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Two Ocean Pass: An Alternative Hypothesis for the Invasion of Yellowstone Lake by Lake Trout, and Implications for Future Invasions

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Abstract: Preventing the interbasin transfer of aquatic invasive species is a high priority for natural resource managers. Such transfers can be made by humans or can occur by dispersal through connected waterways. A natural surface water connection between the Atlantic and Pacific drainages in North America exists at Two Ocean Pass south of Yellowstone National Park. Yellowstone cutthroat trout *Oncorhynchus clarkii bouvieri* used this route to cross the Continental Divide and colonize the Yellowstone River from ancestral sources in the Snake River following glacial recession 14,000 bp. Nonnative lake trout *Salvelinus namaycush* were stocked into lakes in the Snake River headwaters in 1890 and quickly dispersed downstream. Lake trout were discovered in Yellowstone Lake in 1994 and were assumed to have been illegally introduced. Recently, lake trout have demonstrated their ability to move widely through river systems and invade headwater lakes in Glacier National Park. Our objective was to determine if lake trout and other nonnative fish were present in the connected waters near Two Ocean Pass and could thereby colonize the Yellowstone River basin in the past or future. We used environmental DNA (eDNA), electrofishing, and angling to survey for lake trout and other fishes. Yellowstone cutthroat trout were detected at nearly all sites on both sides of the Continental Divide. Lake trout and invasive brook trout *S. fontinalis* were detected in Pacific Creek near its confluence with the Snake River. We conclude that invasive movements by lake trout from the Snake River over Two Ocean Pass may have resulted in their colonization of Yellowstone Lake. Moreover, Yellowstone Lake may be vulnerable to additional invasions because several other nonnative fish inhabit the upper Snake River. In the future, eDNA collected across smaller spatial intervals in Pacific Creek during flow conditions more conducive to lake trout movement may provide further insight into the extent of non-native fish invasions in this stream.

Keywords: cutthroat trout; environmental DNA; invasion risk; nonnative fish; *Salvelinus*; Snake River; national park; wilderness pathway

1. Introduction

Connectivity among drainage basins is especially challenging for managers attempting to prevent dissemination of harmful aquatic invasive species [1,2] and pathogens [3,4] in the United States and elsewhere [5,6]. The number of large infrastructure projects involving the transfer of water from basins considered to have surplus water to those where demand exceeds availability has grown worldwide [7]. Although most interbasin water connections in the United States are anthropogenic, some occur naturally. For example, Eagle Marsh in Indiana is a flood-prone aquatic pathway connecting the

Great Lakes Basin to the Mississippi River; aggressive actions are being taken there to prevent passage by Asian carp (e.g., *Hypophthalmichthys molitrix*) and other invasive species [8]. A similar natural hydrological connection exists along the Minnesota–South Dakota border where the headwaters of the Minnesota River join with those of the Red River of the North in a wetland, connecting the Mississippi and Hudson Bay drainages during periods of high water. This connection is a remnant of the River Warren, which drained glacial Lake Agassiz, carved out the Minnesota River valley, and allowed post-glacial dispersal of fishes northward [9,10]. The additional movement of aquatic species from the upper Mississippi River drainage to the Hudson Bay drainage (United States to Canada) via the Red River of the North has long been a concern for managers [11,12].

The hydrologic oddity known as Two Ocean Pass connects the headwaters of the Atlantic and Pacific drainages in the Bridger-Teton Wilderness of Wyoming south of Yellowstone National Park [13,14]. Here, a broad alpine meadow straddles the Continental Divide at 2478 m elevation and the headwaters of the Columbia and Missouri drainages originate from a single perennial stream; North Two Ocean Creek flows along the Continental Divide and branches into Atlantic Creek, a Yellowstone River tributary flowing to the east, and Pacific Creek, a Snake River tributary flowing to the west (Figure 1, Supplementary Materials: Video S1) [14,15]. No barrier prevents the movement and mixing of fish between Pacific Creek and Atlantic Creek (Figures 2 and A1). Following glacial recession from the region about 14,000 years ago [16], ancestral Yellowstone cutthroat trout *Oncorhynchus clarkii bouvieri* apparently colonized the Yellowstone River drainage from sources in the lower Snake River drainage over Two Ocean Pass [17,18]. They dispersed downstream and were the only trout inhabiting Yellowstone Lake for thousands of years prior to the establishment of Yellowstone National Park. There, they remained isolated and protected, despite the existence of Two Ocean Pass. The watershed of the Yellowstone River upstream of Yellowstone Lake, including Two Ocean Pass, is among the most remote in the contiguous United States and lies largely within protected federal wilderness (Figure A2). Yellowstone Lake is located at the center of the Greater Yellowstone Ecosystem within Yellowstone National Park. The Lower Falls of the Yellowstone River (Figure 1) provide protection from invasion by nonindigenous aquatic species downstream of the lake.

The biogeographic isolation of aquatic systems *sensu* [19] in the Greater Yellowstone Ecosystem began to erode after Yellowstone National Park was established in 1872. Nearly 50% of the surface waters of Yellowstone National Park were originally fishless because natural waterfalls and other features prevented their colonization following glacial recession [20]. The U.S. Commission on Fish and Fisheries sought to stock fish into many of these waters and among the first fish introduced were nonnative lake trout *Salvelinus namaycush* and exotic brown trout *Salmo trutta* into Lewis and Shoshone lakes in the upper Snake River drainage in 1890 [21] (Figure 1). Brook trout *Salvelinus fontinalis* were later stocked into a tributary of Shoshone Lake in 1893. Populations became established and dispersed downstream in the Lewis and Snake rivers.

The invasion of Yellowstone Lake by lake trout is one of the most deleterious results of the reduced isolation of the Greater Yellowstone Ecosystem. Lake trout were first verified in Yellowstone Lake in 1994 after an individual was caught by an angler [22]. The species was assumed to have been introduced illegally because natural movement of lake trout into Yellowstone Lake from other park waters was not thought possible at that time [23]. A lake trout catch-and-kill regulation was implemented immediately. However, subsequent gillnetting confirmed that thousands of lake trout, and perhaps over 10,000, were present in Yellowstone Lake [24]. Following significant input from scientists and managers, a gillnetting program was initiated to suppress the highly piscivorous lake trout to conserve Yellowstone cutthroat trout [23]. Although slowed by the gillnetting program [25], lake trout population growth and expansion continued, resulting in a precipitous, lake-wide decline in Yellowstone cutthroat trout [26] with cascading effects that extended to predatory birds, such as bald eagles *Haliaeetus leucocephalus* and ospreys *Pandion haliaetus*, and land-dwelling animals, such as grizzly bears *Ursus arctos*, black bears *U. americanus*, and river otters *Lutra canadensis* [27]. The lake trout suppression gillnetting effort was augmented significantly by incorporating private-sector contracted gillnetters in 2009 [28]. Overall, more than 3.35 million lake trout were killed by gillnetting during

1995–2019 [29]. Yellowstone National Park and several partners maintain the large-scale lake trout suppression program in Yellowstone Lake (Figure A3).

