heavily managed, highly fluctuating, and serve multiple uses which can influence their Hg dynamics (e.g., Willacker et al., 2016), and biota in reservoirs in particular have been found to be elevated in Hg concentration (Tremblay and Lucotte, 1997; French et al., 1998; Bodaly and Fudge, 1999). These types of systems and operations/management are analogous to those considered here, and Colorado offered a wide range of predictors like high and low elevation and little or no salmonid stocking to heavy salmonid stocking. Thus, the patterns observed in this study are likely present in other systems where comparable lake and reservoir management approaches (e.g., salmonid stocking, irrigation, recreation, etc.) are being applied. Using the RF approach described here could identify important predictors of fish Hg concentrations at other locations and scales across the landscape to inform monitoring and advisory programs in place to protect human health.

4.3. Conceptual and empirical rates of change in sport fish Hg concentrations

Sport fish Hg concentrations have the capacity to respond rapidly and significantly to alterations in Hg bioaccumulation dynamics. The strongest evidence to support this were empirical observations in Colorado during a whole-system experimental manipulation. Individual northern pike Hg concentrations were manipulated (reduced by up to 50% in approximately 50 days) by providing relatively high energy density and low Hg concentration forage in the form of hatchery-raised rainbow trout (Lepak et al., 2012b). The inverse (a cessation of salmonid stocking) occurred in Elkhead Reservoir in 2011, and northern pike Hg concentrations increased by over an order of magnitude, similar to what was predicted by the full RF model when applied to this scenario. Though untested, this change likely occurred over the course of two years, from 2011 (following the management action) to 2013 when the data were collected, indicating the potential for rapid responses in fish Hg concentrations.

Although many factors we evaluated focused on food web structure dynamics, and fish Hg concentrations, this does not discount the importance of changes in Hg emission/deposition. The largest reduction in fish Hg concentrations from hypothetical changes in emissions/deposition was in the MACT scenario where a large (75% of what was being deposited in the area), local source of Hg could be reduced by 91%. However, this scenario considers local Hg sources, and in Colorado, much of what is deposited on the landscape is from outside of the state and region, and would be challenging to control autonomously while being influenced from large external sources like East Asia (Lin et al., 2012; Zhang and Jaeglé, 2013). In general, the other scenarios altering Hg deposition/emissions were expected to result in relatively small changes in fish Hg concentrations over longer periods of time when compared to changes associated with significant shifts in food webs within systems.

We assumed that fish Hg concentrations would respond proportionally and rapidly to hypothetical changes in Hg deposition. This is a significant oversimplification for demonstration purposes; however, fish Hg concentrations have shown responses to experimental changes in Hg deposition (Harris et al., 2007), and we based our assumption on those findings. Although intuitive responses have been observed, we acknowledge Hg concentrations in fish can respond unexpectedly to changes in Hg deposition, and biota Hg concentrations do not necessarily track atmospheric Hg (Wolff et al., 2017; Wang et al., 2019). For example, Brigham et al. (2014) observed reductions in wet Hg deposition by 32% across four study lakes in Voyageurs National Park from 1998 to 2012. During the same time period, Hg concentrations in yellow perch decreased in two systems by approximately 35%. Yet, yellow perch Hg concentrations increased in one system by 80%, and no changes were observed in a fourth system. Brigham et al. (2021) revisited this work and found biota in only one system had responded expectedly to reductions in Hg deposition from 1998 to 2018 and concluded that reductions in sulfate emissions may be a more important driver (under conditions there) of Hg cycling and bioavailability than Hg deposition. The same is true in other well-studied systems in Norway where it has been suggested that five decades of reductions in sulfate deposition (concomitant but independent of reductions in Hg deposition) have been a primary driver of long-term changes in biota Hg concentrations by influencing sulfate-reducing bacteria and their role in making Hg bioavailable (Braaten et al., 2020). Thus, it is often difficult to make direct connections between Hg concentrations in biota and changes in Hg deposition.

Although the trajectories of Hg emissions and sport fish Hg concentrations are uncertain, based on the projections presented here, even with significant domestic regulation, it may be difficult to reach target endpoints in some systems and fish species in the near future (Lin et al., 2012) using only controls on Hg emissions. The global pool of Hg is large and unmanageable with respect to US regulations alone, so international cooperation will be necessary to reduce Hg deposition and bioaccumulation, especially in places where Hg sources are largely outside a political boundary or region (Lin et al., 2012; Zhang and Jaeglé, 2013; Wentz et al., 2014). Although some declines in atmospheric Hg have been observed in some regions, there are also areas where atmospheric Hg has increased (Zhang et al., 2016; Lyman et al., 2020), and due to global Hg pools, biogeochemical cycling, and other factors, Hg in some fish populations may take decades or more to respond to reductions in Hg deposition (Sherman and Blum, 2013). An analysis of historical sport fish Hg data collected nationwide suggests that major legislative acts to control contaminants (e.g., the Clean Water Act and Clean Air Act and Hg emission amendment) may result in the overall reduction of sport fish Hg concentrations, but these changes may occur relatively slowly (Chalmers et al., 2011; Wentz et al., 2014), and in the context of other potential sources of change (e.g., food web shifts or changes in methylation mechanisms), which confounds interpretation.

5. Conclusions

Based on our observations, sport fish Hg concentrations can change rapidly from a variety of system-specific factors. These changes can occur faster than one might expect changes in Hg emissions and deposition from regulation or control to be realized. Thus, simplistic yet flexible approaches to rapidly identify areas or species of concern and communicate findings to anglers and their families (e.g., the case of the Elkhead Reservoir northern pike population) might be the most effective means for addressing human health concerns from Hg contamination in the short-term. In Colorado, it is difficult to characterize Hg deposition because of high variability and a general lack of information (few Hg monitoring sites; n = 2). Based on available atmospheric information (e.g., Lin et al., 2012; Zhang et al., 2016; Lyman et al. 2020), Hg deposition may increase in some areas (including areas of Colorado) in the future. These potential changes coupled with the observed dynamic nature of sport fish Hg concentrations, make the RF approach a useful, predictive tool to prioritize and streamline Hg monitoring efforts. Control of anthropomorphic Hg emissions represents an important component of the ubiquitous issue of Hg contamination in the environment. This is recognized by groups like the Minamata Convention on Mercury (UNEP, 2014), and likely represents one of the few long-term solutions to the problem of Hg significantly exceeding natural concentrations in ecosystems and biota. In the short-term, predictive tools like RF that incorporate food web structures, and their dynamics, are important to understand and provide context for Hg bioaccumulation in sport fish and changes in Hg emission, deposition, and cycling.

Declaration of competing interests

We have no competing interests to declare.

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Tables and Figures

Table 1. Predictive variables of sport fish Hg, the mechanism they operate under and their hypothesized influence on fish Hg concentrations. Development of these covariates is described in detail in section *2.1. Data collection and compilation*. Abbreviations for the various predictive covariates are: system surface area (Area), shortest linear distance to Colorado Interstate 25 (I-25), population density of the county containing the system (Pop dens), whether or not rainbow trout (*Oncorhynchus mykiss*) were stocked from 2003-2013 (RBT), the total mass of salmonids stocked from 2003-2013 (Salm mass), the mean annual stocking density (g/ha) of rainbow trout from 2003-2013 (RBT mass/area), presence or absence of bluegill sunfish (BGL, *Lepomis macrochirus*), presence or absence of cyprinids (CYP), presence or absence of gizzard shad (GSD, *Dorosoma cepedianum*), presence or absence of green sunfish (SNF, *Lepomis cyanellus*), presence or absence of yellow perch (YPE), index of chlorophyll a (Chl a), index of secchi depth (Secc), index of total phosphorous (TP), system elevation (Elev), system latitude (Lat), and index of water level fluctuation (H_2O ↑↓). Mechanism, directional expectation of fish Hg concentrations, and data sources and their respective time frames are provided (CPW: Colorado Parks and Wildlife, CDPHE: Colorado Department of Public Health and Environment).