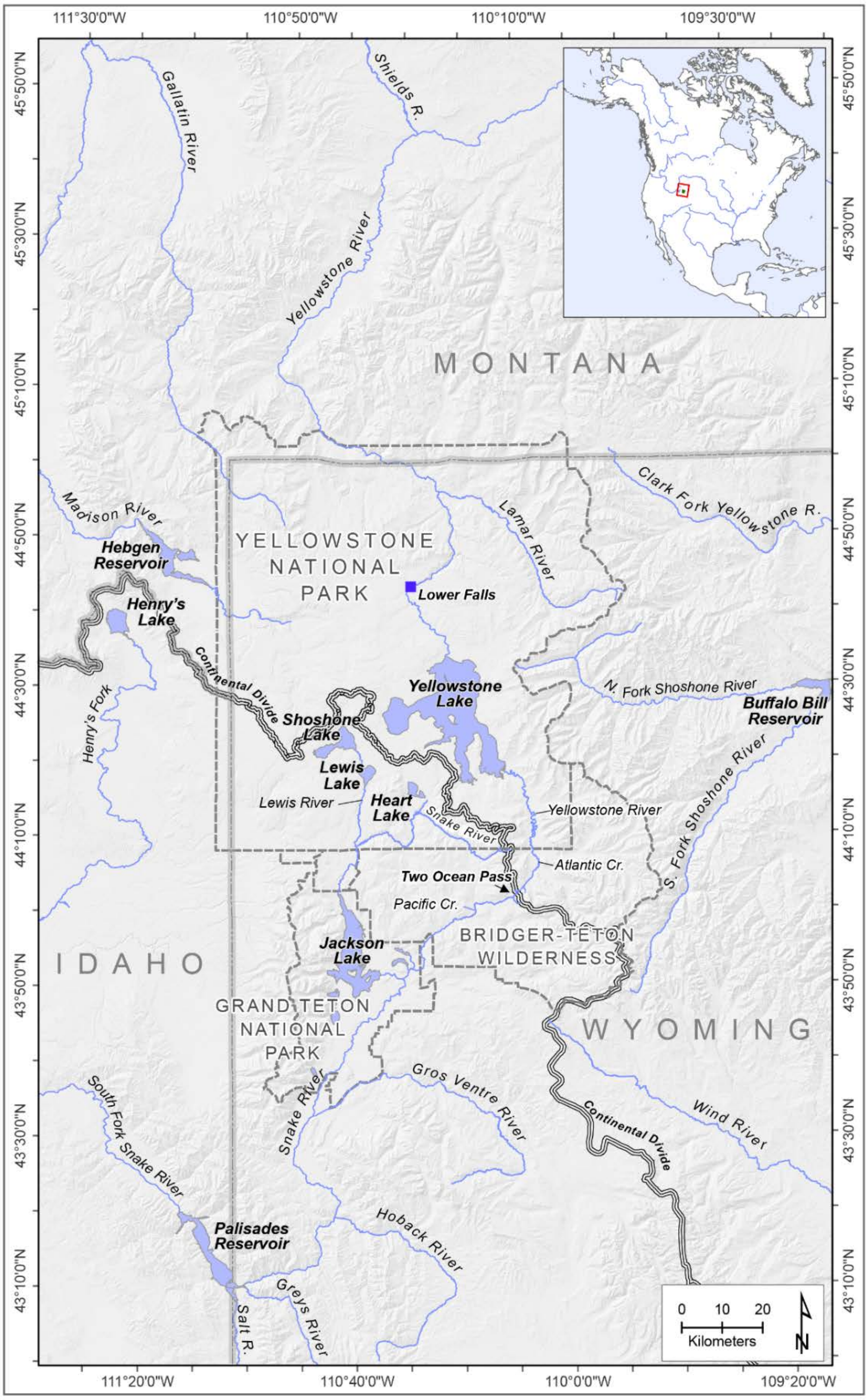

Figure 1. Headwaters of the Atlantic and Pacific drainages in Idaho, Montana, and Wyoming, including the upper Snake and Yellowstone rivers in Yellowstone National Park, Grand Teton National Park, and the Bridger-Teton Wilderness where surface waters of both systems naturally join on the Continental Divide at Two Ocean Pass.

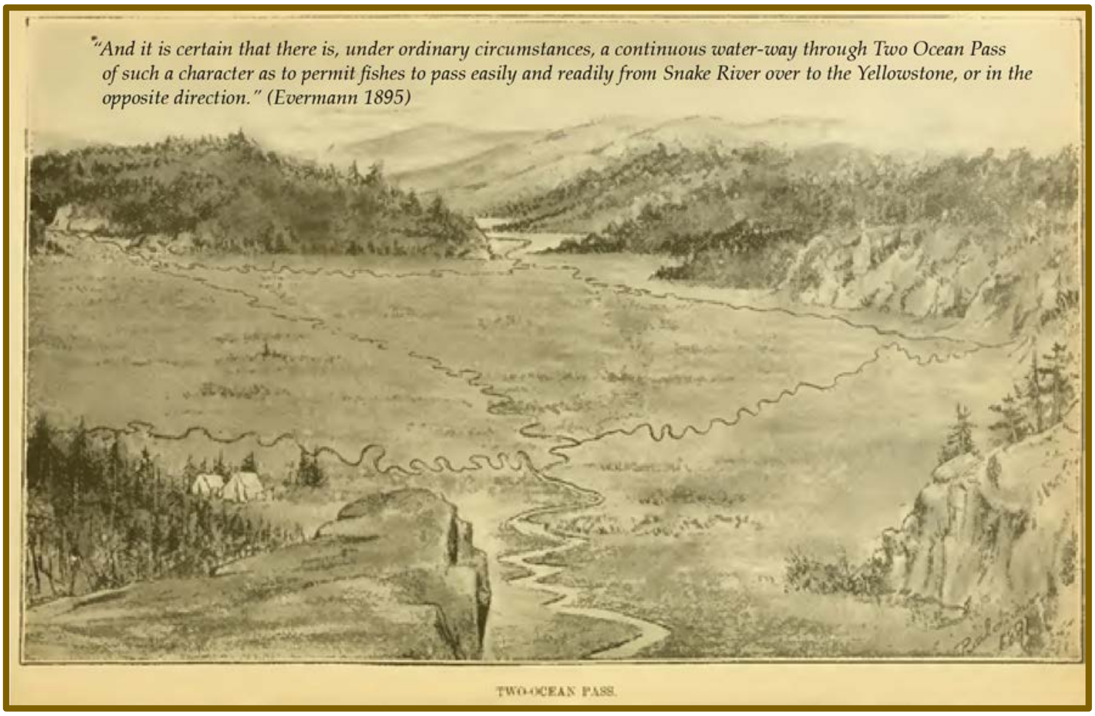

Figure 2. Sketch of Two Ocean Pass with a view to the northeast in 1891, recreated from [14,15]. Atlantic Creek is shown exiting the pass between the hills in the upper part of the image. Pacific Creek exits to the southwest in the lower part of the image. North Two Ocean Creek enters from the left side of the image and divides into its two distributaries. South Two Ocean Creek enters from the right of the image and apparently also divided into two distinct streams at that time. The pass is a nearly level meadow near the center, which is a marsh that becomes a small lake in times of wet weather or snowmelt runoff [14,15].

Following intentional introduction to Lewis and Shoshone lakes by the U.S. Fish Commission in 1890, invasive lake trout dispersed through the Lewis and Snake rivers and colonized Heart Lake in Yellowstone National Park and Jackson Lake in Grand Teton National Park [21] (Figures 3 and 4A–C). The colonization of Heart Lake required upstream dispersal via the Snake River followed by the Heart River, a small, montane outlet stream. Anglers caught lake trout weighing up to 7 kg in Jackson Lake during the early 1900s [30]. In addition to colonization from upstream and natural reproduction in the lake, Jackson Lake was also heavily stocked with hatchery fish. More than 7 million lake trout were stocked into Jackson Lake from 1937 to 2006 to enhance the sport fishery [30]. Lake trout passed over the Jackson Lake Dam, where a popular sport fishery exists in the tailwaters [31], (Figure 4D) and dispersed downstream via the Snake River to populate Palisades Reservoir after its creation in 1957 (Figure 1). Although the stocking of Jackson Lake with lake trout was discontinued in 2006, angling regulations protect them during the autumn spawning period [32,33], and lake trout continue to disperse in the Snake River as far downstream as American Falls Reservoir in southeast Idaho, especially after spring run-off [34–36]. The threat of nonnative fish prompted the National Park Service to emphasize the need for protection of native cutthroat trout in the Snake River downstream of Jackson Lake Dam [33].