Predictor	Mechanism	Expectation	Data source	Time frame
Area	Increased depositional area	+	CPW	2013
I-25	Increased loading/urbanization	-	Google Earth	Constant
Pop dens	Increased loading/urbanization	+	2010 census	2010
RBT	Stocked forage/biomass dilution	-	CPW	2003-2013
Salm mass	Stocked forage/biomass dilution	-	CPW	2003-2013
RBT mass/area	Stocked forage/biomass dilution	-	CPW	2003-2013
BGL	Forage base/biomass dilution	-	CPW	2003-2013
CYP	Forage base/biomass dilution	-	CPW	2003-2013
GSD	Forage base/biomass dilution	-	CPW	2003-2013
SNF	Forage base/biomass dilution	-	CPW	2003-2013
YPE	Forage base/biomass dilution	-	CPW	2003-2013
Chl a	Nutrients/biomass dilution	-	CDPHE	2000-2014
Secc	Nutrients/biomass dilution	+	CDPHE	2000-2014
TP	Nutrients/biomass dilution	-	CDPHE	2000-2014
Elev	Growing season/biomass dilution	+	CPW	Constant
Lat	Growing season/biomass dilution	+	CPW	Constant
H_2O ↑↓	Methylation/bioavailability	+	CPW	2003-2013