Given the demonstrated ability of lake trout to move widely through interconnected streams, rivers, and lakes, we hypothesize that lake trout became established in Yellowstone Lake by swimming there of their own volition, invading from the Snake River over Two Ocean Pass much like ancestral Yellowstone cutthroat trout. Lake trout commonly traverse similar distances and gradients in the Flathead River system [37]. Connected waters at Two Ocean Pass may therefore allow lake trout invasion of the upper Yellowstone River system, especially following spring runoff when waters are cold and the Two Ocean Pass plateau is flooded (Figure A4), broadly connecting the Pacific and Atlantic drainages.

We contend that the assumption that lake trout were introduced illegally into Yellowstone Lake may be incorrect. We propose instead that decreased biogeographic isolation of the Greater Yellowstone Ecosystem allowed lake trout to move through Two Ocean Pass into the upper Yellowstone River drainage and invade Yellowstone Lake. Recent characterizations of the movement and colonization patterns of lake trout in montane ecosystems provide a basis for this hypothesis. Moreover, we contend that potential exists for additional invasions of Yellowstone Lake and connecting streams by nonindigenous aquatic species via this route. Our objective was to determine if lake trout and other nonnative fish were present in the connected waters near Two Ocean Pass and could thereby colonize the Yellowstone River basin in the past or future.

2. Materials and Methods

2.1. Study Area

Rivers and streams were chosen for sampling based on connectivity and proximity to North Two Ocean Creek. The study area extended from the Jackson Lake Dam to Yellowstone Lake (Figure 3). Pacific Creek joins the Snake River just 9 km downstream of Jackson Lake Dam and is an open system, has a relatively low gradient (7 m km^{-1}), and poses no physical barrier to upstream movement by lake trout or other nonnative fishes (Figure 4E–G). Pacific and Atlantic creeks diverge at Two Ocean Pass 62 km from Jackson Lake (Figure 4H). The distance down Atlantic Creek from Two Ocean Pass to Yellowstone Lake (via the upper Yellowstone River) is 68 km (Figure 4I–L). The total distance from the Jackson Lake Dam to Yellowstone Lake is 130 km.

2.2. Sample Collection and Analysis

Fishes in rivers and streams of our study area have historically been sampled (intermittently) by biologists using electrofishing gear, angling, or surveys of the angling public [31,38]. We used environmental DNA (eDNA) [39,40] as a primary tool to infer fish species presence by detecting its genetic material in water samples. Environmental DNA sampling provides a viable alternative to electrofishing or netting for determining species occupancy [41–43]. The use of eDNA is an efficient, non-invasive approach for the broad-scale detection of (potentially) rare or invasive fish species [44–46] in large rivers and headwater streams such as those near Two Ocean Pass. A total of 10 eDNA sampling sites were located on the Snake River (downstream of the Jackson Lake Dam), Pacific Creek, North Two Ocean Creek, Atlantic Creek, and the upper Yellowstone River (Sites 1–6 and 8–11; Figure 3). An additional site (Site 7) was used to obtain an eDNA control sample from a fishless reach upstream of a large impassible waterfall on North Two Ocean Creek. The eDNA samples were collected using established protocols [47] during 6–11 July 2019 using individual sterile sample kits and a peristaltic pump (Geotech Environmental Equipment, Inc) to filter about 5 L of water through a 1.5-µm glass microfiber filter (Whatman®1827-047 Glass Microfiber Binder-Free Filter) for each sample. The eDNA samples were extracted and then analyzed in triplicate using quantitative polymerase chain reaction (qPCR) to detect DNA from the target species. All samples were analyzed using established protocols, including the use of a negative control on each PCR plate, at the National Genomics Center, United States Forest Service, Rocky Mountain Research Station in Missoula, Montana, USA [44]. The amplification was done by a single-species approach using specific primers [48] for each, including cutthroat trout, brook trout, brown trout, lake trout, and rainbow trout *O. mykiss*, a harmful nonnative that occurs in the Snake River system downstream of Jackson Lake Dam [49]. We assumed presence based on minimum genetic evidence. A sample was considered positive if at least one reaction in the triplicate was positive for the target eDNA after 45 PCR cycles [50]. With the exception of lake trout, samples were not run with a standard curve [48,51].