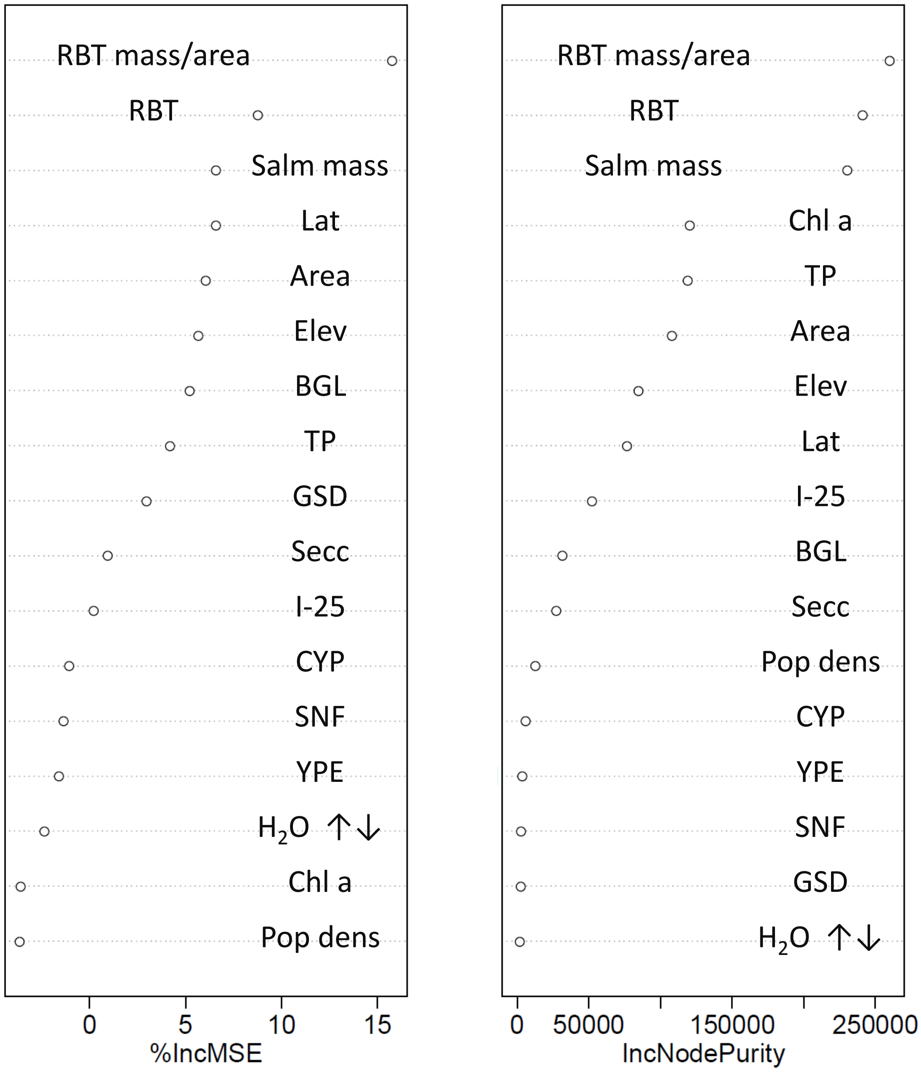

Figure 1. Random forest variable importance indicators for the full model (all variables) predicting 864 mm northern pike Hg concentrations from thirteen Colorado Reservoirs. Variables are listed top to bottom in order of relative predictive value from most to least, respectively. Abbreviations for the various predictive covariates are provided in Table 1.

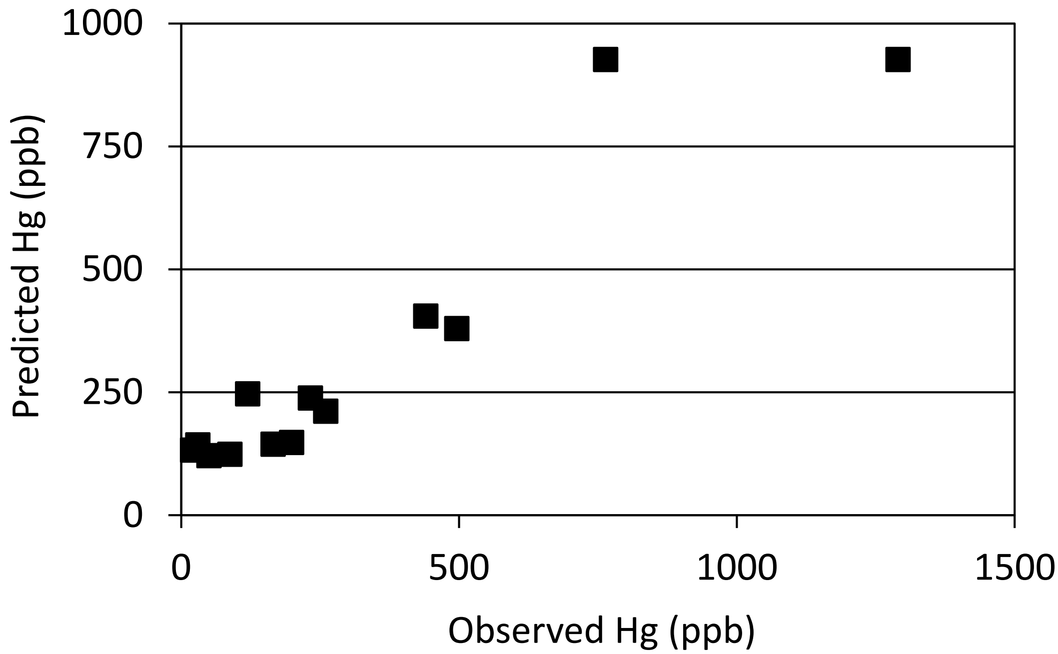

Figure 2. Predicted Hg concentrations of 864 mm northern pike (*Esox lucius*). Estimated northern pike Hg concentrations from thirteen Colorado reservoirs are plotted as a function of their Hg concentrations from empirical data regressions. The reduced model (the three salmonid stocking metrics only) was used in this analysis.

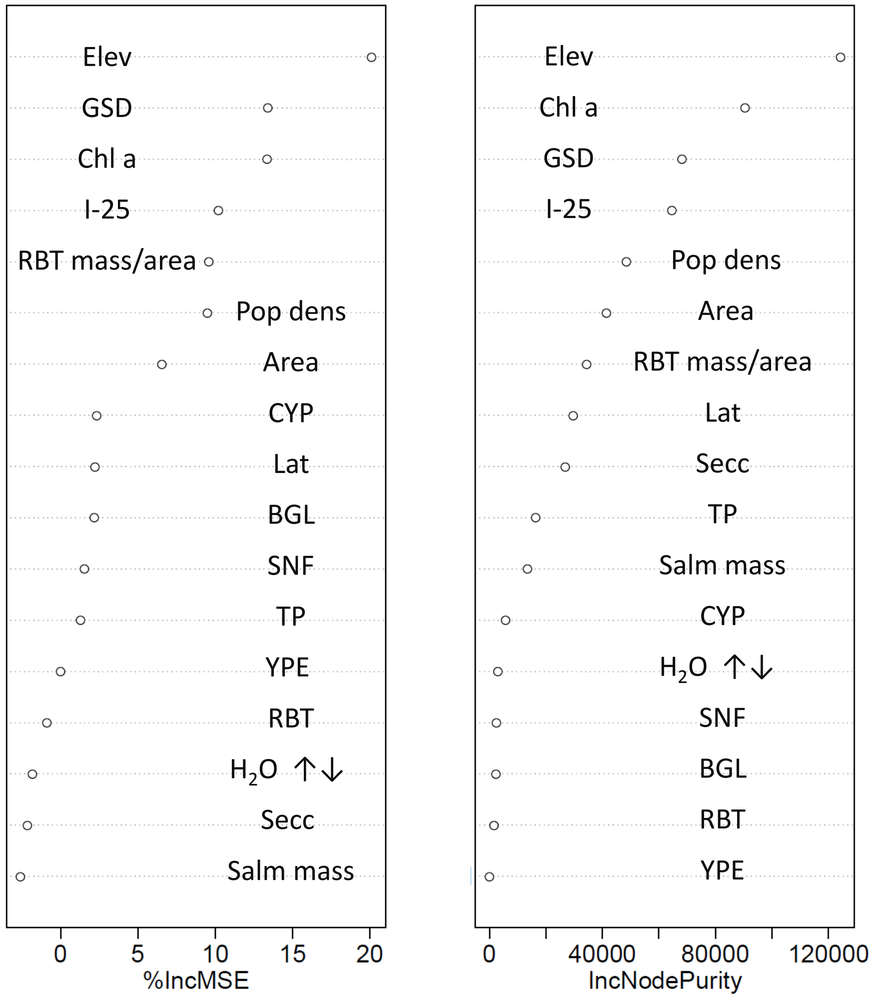

Figure 3. Random forest variable importance indicators for the full model (all variables) predicting 381 mm smallmouth bass (*Micropterus dolomieu*) Hg concentrations from fifteen Colorado Reservoirs. Variables are listed top to bottom in order of relative predictive value from most to least, respectively. Abbreviations for the various predictive covariates are provided in Table 1.