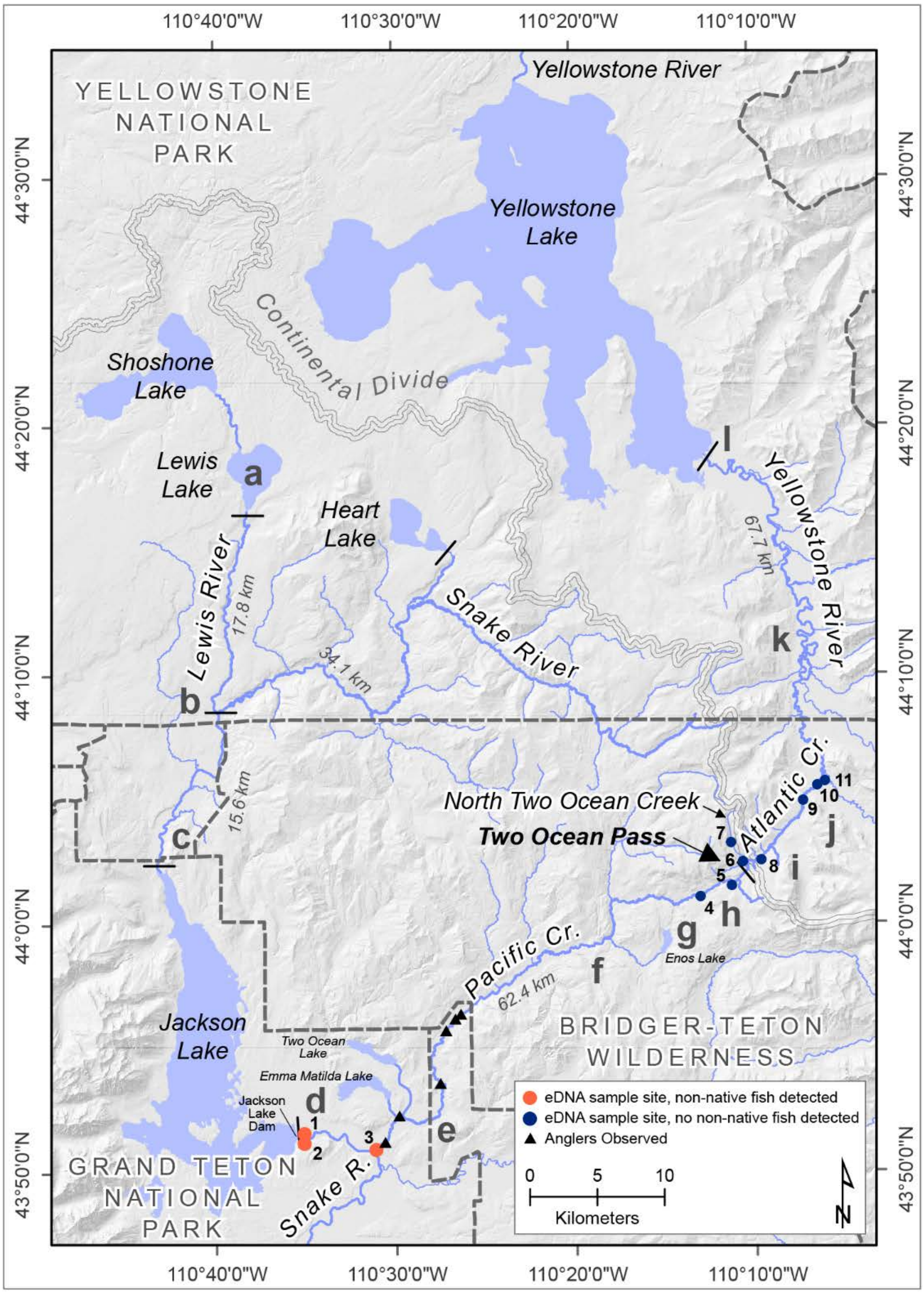

Figure 3. Headwaters of the Atlantic and Pacific drainages where they unite on Two Ocean Pass within the Bridger-Teton Wilderness, Wyoming. North Two Ocean Creek flows down the Continental Divide and branches into Atlantic Creek, a Yellowstone River tributary flowing to the east, and Pacific Creek, a Snake River tributary flowing to the west. No barriers to the movement and mixing of fish between Atlantic and Pacific creeks exist. The distances (km) are river lengths between hash marks (—). The letters (**a**–**l**) are locations of aerial photographs shown in Figure 4.

To validate the results of the eDNA survey, we electrofished 205- and 437-m reaches using a backpack electrofishing unit (Smith-Root, Inc., Vancouver, WA, USA, Model LR-24) at sites 4 and 5, respectively, on upper Pacific Creek near Two Ocean Pass. Angling was used on the Snake River, upper and lower Atlantic Creek, and the Yellowstone River as an additional validation method for fish because those waters were too large to be effectively sampled with a backpack electrofishing unit.

In addition, on 8 and 11 July 2019, aerial surveys for anglers were conducted by helicopter to record the GPS locations and number of anglers throughout the study area, including waters upstream of eDNA sampling sites. The aerial surveys for anglers were conducted to assess the potential for contamination that could confound eDNA results, because PCR screening could potentially detect eDNA shed from waders or other angling gear after previous use elsewhere in waters containing nonnative fish.

3. Results

Historical fishery investigations have repeatedly documented the presence and dominance of native Yellowstone cutthroat trout in the rivers and streams of our study area on both sides of the Continental Divide. In contrast, nonnative brown trout, lake trout, and rainbow trout have only been found previously in the Snake River (Figure 1). Brook trout have only been found in our study area in the Snake River and in the Two Ocean Lake outlet stream, a small tributary of lower Pacific Creek near its confluence with the Snake River (Figure 3) Environmental DNA was not detected in the negative control sample obtained from a fishless headwater reach of North Two Ocean Creek on the Continental Divide (Site 7; Figure 3; Table 1). Negative controls used during analysis did not amplify, indicating little possibility of contamination in the field or laboratory. Yellowstone cutthroat trout DNA was detected at all sites in our study area except Site 4 on Pacific Creek and Site 6 on North Two Ocean Creek. Electrofishing at Site 4 on Pacific Creek also did not detect fish presence. Yellowstone cutthroat trout were sampled by electrofishing at the uppermost site on Pacific Creek (Site 5) and by angling on Atlantic Creek and the upper Yellowstone River (Sites 8–11), further confirming eDNA results. North Two Ocean Creek was not sampled by methods other than eDNA because of logistical constraints in the backcountry.

Table 1. Presence of environmental DNA (eDNA) for nonnative fish, including lake trout (LKT), brown trout (BRN), brook trout (BKT), rainbow trout (RBT), and native Yellowstone cutthroat trout (YCT) in streams near Two Ocean Pass. The site numbers refer to locations on Figure 3. The number in each species column represents the number of PCR wells out of 3 that tested positive for each sample. The numbers in red and green indicate sites where nonnatives and native cutthroat trout were detected, respectively. Lake trout samples were run with a standard curve, generating a concentration in DNA copies per liter for Site 1 (67.92 copies/L), Site 2 (209.83 copies/L) and Site 3 (2.05 copies/L).

Stream	Site	LKT	BRT	BKT	RBT	YCT
Snake River	1	**3/3**	**2/3**	0/3	0/3	**3/3**
	2	**3/3**	**3/3**	0/3	0/3	**3/3**
Pacific Creek	3	**2/3**	0/3	**3/3**	0/3	**3/3**
	4	0/3	0/3	0/3	0/3	0/3
	5	0/3	0/3	0/3	0/3	**3/3**
North Two Ocean Creek	6	0/3	0/3	0/3	0/3	0/3
	7 [1]	0/3	0/3	0/3	0/3	0/3
Atlantic Creek	8	0/3	0/3	0/3	0/3	**3/3**
	9	0/3	0/3	0/3	0/3	**1/3**
	10	0/3	0/3	0/3	0/3	**1/3**
Yellowstone River	11	0/3	0/3	0/3	0/3	**1/3**
	Mean [2]	0.89	0.83	1.00	N/A	0.75

[1] Site 7 was used to obtain an eDNA negative control sample from a fishless reach above a waterfall on North Two Ocean Creek. [2] The mean number of PCR wells out of 3 for samples that tested positive.

Figure 4. Connected waters of the upper Snake and Yellowstone River systems in Yellowstone National Park, Grand Teton National Park, and the Bridger-Teton Wilderness in northwestern Wyoming, including (**A**) Lewis Lake; (**B**) the confluence of the Lewis and Snake rivers; (**C**) Snake River confluence with Jackson Lake; (**D**) Jackson Lake and dam; (**E**) lower Pacific Creek; (**F**) middle Pacific Creek; (**G**) upper Pacific Creek; (**H**) Two Ocean Pass and the confluence of North Two Ocean Creek with Pacific and Atlantic creeks; (**I**) middle Atlantic Creek; (**J**) the confluence of Atlantic Creek and the Yellowstone River; (**K**) Yellowstone River upstream of Yellowstone Lake; and (**L**) Yellowstone River confluence with Yellowstone Lake.

Nonnative lake trout and brown trout DNA were detected in the Snake River downstream of the Jackson Lake Dam (Sites 1 and 2; Figure 3; Table 1). Angling also documented the presence of lake trout in the Snake River as one was caught immediately upstream while the eDNA water sample was being filtered. Lake trout and brook trout DNA were detected at the lowermost site on Pacific Creek (Site 3), a stream that biologists have not documented these species from previously. Professional outfitters, however, have provided anecdotal information regarding lake trout catches by angling on middle Pacific Creek [52]. Brook trout have been found previously by electrofishing in the Two Ocean Lake outlet, which joins Pacific Creek upstream of Site 3 (Figure 3). Aerial surveys conducted by helicopter documented the presence of anglers at six locations on Pacific Creek (Figure 3). Anglers were not observed anywhere else in the study area.