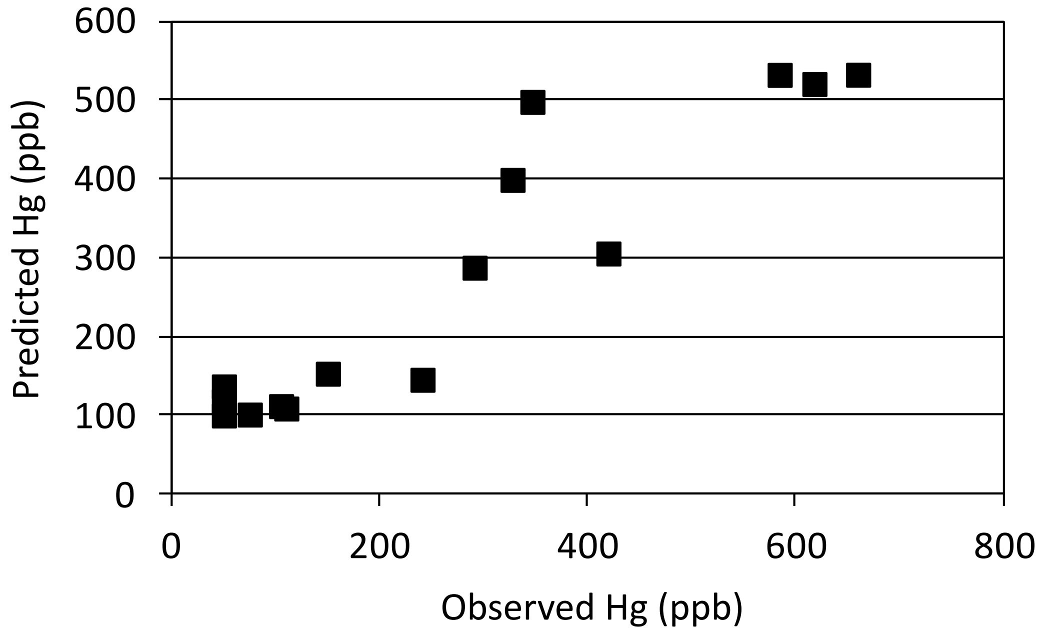

Figure 4. Predicted Hg concentrations of 381 mm smallmouth bass (*Micropterus dolomieu*). Estimated smallmouth bass Hg concentrations from fifteen Colorado reservoirs are plotted as a function of their Hg concentrations from empirical data regressions. The reduced RF model (Elev, GSD, and Chl a) was used in this analysis.