4. Discussion

Surface waters of the Atlantic and Pacific drainages in North America join on the Continental Divide at Two Ocean Pass. Environmental DNA of native Yellowstone cutthroat trout, a fish that used this pathway to naturally access the Yellowstone River from ancestral sources in the lower Snake River, was detected at nearly all sites sampled on both sides of the divide. Invasive lake trout, which are the most abundant fish in all large lakes of the upper Snake River, were detected by eDNA in the Snake River downstream of the Jackson Lake Dam and in Pacific Creek near its confluence with the Snake River. Lake trout presence in the Snake River was also confirmed by angling. The likely source of lake trout in these fluvial habitats is Jackson Lake, which supports a popular sport fishery for this species. We did not detect lake trout DNA in upper Pacific Creek or other waters of our study area east of the divide, possibly because our sampling occurred during July, a period of low stream discharges and high water temperatures not conducive to lake trout movement [37,53]. Other nonnative fishes, including brown trout and brook trout, were detected in the Snake River and Pacific Creek, respectively. Although these species are common in many waters of the upper Snake River, the detection of brook trout in the Pacific Creek mainstem is cause for serious concern given their proven ability to outcompete and displace native cutthroat trout throughout the Intermountain West.

Detections of eDNA may occur without the target species being present. For example, a false-positive signal may be obtained when the sample does not contain any DNA but PCR amplification inadvertently occurs in the laboratory [54]. Because our negative controls failed to detect DNA from any species, little possibility exists that contamination in the laboratory resulted in a false-positive detection from our samples. Additionally, samples may contain DNA without the target species being present because of the activities of anglers, bears, or other wildlife upstream of the sampling site. Anglers observed on Pacific Creek during aerial surveys may have contaminated waters with uncleaned gear resulting in eDNA detections. The detectability of eDNA in streams is affected by proximity to target individual(s) [55], stream flow rates [56], water temperature [57], seasonal movement [58], other environmental conditions, and time after the removal of the species source [59]. Signals of past site occupancy, however, are long-lasting in sedimentary eDNA and its resuspension by anglers or wildlife could result in its detection [60]. Angler- or wildlife-caused DNA detections would be additional signs of the close proximity of invasive lake trout and brook trout to Two Ocean Pass. Additional samples collected across smaller spatial intervals on Pacific Creek during flow conditions conducive to lake trout movement may provide further insight into the extent of non-native fish invasions in this stream [61,62].

The origin of the lake trout in Yellowstone Lake remains uncertain. Lake trout were present in low densities in Yellowstone Lake for an unknown number of years prior to being verified in 1994 [22]. Experienced anglers reported catching them many years earlier. The otolith microchemistry of several large lake trout netted during 1996–1997 suggested that fish were introduced into Yellowstone Lake during 1989 and 1996 (two years after their discovery by an angler) [63]. However, others had spent their entire lives in Yellowstone Lake, raising questions about their parental origin and abundance. Although genetic analyses identified Lewis Lake as a probable source, a significant number of fish appeared to be from another unidentified source, possibly one that had been colonized by fish dispersing from Lewis Lake [64]. The otolith microchemistry and genetics studies did not examine Jackson Lake fish or consider it as a potential source. Although some lake trout may indeed have been illegally introduced in the 1980s, opportunistic invasions by lake trout over Two Ocean Pass may have also been occurring throughout the 20th century. The original assumption that lake trout were introduced illegally was based on the belief that they could not have moved into Yellowstone Lake from other park waters on their own [23]. We now know that assumption was incorrect.

4.1. Invasive Tendencies of Lake Trout in the Western United States

Lake trout have successfully populated western North American lakes and reservoirs outside of their native range because they are adapted to similar deep, cold oligotrophic lakes of Canada and the northeastern United States [65]. They have been widely introduced and now occur in over 200 waters in the western United States following intentional or illegal introductions or invasive movements among lakes via river networks [32]. In Montana, lake trout from the Great Lakes were stocked in 1905 into Flathead Lake [66], the largest lake in the western United States. Lake trout abundance in Flathead Lake remained relatively low until the 1980s, but then increased rapidly when opossum shrimp *Mysis diluviana* invaded the lake and caused abrupt, cascading changes to the food web [66,67]. Following rapid population growth and expansion within Flathead Lake, lake trout's use of the Flathead River system upstream of the lake greatly increased. Lake trout dispersed throughout stream networks of the upper Flathead River basin and invaded headwater lakes in Glacier National Park, causing reductions in native bull trout *S. confluentus* populations [68,69]. Lake trout progressively invaded Lake McDonald (in 1959), Kintla and Bowman lakes (1962), Logging Lake (1984), Harrison Lake (2000), lower Quartz Lake (2003), and Quartz and Rogers lakes (2005) [70,71]. Lake trout invaded Swan Lake and its headwaters in 1998 [72].

Distances moved and gradients ascended by lake trout in the Flathead River and its tributaries as they invaded headwater lakes were extensive. Telemetered lake trout used rivers during autumn, winter, and spring when water temperatures were cool, and avoided rivers during the warm summer months [37]. Upstream movements occurred primarily in October, concurrent with prey fish migrations. Although the gradient of the Flathead River is relatively low (2 m km^{-1}), the gradients of tributaries that lake trout ascended were high (15–23 m km^{-1}). Radio-tagged lake trout moved up to 230 km throughout the Flathead River system [37]. The colonization and proliferation upstream of Flathead Lake is ongoing [73]. Lake trout are typically considered a deep lake dwelling species. Before their extinction in the 1950s, however, river spawning populations capable of ascending steep rapids (such as in the Dog and Montreal rivers, Ontario) used the tributaries of Lake Superior along the Canadian shoreline [74,75]. In the extreme northern part of their native range, lake trout also occupy rivers and shallow lakes [76]. The substitution of altitude for latitude in montane systems may further allow lake trout to traverse small, high gradient lake outlets to invade high-elevation lakes.

4.2. Future Threat of Yellowstone Lake Invasion by Nonnative Fish

Although native Yellowstone cutthroat trout are the most common trout in the upper Snake River [77], nonnative brown, brook, and rainbow trout, and rainbow × cutthroat trout hybrids also occur in the system. In Yellowstone National Park, brook and brown trout are abundant in Lewis and Shoshone lakes and their tributaries and are common in the Lewis and Snake rivers [78]. Brook, brown, and rainbow trout and rainbow × cutthroat trout hybrids occur downstream of Jackson Lake in the Snake River and large tributaries, including the Gros Ventre, Hoback, Greys, and Salt rivers [49] (Figure 1). Levels of hybridization are highest in the Gros Ventre River, which is a source of rainbow trout genes in the upper Snake River [49]. In addition to nonnative fish, the Utah chub *Gila atraria* is a common native species in the upper Snake River drainage [79] that does not occur in the Yellowstone River drainage. Utah chub are abundant in Heart, Lewis, and Jackson lakes of the upper Snake River, and they also occur in Emma Matilda, Enos, and Two Ocean lakes within the Pacific Creek watershed (Figure 3). The elevation of Enos Lake is 2390 m and the distance to Two Ocean Pass (+ 88 m elevation) is 26 km (Figure A5). The presence of Utah chub and multiple other nonnative fish species in the Snake River system raises concern that these species could also eventually invade Yellowstone Lake via Two Ocean Pass and establish reproducing populations. Limited fish sampling in the remote and expansive upper Yellowstone River [38] precludes knowledge of the degree to which these nonnatives may have already sporadically accessed the basin.

Nonnative fish introductions are a primary cause of extirpations of Yellowstone cutthroat trout populations throughout their native range [18]. Most remaining populations are either hybridized or sympatric with nonnative species [80]. Nonnative rainbow trout have invaded Yellowstone cutthroat trout habitats in the Greater Yellowstone Ecosystem resulting in hybridization and loss of genetically pure, large-river migratory Yellowstone cutthroat trout in the lower Lamar River in Yellowstone National Park, the North Fork Shoshone River in Wyoming, the Shields and Yellowstone rivers in Montana, and the South Fork Snake River in Idaho [18,81,82] (Figure 1). Other nonnative salmonids are expanding their ranges and displacing native fish in many waters of the Intermountain West. Brook trout have replaced native cutthroat trout in much of their historical range and continue to invade cutthroat trout habitats [83]. Brown trout are actively invading streams in western Montana and negatively affecting native bull trout [84] and Yellowstone cutthroat trout [85]. Brown trout are increasing in abundance and dispersing downstream in the Colorado River below Glen Canyon Dam, where they are threatening native fishes [86,87]. Brown trout in Jackson Lake in the upper Snake River drainage have been increasing since the mid-1990s [88]. Moreover, changing climatic conditions resulting in increased stream temperatures and altered flow regimes are predicted to benefit some nonnative fish over native species, further exacerbating nonnative fish invasions [89,90]. As stream temperatures warm, the amount of thermally suitable habitat for Yellowstone cutthroat trout may be reduced considerably in some areas [91]. Nonnative species such as brook, brown, and rainbow trout are predicted to benefit from the climate-change driven warming of surface waters and altered timing of spring snowmelt run-off [92]. The invasive hybridization of cutthroat trout by rainbow trout may also increase [93]. Although currently limited, increased abundances and range expansions of nonnative and hybridized salmonids should be expected within the upper Snake River watershed, including Pacific Creek, as thermal and flow regimes shift to favor them in future years [94].

The Utah chub is an abundant and widespread species that occupies a broad range of ecological environments within its native range, including springs, marshes, shallow- and deep-water lakes, small creeks, and large rivers [79]. In lakes within its native range, Utah chub often make up a large proportion of the total fish biomass. Following introductions elsewhere in the western United States, Utah chub have competed directly with trout for zooplankton and other foods, resulting in declines in trout abundances [95,96]. Utah chub are also a primary prey of lake trout in all large lakes of the upper Snake River drainage [21] and other large waters in the western United States where these species coexist [97,98].

4.3. Implications of Additional Yellowstone Lake Invasions

Additional nonnative fish invasions of the upper Yellowstone River system would have significant implications for Yellowstone Lake, the Yellowstone River, and tributaries downstream. Rainbow trout would interbreed with the Yellowstone cutthroat trout of Yellowstone Lake, resulting in the loss of what is currently the largest genetically pure population of cutthroat trout remaining in the United States. Brook and brown trout would prey on and compete with Yellowstone cutthroat trout in Yellowstone Lake. Moreover, brook, brown, and rainbow trout would colonize the Yellowstone River and tributary streams and negatively affect Yellowstone cutthroat trout therein, unlike lake trout, which primarily inhabit Yellowstone Lake. The establishment of Utah chub in Yellowstone Lake and connecting streams could have significant, irreversible ecological consequences because they would probably compete directly with Yellowstone cutthroat trout for food and habitat and would serve as additional prey for the invasive lake trout. Species invading Yellowstone Lake would also disperse downstream over the Lower Falls of the Yellowstone River (Figure 1) and into the Lamar and lower Yellowstone rivers [82]. No barriers prevent invasive fish from becoming established in the Lamar River after accessing the drainage. The upper Lamar River (upstream of Cache Creek) is the largest remaining contiguous riverine system where Yellowstone cutthroat trout persist in isolation from most nonnative fish [81].

Additional investigations should resample eDNA study sites to confirm results and include analysis using specific primers for Utah chub. Future studies could also determine the source of lake trout and the dates of their introduction to Yellowstone Lake; otolith microchemistry and genetics technologies have advanced greatly since initial investigations were conducted two decades ago [63,64]. To determine if lake trout invaded via Two Ocean Pass, the chemical characteristics of water and the otolith microchemistry and genetics of lake trout from Jackson Lake should be compared to those of Heart, Lewis, and Yellowstone lakes. To further examine the potential that lake trout were illegally introduced, fish from Buffalo Bill Reservoir in the Shoshone River system of Wyoming should be included as it supports a lake trout fishery only 95 km east of Yellowstone Lake (Figure 1). Additionally, genetic molecular methods now allow for comprehensive analysis of the Yellowstone Lake population, including estimation of the number of fish that founded the population and the length of time that they have been present [99].

5. Conclusions

The environmental DNA of native Yellowstone cutthroat trout was detected at nearly all sites sampled on both sides of the Continental Divide. In contrast, the eDNA of invasive lake trout was detected in the Snake River and in Pacific Creek near its confluence. National parks and wilderness are not typically considered areas with a high risk of aquatic invasive species dissemination among drainage basins, but Two Ocean Pass may pose a significant risk to the upper Yellowstone River system if it affords access to nonnatives moving from the Snake River drainage. If this is a legitimate pathway, such invasions would greatly complicate on-going restoration efforts and jeopardize the long-term persistence of Yellowstone cutthroat trout. Reciprocal movements could threaten the upper Snake River. For example, *Myxobolus cerebralis*, the parasite that causes whirling disease, is present in Yellowstone cutthroat trout in Yellowstone Lake [100], and dreissenid mussels may have invaded the upper Missouri River in Montana [101]. Continued inspections and decontaminations of boats accessing Yellowstone National Park are needed to curtail possible future threats.

Preventing future invasions of the upper Yellowstone River system by nonnative fish via Two Ocean Pass would require creative solutions by multiple management agencies working on an ecosystem scale. A pathway assessment similar to those being conducted in the Midwest [8] should be completed to confirm that a viable aquatic pathway exists and to determine which species pose the greatest threat. The Snake River below Jackson Lake Dam and Pacific Creek should be closely monitored for the presence of invading nonnative fishes. We contend that, because these species are present at low abundances, the potential expansion of lake trout and other invading nonnatives in the Pacific Creek watershed can best be monitored by frequent sampling of eDNA as described in this paper [62]. Artificial barriers are commonly used in the western United States to prevent the upstream movement of nonnative fish and protect headwater native fish restoration areas [102–104], but no natural waterfalls or other features exist on Pacific Creek that could be modified to curtail upstream movement by nonnative fish. Given that Pacific Creek lies largely within federally protected wilderness, the isolation of its headwaters by the construction of an artificial barrier may not be possible or practical. The selective removal of nonnative fish by electrofishing, piscicides, angling, or some combination thereof has reduced their prevalence in the Lamar River drainage of Yellowstone National Park [82,105]. The proactive selective removal of nonnative fish in the upper Snake River system [106,107] may be needed to reduce the probability of the invasion of Yellowstone Lake via Two Ocean Pass.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4441/12/6/1629/s1. Video S1. Two Ocean Pass south of Yellowstone National Park, Wyoming.