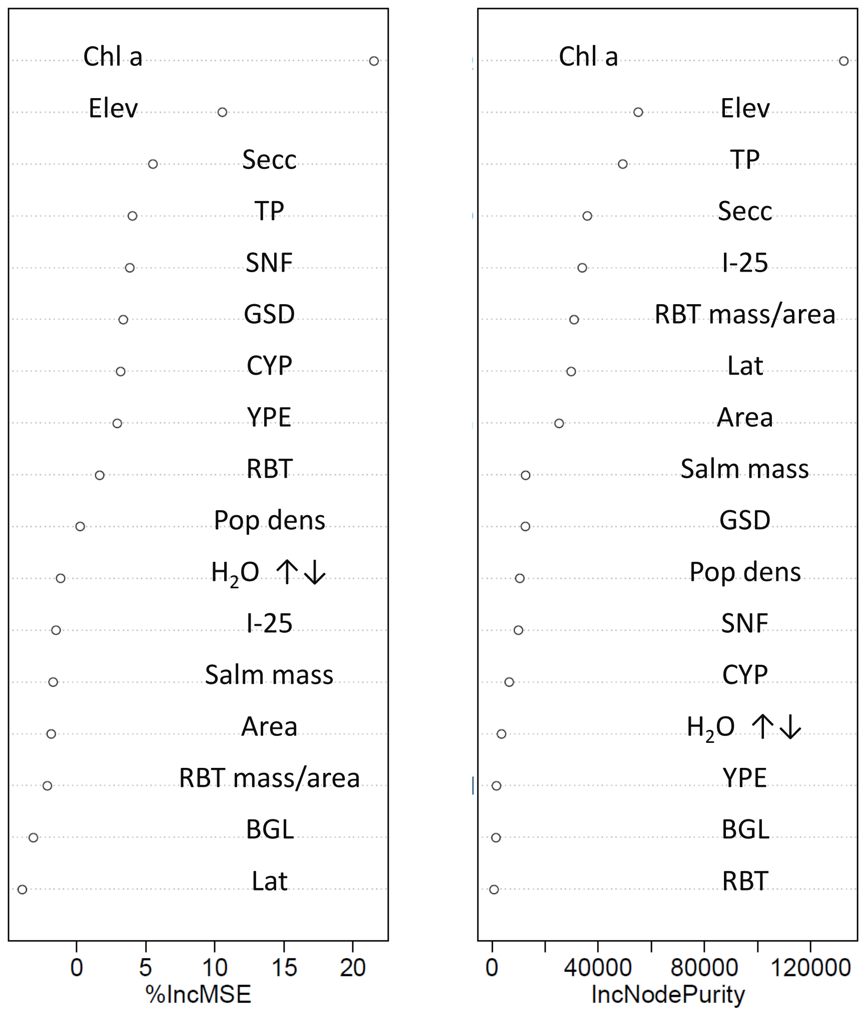

Figure 5. Random forest variable importance indicators for the full model (all variables) predicting 381 mm walleye (*Sander vitreus*) Hg concentrations from 26 Colorado Reservoirs. Variables are listed top to bottom in order of relative predictive value from most to least, respectively. Abbreviations for the various predictive covariates are provided in Table 1.

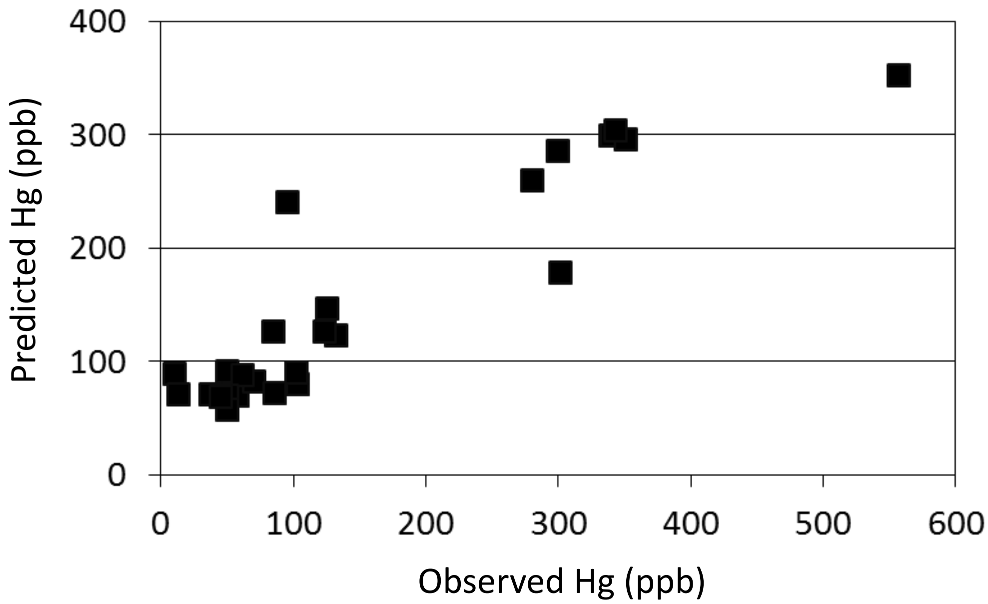

Figure 6. Predicted Hg concentrations of 381 mm walleye (*Sander vitreus*). Estimated walleye Hg concentrations from 26 Colorado reservoirs are plotted as a function of their Hg concentrations from empirical data regressions. The reduced model (Chl a, Elev, Secc, and TP) was used in this analysis.