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Appendix A

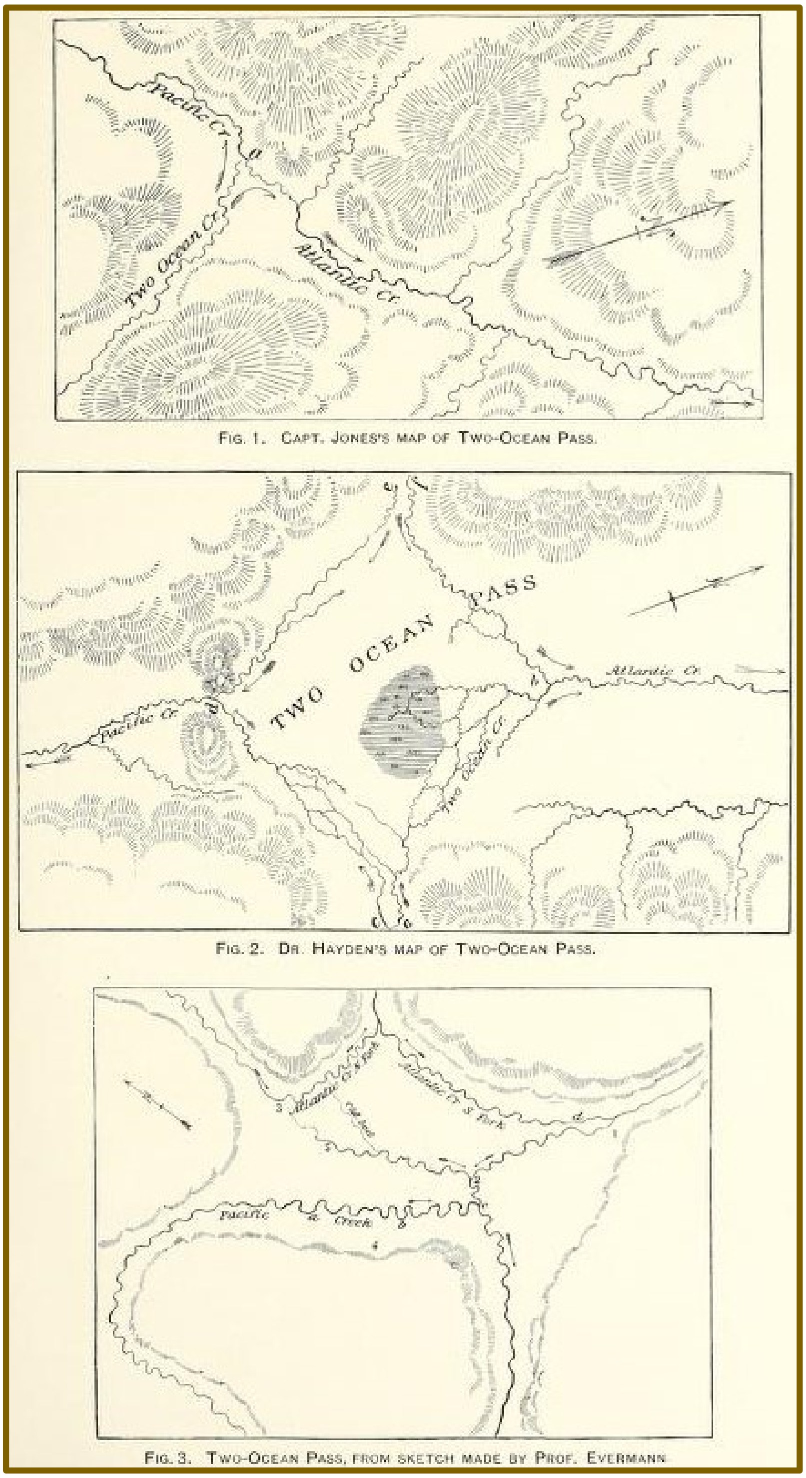


Figure A1. After first being described in an 1868 report on early explorations of the upper Yellowstone River region by the United States Secretary of War [108], hand-drawn maps of Two Ocean Pass were created in 1873 (Figure 1 Jones), 1878 (Figure 2 Hayden), and 1891 (Figure 3 Evermann), recreated here from [15].

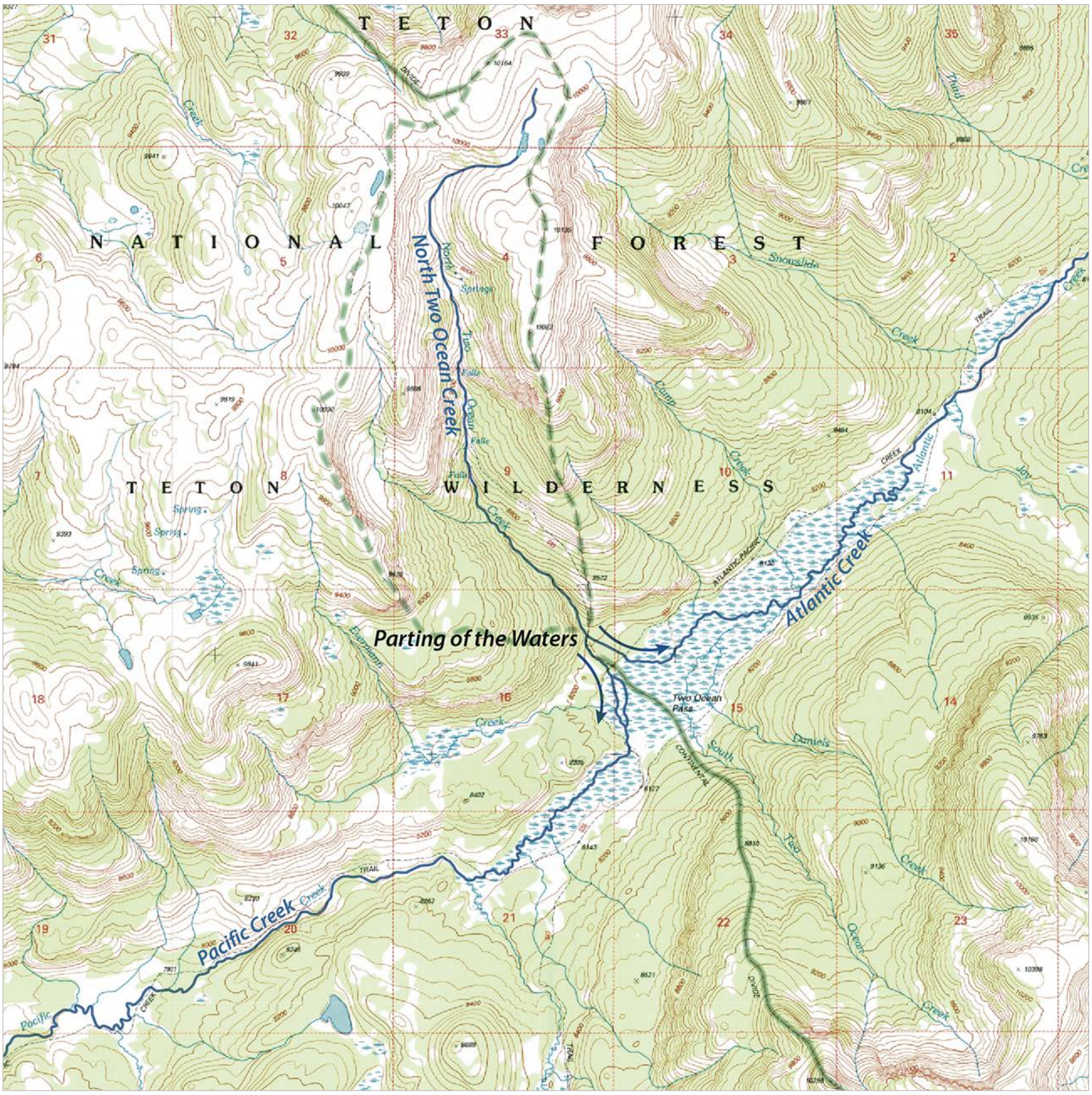


Figure A2. Modified portion of the U.S. Geological Survey, Two Ocean Pass quadrangle map for 2007 [109] showing the *Parting of the Waters* hydrological feature. North Two Ocean Creek flows along the Continental Divide and branches into Atlantic Creek, a Yellowstone River tributary flowing to the east, and Pacific Creek, a Snake River tributary flowing to the west. Scale (squares) is U.S. miles (1 mi = 1.61 km, 1 mi^2 = 2.59 km^2).

(**a**)

(**b**)

Figure A3. (**a**) Yellowstone Lake in Yellowstone National Park has been the site of intensive actions to suppress (**b**) invasive predatory lake trout [26] and restore native Yellowstone cutthroat trout and natural ecosystem function [24].

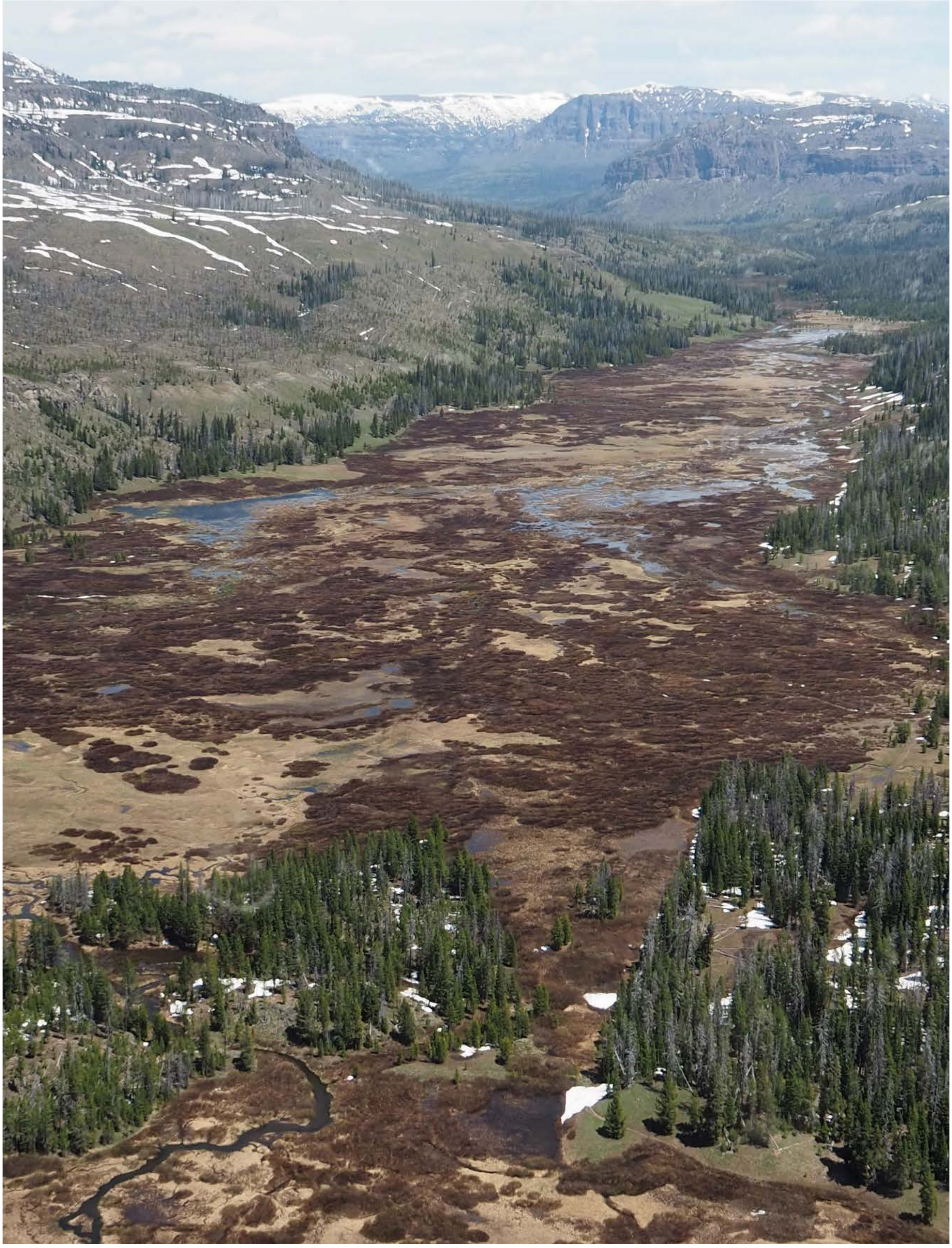
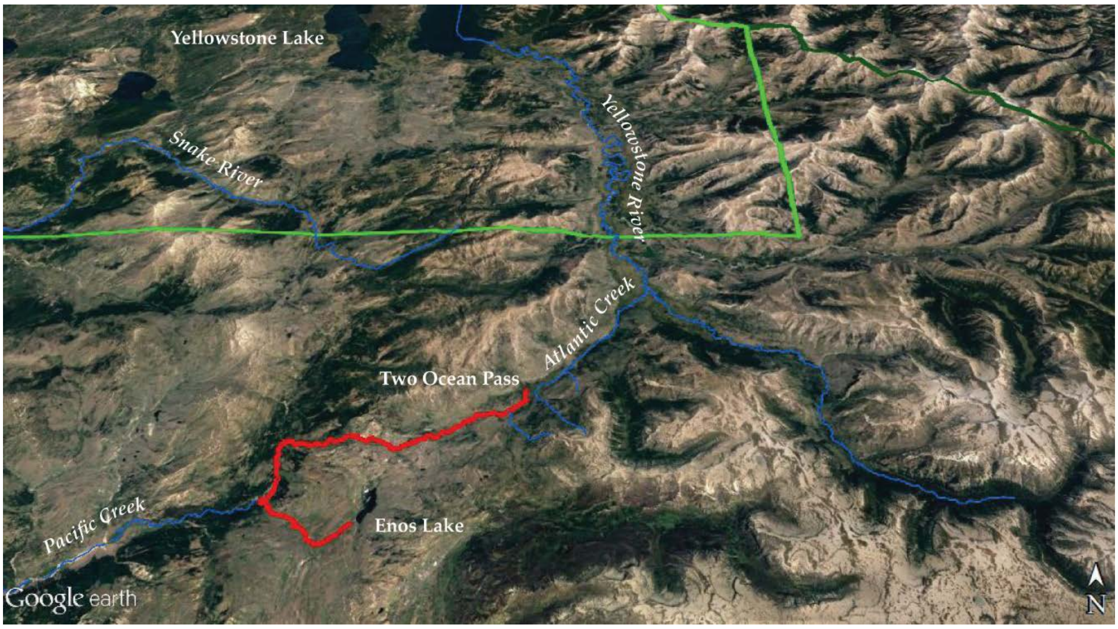


Figure A4. Two Ocean Pass partially flooded following snowmelt run-off in June 2018. Photo is looking east, with Pacific Creek in the foreground and with Atlantic Creek and the Thorofare region (Hawks Rest and the Trident Plateau) of the upper Yellowstone River system in the distance.

(a)



(b)

Figure A5. (**a**) Lewis Lake (Figures 3 and 4A) in the headwaters of the Snake River drainage in Yellowstone National Park was isolated by impassable waterfalls and naturally fishless when the park was first established in 1872 [15]. Nonnative lake trout (Lake Michigan, Lawrentian Great Lakes origin) and exotic brown trout (Loch Leven Scotland origin) were among the first nonnative fish stocked to the park when they were introduced to Lewis Lake by the U.S. Commission on Fish and Fisheries in 1890. The popular sport fishery resulted in Utah chub being introduced to the lake by the 1950s, likely as baitfish. Utah chub became the most abundant fish in Lewis Lake, as corroborated by this gillnet catch in August 2019. (**b**) Utah chub are abundant in Enos Lake within the Pacific Creek watershed, a stream distance of 26 km (in red) from Two Ocean Pass.

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