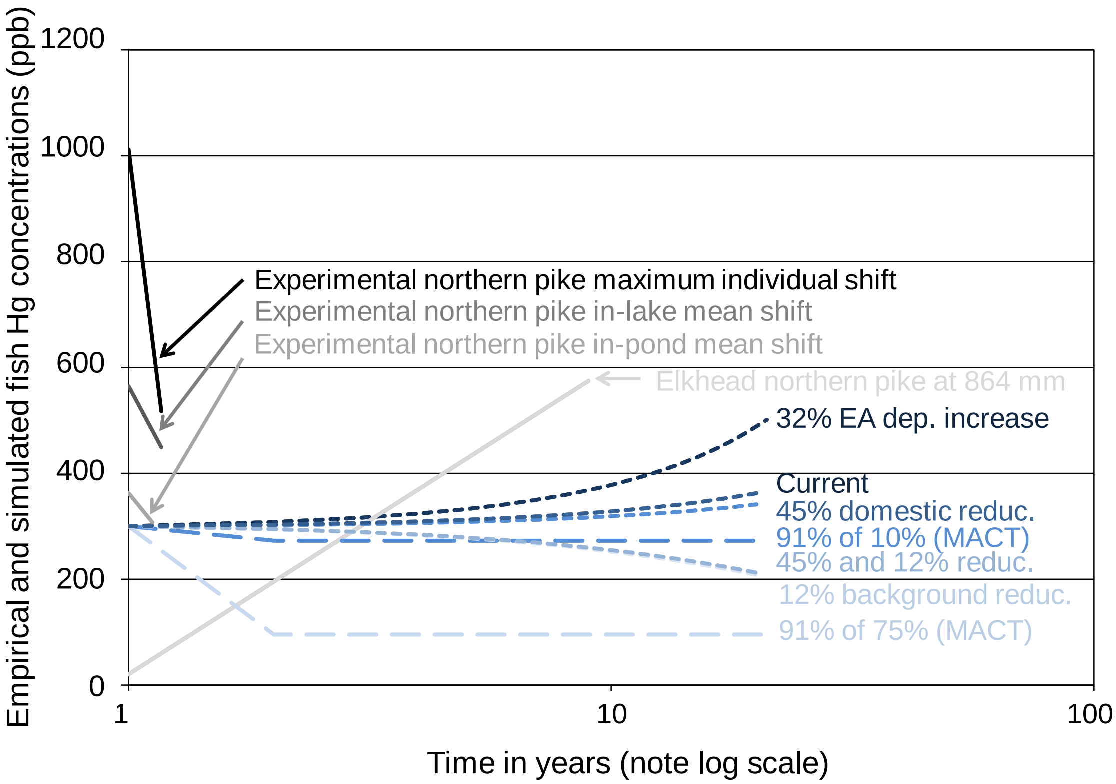

Figure 7. Empirical and estimated sport fish Hg concentration responses to food web shifts and changes in Hg deposition. Label coloration matches the coloration of the corresponding data. Empirical experimental results from before and after northern pike were provided stocked rainbow trout as forage by Lepak et al. (2012) are represented by solid grayscale lines ending with arrows. Included are the largest Hg shift observed in an individual northern pike (Experimental pike maximum individual shift), the mean shift in northern pike Hg in the lake (Experimental pike in-lake mean shift), and the mean shift in northern pike Hg in the ponds (Experimental pike in-pond mean shift). Elkhead northern pike (at 864 mm) Hg concentrations before and after the cessation of rainbow trout stocking (Elkhead pike at 864 mm) are shown with a solid grayscale line without arrows. Short dashed blue lines represent five different volume-weighted wet deposition scenarios in the West modeled by Zhang and Jaeglé (2013); a 32% increase East Asian Hg emissions (32% EA dep increase), current conditions in the West and Buffalo Pass specifically (Current), a 45% reduction in domestic Hg emissions (45% domestic reduc.), a 45% reduction in domestic Hg emissions and a 12% reduction in background atmospheric Hg (45% and 12% reduc.), and a 12% reduction in background atmospheric Hg. Long dashed lines represent a 91% reduction (from EPA maximum attainable control technology) in the estimated 10% of wet and dry Hg emissions coming from within the borders of the southwest region (91% of 10% (MACT), and a 91% reduction (from EPA maximum attainable control technology) in the estimated 75% of wet and dry Hg emissions possible from local Hg sources (91% of 75% (MACT) (Lin et al. 2012).

Credit Author Statement:

Author contributions include: Lepak (conceptualization, investigation, writing original draft, funding acquisition), Johnson (conceptualization, review and editing, funding acquisition, resources), Wolff (Data curation, project administration, review and editing), Hooten (conceptualization, formal analysis, review and editing), Hansen (resources, review and editing).

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: