# USE OF eDNA TO ESTIMATE ABUNDANCES OF SPAWNING YELLOWSTONE CUTTHROAT TROUT IN YELLOWSTONE NATIONAL PARK, WYOMING, USA

by

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# ABSTRACT

Invasive Lake Trout *Salvelinus namaycush* and whirling disease have reduced the abundance of native Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri* (YCT) in Yellowstone Lake, Yellowstone National Park, thereby disrupting the Yellowstone Lake ecosystem. One indication of the YCT population decline is the decrease in the number of adults returning to tributaries to spawn each spring. Yellowstone National Park implemented a gillnetting program to remove Lake Trout starting in 1995 to restore YCT abundance and size structure and thereby conserve the Yellowstone Lake ecosystem. An important metric for evaluating the success of the program is the number of YCT ascending spawning tributaries each year. Annually, 9 to 11 of these tributaries are visually surveyed on a weekly basis from May through July for the presence of spawners, but these surveys are time consuming. The use of environmental DNA (eDNA) has become increasingly common for determining presence of aquatic species and may provide managers with a more efficient tool for estimating abundances of YCT spawners. The primary objective of my study was to evaluate the efficacy and accuracy of using eDNA to detect the presence and estimate abundance of YCT spawners by collecting eDNA samples from spawning tributaries to Yellowstone Lake in conjunction with visual surveys of YCT spawners. A secondary objective was to evaluate whether terrestrial or semi-terrestrial species such as grizzly bear *Ursus arctos horribilis* and North American river otter *Lontra canadensis* could be detected in a water sample from YCT spawning tributaries. Environmental DNA quantities were more effective for determining presence of YCT spawners than for predicting their abundances, but eDNA quantities were positively related to spawner abundances. The difference between eDNA rates when spawners are present versus absent may provide managers with an efficient method for monitoring YCT in tributaries throughout Yellowstone Lake basin. I also demonstrated that DNA from a terrestrial species, grizzly bear, can be detected in water samples. Incorporation of eDNA sampling with existing methods for monitoring YCT spawners in Yellowstone Lake tributaries would facilitate an increased scale of assessment and allow for detection and quantification of multiple species of current and future interest from single samples.

# EVALUATING THE EFFICACY OF USING eDNA TO ESTIMATE ABUNDANCES OF SPAWNING YELLOWSTONE CUTTHROAT TROUT IN TRIBUTARIES TO YELLOWSTONE AND TROUT LAKES

## Introduction

### Yellowstone Cutthroat Trout Life History

Yellowstone Lake, Yellowstone National Park (YNP), Wyoming, is home to the largest population of genetically pure Yellowstone Cutthroat Trout *Oncorhynchus clarkii bouvieri*; (YCT) in existence (Gresswell 2011). The Yellowstone Cutthroat Trout is one of only two native fish species in the lake and the only native trout species to evolve in the Yellowstone Lake ecosystem. The life history characteristics of this Cutthroat Trout subspecies are the result of a unique ecosystem and thousands of years of isolation (Varley and Gresswell 1988). Yellowstone Cutthroat Trout not only play a key role in the Yellowstone Lake ecosystem, but they are an important cultural component of the past, present, and future of Yellowstone National Park. However, this population has declined in recent decades because of non-native species introduction, whirling disease, and drought (Koel et al. 2005).

The historic range of YCT included parts of Montana, Wyoming, Idaho, and Utah. The current range includes only a portion of the original range in each of these states (Al-Chokhachy et al. 2018), but additional YCT populations were established outside of their historic range in at least eight western states (Varley and Gresswell 1988); over 818 million eggs and fry were shipped from YNP (mainly from Yellowstone

Lake) to locations throughout the United States and Canada between 1899 and 1957 (Varley and Gresswell 1988).

Yellowstone Cutthroat Trout in the greater Yellowstone ecosystem have adapted to diverse environments. For example, the documented pH range in habitat occupied by YCT in the Yellowstone ecosystem is 5.6 to 10 (Varley and Gresswell 1988). Because of geothermal influences, some waters in YNP are consistently warmer than typical trout streams and YCT have adapted to survive these conditions. The optimal water temperature for YCT is about 15°C, but they can survive in waters up to 26°C in thermally influenced areas and have been documented actively feeding in Yellowstone Lake under 1 m of ice at temperatures below 4°C (Varley and Gresswell 1988).

Yellowstone Cutthroat Trout spawning patterns and timing are dependent on several environmental factors and vary throughout the Yellowstone Lake ecosystem (Gresswell et al. 1997). Yellowstone Cutthroat Trout occupy the main basin of the lake, the inlet and outlet (i.e., the Yellowstone River), and many tributaries around the lake (Gresswell 1994). The fish are residents in some tributaries whereas in others adult YCT migrate into the streams or the outlet to spawn and then return to the lake (Gresswell et al. 1994; Kaeding et al. 2001). Yellowstone Cutthroat Trout migrate to spawning tributaries on the descending limb of the hydrograph, between mid-May and mid-July and spawn at water temperatures between 5.5 and 15.5°C (Varley and Gresswell 1988). Spawning tributaries are often low gradient (3% or less), groundwater or snowmelt fed, and with gravel 12 to 85 mm in diameter (Varley and Gresswell 1988). Yellowstone Cutthroat Trout in Yellowstone Lake exhibit spawning site fidelity and usually return to

their natal streams to spawn each year (Varley and Gresswell 1988). Juvenile YCT emerge from the substrate about two weeks after hatch and usually migrate to the lake shortly thereafter, but some may stay in their natal streams for extended periods before migrating to the lake (Varley and Gresswell 1988). Although the timing of YCT spawning varies among tributaries because of differing elevations and temperatures around Yellowstone Lake, the preferred habitat and optimal conditions are consistent.

Yellowstone Cutthroat Trout Importance

The Yellowstone Lake population of YCT is a large source of genetically pure YCT that could be used to augment declining populations or restore the species to waters where it has been extirpated. Climate change is potentially the greatest range-wide threat to the persistence of YCT because it will probably exacerbate the effects of nonnative species invasions and alter the amount of habitat (Al-Chokhachy et al. 2013; Al-Chokhachy et al. 2017). As global air temperatures warm, the ranges of species such as YCT are predicted to move northward in latitude and upward in elevation (Gresswell 2011). Warmer temperatures, particularly in the summer months, will probably also affect the connectivity of spawning streams to lakes, increase the susceptibility of YCT to disease, and increase the mortality rate from angling (Al-Chokhachy 2017). However, the risk of the high-elevation YCT population of Yellowstone Lake and its associated tributaries to climate change is lower than that of lower elevation populations (Gresswell 2011). As climate change, nonnative species invasions, and other anthropogenic effects continue to limit the range of, and in some places extirpate, YCT populations,

maintaining a healthy population of genetically pure YCT in Yellowstone Lake will become increasingly important.

Yellowstone Cutthroat Trout are a cultural and economic resource that has long been valued by anglers, wildlife viewers, Yellowstone National Park employees, and visitors. About 42,000 to 43,000 YNP fishing permits were issued annually from 2012-2014 (Koel et al. 2015) and YCT have been and continue to be a sought-after sport fish in YNP (Varley and Schullery 1995, 1998; Koel et al. 2015); in 2014, 59% of fish caught were YCT (Koel et al. 2015). The economic value of the sport fishery of the Yellowstone Lake ecosystem alone was estimated to be about US$36 million in 1994, the same year that invasive Lake Trout *Salvelinus namaycush* (LKT) were discovered in Yellowstone Lake (Varley and Schullery 1995). An abundant YCT population also provides valuable wildlife-watching opportunities; in the early 1990s, about 10% of Yellowstone National Park visitors watched spawning YCT at the iconic Fishing Bridge and LeHardy's Rapids (Varley and Schullery 1995). The combination of these genetic, ecological, and cultural factors makes the YCT population of Yellowstone Lake an important resource.

Introduction of Lake Trout and the Effects on YCT

The decline in YCT abundance in the Yellowstone Lake ecosystem is largely attributed to the inadvertent introduction of piscivorous Lake Trout, the presence of whirling disease, and ongoing drought beginning in the early 2000s (Koel et al. 2005). Since the initial discovery of non-native LKT in Yellowstone Lake, Yellowstone National Park managers have implemented an aggressive removal program. Despite these efforts, the effects of non-native LKT on the YCT population have been substantial.

Lake Trout were first introduced to Yellowstone National Park in nearby Lewis and Shoshone lakes by the U.S. Fish Commission in 1890 (Varley 1981). These lakes are not connected to Yellowstone Lake. The initial reports of LKT in Yellowstone Lake in the 1980s (Varley and Schullery 1998) were scarce and unconfirmed; the first documented LKT in Yellowstone Lake was caught by a fishing guide in 1994 (Kaeding 1995; Varley and Schullery 1998). Based on subsequent angling and gillnetting and the number of year classes that were caught, the population of LKT had been reproducing in Yellowstone Lake since at least 1989 (Kaeding 1995). Microchemistry analysis of LKT otoliths and genetic analyses suggested some LKT were introduced into Yellowstone Lake from Lewis Lake in the 1980s and other LKT potentially earlier, possibly from nearby connected sources (Munro 2005; Koel et al. 2020a).

Yellowstone National Park managers have used various methods to target and assess the LKT population since their discovery in Yellowstone Lake. Lake Trout were targeted with gillnets and angling and aged using scale samples, and visual surveys were conducted for potential spawning habitat soon after their discovery in 1994 (Kaeding 1995). Targeted gillnetting for LKT suppression continued the next year. By 2014, gillnetting effort had increased, acoustic telemetry was being used to improve gillnetting efficiency, and alternative methods such as embryo suppression on spawning areas were being explored (Koel et al. 2015). Overall, more than 3.35 million lake trout were gillnetted (1995-2019) to allow for YCT recovery (Koel et al. 2020b). The effectiveness of suppression efforts is evaluated annually through long-term gillnetting assessments of YCT and LKT, angler catch rates, and monitoring of YCT spawner abundances in

tributaries around the lake (Koel et al. 2020b). Lake Trout suppression continues to be a priority for Yellowstone National Park (Koel et al. 2020b).

The changes in abundance of YCT concurrently affected trophic dynamics of the Yellowstone Lake ecosystem. Yellowstone Cutthroat Trout are an important prey for a variety of species including black bear *Ursus americanus*, grizzly bear *Ursus arctos horribilis*, North American river otter *Lontra canadensis*, and many bird species (Reinhart and Mattson 1990; Baril et al. 2013; Crait et al. 2015; Koel et al. 2019). The number of bears observed fishing in tributaries to Yellowstone Lake has been linked to the number of YCT spawners observed in these tributaries and has declined in concert with the decline in YCT numbers (Reinhart and Mattson 1990; Haroldson et al. 2005; Teisberg et al. 2014). River otters in the Yellowstone Lake area also rely on YCT, particularly during the spawning season when YCT move into tributaries to spawn (Crait and Ben-David 2006). At least 16 species of birds prey or scavenge on YCT in Yellowstone Lake and its tributaries. Of these, osprey *Pandion haliaetus*, American white pelicans *Pelecanus erythrorhynchos*, Caspian terns *Hydroprogne caspia*, and double-crested cormorants *Phalacrocorax auritus* are the most dependent on YCT (Baril et al. 2013). Because these species prey on YCT primarily during the spawning season when YCT are readily accessible and have a high energy value, YCT are not functionally replaced by LKT, which inhabit deeper water and do not spawn in tributaries (Crait et al. 2015; Koel et al. 2020b). As a result, the abundance and feeding habits of species that formerly depended on YCT have subsequently been altered (Teisberg et al. 2014; Crait et al. 2015; Koel et al. 2017). The introduction of LKT as a new top predator also affected

lower levels of the food chain within Yellowstone Lake by increasing zooplankton biomass, decreasing zooplankton production, lowering phytoplankton biovolume, and lowering chlorophyll *a* concentrations (Koel et al. 2019). Yellowstone National Park biologists and outside researchers continue to study and monitor the far-reaching consequences of the invasive Lake Trout population in Yellowstone Lake.

Whirling disease and persistent drought also contributed to the decline of the YCT population in Yellowstone Lake (Koel et al. 2005). Whirling disease, caused by the parasite *Myxobolus cerebralis*, was first discovered in Yellowstone Lake YCT in 1998 (Koel et al. 2006). Following the discovery of *M. cerebralis*, the Yellowstone River downstream of Fishing Bridge, Clear Creek, and Pelican Creek all tested positive, with varying levels of severity (Koel et al. 2006). Of these tributaries, Pelican Creek, the second largest tributary to Yellowstone Lake, has consistently tested positive with the most severe level of infection across all years (Koel et al. 2015). Factors that may affect infection prevalence and severity include stream flow, temperature, and substrate material (Murcia et al. 2015). Fisheries managers currently assess the status of whirling disease around Yellowstone Lake and its tributaries once every 5 years (Koel et al. 2015). Persistent drought has also contributed to the decline in YCT abundance (Koel et al. 2020b). Lower water levels in tributaries, particularly in late summer and early autumn, may preclude YCT fry from migrating back to the lake (Koel et al. 2020b). Whirling disease and drought thereby exacerbate the effects of invasive LKT on the YCT population.

Current spawning stream monitoring efforts

The abundance of YCT migrating into spawning streams each spring is an important metric for evaluating the success of YCT restoration efforts. Spawning streams were monitored for years using a combination of visual surveys, fish weirs, a sonar counting unit, and electronic counting banks. Each method has advantages and disadvantages.

Visual surveys of YCT spawning streams have been conducted by the Yellowstone Resource Management Program (1988 to 2011) and Bear Management Office (BMO, 2012 to present) (both programs hereafter collectively referred to as the "BMO"). Surveys were conducted on 9 to 11 front-country tributaries (hereafter "historically sampled" streams or tributaries) around Yellowstone Lake every spring since 1988 (Koel et al. 2019). The numbers of spawning-sized YCT are counted and any sign of bear activity at each stream such as tracks, scat, or fish remains is recorded during each survey; attempts were made to distinguish between grizzly and black bear sign, but this was often not possible. Spawning runs occurred in 59 of the 124 known tributaries to Yellowstone Lake in 1985, with evidence of bears fishing in 36 of those streams (Reinhart and Mattson 1990). Between 1985 and 2000, the frequency of bear visits to YCT spawning streams was related to the density of spawning fish in each stream and in most cases the number of visits by bears decreased with the decline in the YCT population (Reinhart and Mattson 1990). Bear use of spawning streams increased after the early 1970s, probably in response to a decrease in the availability of human foods and an increase in the numbers of large YCT as a result of changes to Yellowstone National Park regulations and fisheries management policies (Reinhart and Mattson 1990).

Although grizzly and black bears cohabit in the Yellowstone Lake basin, grizzly bears are known to exclude black bears from high-quality foods like spawning YCT and are therefore more often associated with fishing on spawning tributaries (Teisberg et al. 2014). After Lake Trout introduction (1997 to 2000), both YCT abundance and grizzly bear activity declined at a subset of 25 tributaries, except in those along the west shore of Yellowstone Lake (Haroldson et al. 2005). Subsequently (2007 to 2009), spawning YCT were observed in 16 of the 22 tributaries where spawning activity was documented from 1997 to 2000 and the median peak count of spawning fish decreased by 98.4%; grizzly bear use of YCT streams also decreased (Teisberg et al. 2014). When visual surveys began on the historically sampled streams in 1988, about 70 spawning YCT were seen at each tributary; by 2014, as few as 1 or 2 fish were seen at most sites (Koel et al. 2019). These monitoring programs informed management objectives by providing data on abundance of spawning YCT from before and after the LKT invasion, the discovery of whirling disease, and the effects of ongoing drought.

Although these visual surveys were conducted for several decades using established protocols, they had significant drawbacks. Walking the length of a stream looking for YCT spawners is time and personnel intensive (3 people are required for bear safety). This method is also impractical for consistent monitoring of remote tributaries. Managers therefore wanted more efficient ways to assess the abundances of YCT spawners and associated grizzly bear activity in tributaries to Yellowstone Lake.

The Native Fish Conservation program in YNP has used weirs, sonar units, and electronic counting banks to monitor spawning tributaries. The fisheries program

maintained a weir on Clear Creek, a prominent spawning tributary, from 1945 until the structure washed out in high water in 2008 (Koel et al. 2015). More than 55,000 YCT spawners ascended Clear Creek in 1988, but only 538 were observed during the last complete count in 2007 (Koel et al. 2008). In 2012, the bulkhead that supported the weir was rebuilt, the weir structure was removed, and the sections of streambank damaged by erosion were restored (Koel et al. 2015). Instead of reconstructing the weir, which had caused bank erosion and required several personnel stationed on site to operate, a DIDSON sonar fish counting system (Sound Metrics Corporation, Bellevue, Washington; model Aris 3000) was installed in 2013, but it was only partially operational during the 2013 to 2018 spawning seasons and a complete count was never made. An electronic counter bank (Pulsar Electronics, Vancouver, British Columbia; model 550C Fish Counter System) that operated on Bridge Creek from 1999 through 2004 documented the decline in YCT ascending the tributary to spawn from 2,363 individuals in 1999 to a single fish in 2004 (Koel et al. 2005). These monitoring methods provided managers with valuable information on trends of YCT spawners ascending tributaries around Yellowstone Lake and continue to play a valuable role in the management and recovery of YCT in the Yellowstone Lake ecosystem. However, these methods also are time and personnel intensive, may cause damage to stream banks through erosion, and are prone to technical problems that require time and expertise to solve, resulting in inaccurate or incomplete counts in some years. Moreover, relatively few streams, especially those that are remote, can be monitored. Efficient assessment of more spawning streams with new

techniques, such as environmental DNA, would contribute to a more complete and accurate assessment of the YCT population within Yellowstone Lake.

Environmental DNA

Environmental DNA (eDNA) is genetic material obtained directly from soil, sediment, water, or ice cores without any obvious signs of the organism from which it originated (Thomsen and Willerslev 2015). Organisms shed DNA into their environment in skin cells, mucus, feces, or tissues (Thomsen and Willerslev 2015). Aquatic eDNA is filtered from water in the field and extracted and identified in the laboratory. Studies of the accuracy, reliability, limitations, and usefulness of eDNA analysis have become increasingly commonplace in recent years and have examined the limitations, applications, and effectiveness of eDNA as a monitoring tool (Bohmann et al. 2014; Wilcox et al. 2016).

Limitations. Despite continuing advances in eDNA sampling and extraction methods, this technology has limitations. The DNA may be transported or degraded at different rates in different aquatic environments (Denier and Altermatt 2014; Jane et al. 2015). Stream discharge (Wilcox et al. 2015), the rate at which an organism sheds DNA (Maruyama et al. 2014), and the diet and movement of an organism can affect eDNA concentrations (Dunn et al. 2017); contamination and differences in laboratory procedures can affect sample analyses. Laboratory and field studies continue to inform which sampling and analysis protocols produce the most accurate and consistent results (Lacoursière-Roussel et al. 2016a; Willoughby et al. 2016; Davison et al. 2016; Hinlo et al. 2017).

Environmental conditions and the interactions of aquatic organisms with the environment vary spatially and temporally and DNA shed rates, transport distances, and degradation rates may cause variation in the amount of eDNA that persists in a stream. Stream morphology, discharge (Deiner and Aletermatt 2014; Wilcox et al. 2015), and substrate (Buxton et al. 2017) may affect transport distance. Furthermore, eDNA may not be distributed or transported uniformly in flowing water (Shogren et al. 2017). Environmental DNA may be temporarily absorbed into organic or inorganic materials and detected for some time after the source of eDNA is removed (Dejean et al. 2011; Balasingham et al. 2017) and eDNA that has settled into streambed sediment may be resuspended by anglers or wildlife and subsequently detected in surface waters long after the target organism has left the system (Turner et al. 2015). The degradation of eDNA varies (Barnes et al. 2014) and may be affected by environmental factors such as temperature, which accelerates eDNA degradation (Strickler et al. 2015), and discharge rates, which may affect the concentration of eDNA detected (Jane et al. 2015). The rates at which organisms shed DNA may be affected by life stage, diet, temperature (Maruyama 2014; Klymus et al. 2015; Sassoubre 2016), and spawning activity (Bylemans et al. 2017; Bracken et al. 2019). Amplification of DNA during PCR can be inhibited by common in-stream substances such as leaf litter, thereby reducing or precluding detection of eDNA (Jane et al. 2015; Wilcox et al. 2018). Inhibition detected during PCR can often be mitigated through use of an inhibitor remover (McKelvey et al. 2016). Because significant variation exists among different environments and seasons,

research is needed to understand the effects of these variables on eDNA concentration within an aquatic system.

Sample contamination can occur in both the field and laboratory, leading to inaccurate results. Contamination in the laboratory or imprecise assays, used to target DNA from specific species, can result in false positives (Wilcox et al. 2013). Environmental DNA from a target species that is not actually present may also be detected when eDNA is transported by vectors such as bird feces and boat hulls (Merkes et al. 2014), or when waders and other sampling equipment are used at multiple sites throughout the sampling season. Sample contamination can be reduced by carefully choosing sample locations, avoiding contamination during the sampling process, and implementing rigorous laboratory protocols (Carim et al. 2016).

Applications. Aquatic eDNA has many management applications, including determining the presence of species, estimation of abundances and biomass of aquatic organisms (Lacoursière-Roussel et al. 2016b), efficient delineation of species distributions (McKelvey et al. 2016), detection of semi-terrestrial organisms (Padgett-Stewart et al. 2015), and surveying of entire aquatic communities from one sample (metabarcoding) (Elbrecht et al. 2017). Although eDNA sampling is unlikely to replace traditional sampling methods, in some instances it is more cost effective and less personnel intensive (Sigsgaard et al. 2015). Additional applications will probably become apparent as knowledge of this technology progresses and more studies are conducted.

Small amounts of aquatic eDNA can be used to accurately confirm the presence of an aquatic species (e.g., Jerde et al. 2011; Olson et al. 2012; Baldigo 2016; McKelvey

et al. 2016). Environmental DNA can detect small numbers of organisms (Sigsgaard et al. 2015), which is useful for detecting endangered or at-risk species (Sipendorfer et al. 2016), species with secretive life history traits (Spear et al. 2015), and newly invasive species (Goldberg et al. 2013). Environmental DNA can also inform managers on the success or failure of invasive species removal projects (Dunker et al. 2016; Davison et al. 2017; Carim et al. 2020).

The use of eDNA to verify the presence of aquatic species is well established, but the relationship between eDNA concentration and species abundance is less well understood. However, Brook Trout *Salvelinus fontinalis* abundance was positively correlated with eDNA concentration in an observational study conducted in natural stream systems (Wilcox et al. 2016) and eDNA has been used to predict daily counts of adult Sockeye Salmon *Onchorhynchus nerka* and Coho Salmon *O. kisutch* migrating upstream as well as out-migrating Sockeye Salmon smolts (Tillotson et al. 2018; Levi et al. 2019). Furthermore, eDNA concentrations may allow estimation of abundances (or biomass) of other aquatic species such as amphibians (Pilliod et al. 2013). Common Carp *Cyprinus carpio* biomass was positively related to eDNA concentrations in aquarium and pond settings (Takahara et al. 2012) and eDNA concentrations were positively correlated with Lake Trout relative abundances (Lacoursière-Roussel et al. 2016b) and stream dwelling fish biomass in field experiments (Doi et al. 2017). Bighead Carp *Hypophthalmichthys nobilis* egg density (as an indicator of spawning activity) was not related to eDNA in the Wabash River, Indiana, but a relationship did exist among eDNA, stream discharge, and Bighead Carp movement, providing support for the potential of

using eDNA sampling to monitor relative abundance (Erickson et al. 2016). Environmental DNA was used to detect spawning activity of Macquarie Perch *Macquaria australasica* by comparing the relative abundances of mitochrondrial and nuclear eDNA over time in both experimental and field studies (Bylemans et al. 2017). Use of eDNA to determine that fish are actively spawning and to estimate the abundances of spawners in a particular stream would provide managers with a valuable tool for monitoring both native and invasive fish populations.

Combining eDNA and traditional sampling can allow for more efficient determination of the geographic extent of aquatic species (McKelvey et al. 2016; Katano et al. 2017). Environmental DNA sampling is simple and quick (Biggs et al. 2015), allowing large geographical areas to be surveyed efficiently as well as the determination of the upper extent of a species within a drainage (Laramie et al. 2015). It can be used in conjunction with traditional sampling to accurately determine the extent of non-native species invasions and thereby inform containment actions such as exclusion barriers (Bylemans et al. 2016) or as an early detection tool for the Bighead and Silver Carp invasions in the Great Lakes (Jerde et al. 2013) and the zebra mussel invasion in Lake Winnipeg, Manitoba (Gingera et al. 2017). Because eDNA sampling is non-invasive, it is also useful for detection and range delineation of threatened or endangered species such as Bull Trout, which are rare but widely distributed in western Montana (McKelvey et al. 2016).

Environmental DNA may also provide insight on the use of streams and other water sources by terrestrial and semi-terrestrial organisms. Metabarcoding of water

samples collected in zoo animal enclosures in Yokohama, Japan, successfully detected eDNA of 10 mammal species and field samples from forest ponds detected DNA from house mouse *Mus musculus*, grey red-backed vole *Myodes rufocanus*, raccoon *Procyon lotor*, brown rat *Rattus norvegicus*, long-clawed shrew *Sorex unguiculatus*, and sika deer *Cervus nippon* (Ushio et al. 2017). Environmental DNA of amphibians, insects, Eurasian otter *Lutra lutra*, birds, fish, and crustaceans was detected from samples of streams, lakes, and ponds in Europe (Thomsen et al. 2012). The North American river otter has been successfully detected in water samples from a zoo enclosure, but neither North American river otter nor grizzly bear eDNA detection has been studied in-situ in streams. The development of eDNA assays for species such as these will allow detection of terrestrial and semi-aquatic organisms using water samples collected expressly for that purpose or in combination with samples collected for fish detection (Piaggio et al. 2014. Samples can be archived and analyzed in the future to retrospectively detect presence of multiple species of interest across the landscape.

Water sampling for eDNA analysis may be more cost effective and have less effect on the environment than traditional sampling; it can be less time and labor intensive and require less training (Sigsgaard et al. 2015). Populations can be surveyed without handling individuals and sampling need not involve walking through stream habitat, potentially disturbing active spawning redds, as can occur with traditional methods such as electrofishing (Pfleger et al. 2016). Whereas eDNA sampling will not fully replace traditional sampling methods, in some instances it may be more efficient, practical, and effective.

Although extensive research into the various applications of eDNA has been conducted, the relationship between eDNA and spawning trout abundance has not previously been studied. Therefore, the objectives of my study were to evaluate the efficacy and accuracy of eDNA as a tool for 1) detecting the presence of YCT spawners, 2) detecting temporal changes in YCT spawner or age-0 abundance, 3) determining if a positive relationship exists between eDNA and YCT spawner abundances, and 4) demonstrating presence and use of streams by terrestrial and semi-terrestrial species such as grizzly bears and river otters. Monitoring YCT spawning tributaries with eDNA sampling may allow the National Park Service (NPS) to more efficiently sample a wider range of tributaries throughout the Yellowstone Lake basin. Because a single eDNA sample can be analyzed for multiple species, eDNA sampling may also allow managers to collect information on the use of these streams by both aquatic and terrestrial organisms, thereby documenting other indicators of YCT recovery in the Yellowstone Lake ecosystem.

## Study Area

Yellowstone Lake is located within the Yellowstone River basin in the southeastern corner of Yellowstone National Park, Wyoming (Figure 1). With a surface area measuring 36,000 hectares, a mean depth of 48.5 meters (m), and maximum depth of 107 m, Yellowstone Lake is the largest lake above 2,000 m in North America (Kaplinski 1991). The lake is typically covered in ice and tributaries are covered in snow from mid-December to late May or early June. Water temperatures range from 5 to 18°C in the lake basin and from 0 to 20°C in the tributaries from May through October (Koel et al.

2007). Two native fish species, YCT and Longnose Dace *Rhinichthys cataractae*, and four non-native species, Longnose Sucker *Catastomous catostomus*, Redside Shiner *Richardsonius balteatus*, Lake Chub *Couesius plumbeus*, and Lake Trout, are established in Yellowstone Lake (Varley and Schullery 1998). Yellowstone Lake is surrounded by 124 identified tributaries, 59 of which had documented YCT spawning runs in 1985 (Reinhart and Mattson 1990). I sampled eDNA and visually surveyed YCT abundance at a total of seven Yellowstone Lake tributaries (2 to 6 tributaries each year) (Figure1).

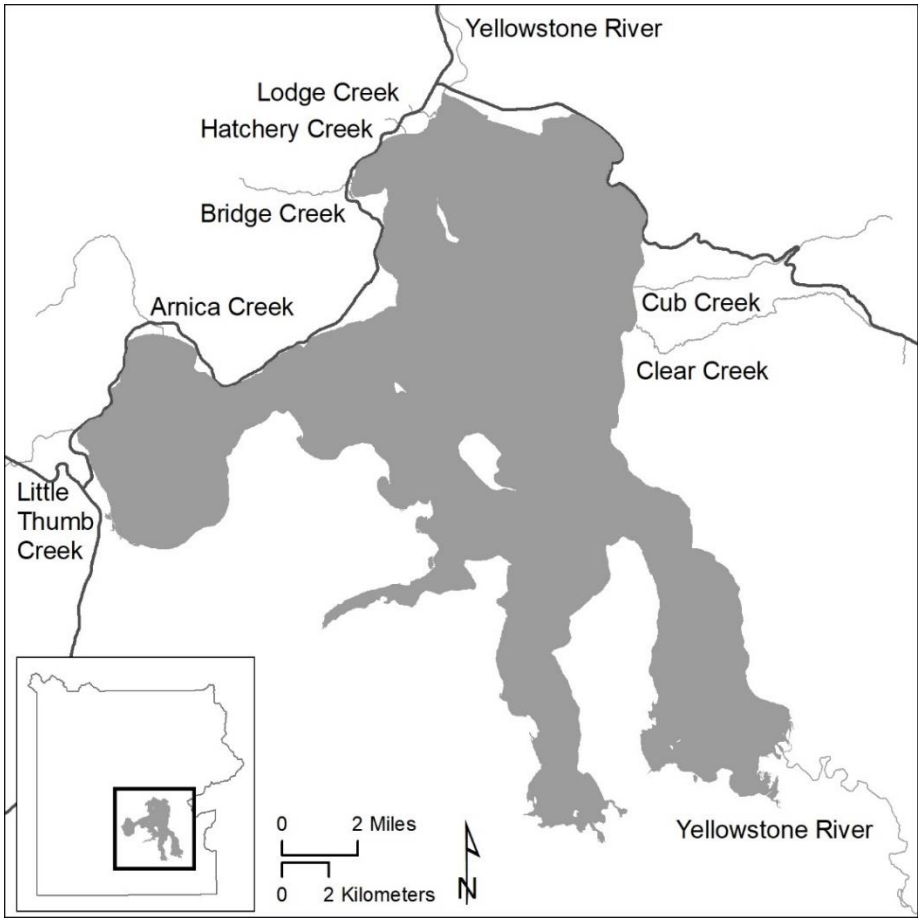


Figure 1. The seven tributaries (light gray lines) to Yellowstone Lake, Yellowstone National Park, where eDNA samples were collected. The Yellowstone River is identified for reference but was not sampled. Dark gray lines represent major roads.

Sample Site Selection and Frequency.

Two or three sample sites were selected on each tributary based on stream attributes and known spawning distributions. The spawning reaches on Yellowstone Lake tributaries were determined from visual survey data collected on Yellowstone Lake tributaries by the BMO in YNP since 1988 (Reinhart and Mattson 1990; Reinhart et al. 1995 cited by Koel et al. 2015). The Trout Lake spawning reach was determined by habitat as a barrier to upstream fish movement is located 0.15 km from the mouth of the inlet. The most downstream sample on each tributary (sample site 1) was taken 50 to 100 m upstream from the mouth of the tributary. Sample site 1 on each tributary was chosen such that eDNA samples would represent fish that had moved upstream to spawn rather than fish that were staging near the mouth of the stream in preparation for spawning. The next upstream sample site on each tributary (sample site 2) was roughly equidistant between the first sample site and the upstream end of the known spawning distribution. The third sample site on each tributary (sample site 3) was located at the upstream end of the known spawning distribution. Samples farther upstream were collected occasionally to document the presence or absence of spawning or resident YCT or both above the known spawning reach. Fewer sites on each tributary were sampled when accessibility was difficult (presence of deep snow or grizzly bears) and during low water levels at the beginning and end of the field season. A single eDNA sample was collected at each sample site on a given date. Samples from stream, site, and year combinations where stream discharge calculations or estimates were unavailable, no corresponding visual

survey was conducted, or spawners were visually observed on fewer than three sampling occasions in a single spawning season were ultimately excluded from the analyses.

Yellowstone Lake YCT spawning tributaries

Clear Creek. Located on the northeastern shore of Yellowstone Lake 4.8 km from a trailhead, Clear Creek is most easily accessible by boat after ice-out (Figure 1). Clear Creek has a history of annual visual (since 1945) and weir trap (1945 to 2008) spawning surveys (Koel et al. 2005). A sonar fish counting unit was installed in 2013 but has not been fully functional in any year. Clear Creek is the largest tributary in this study with a mean wetted width of 10.91 m (Table 2). YCT spawners were visually observed in Clear Creek during visual surveys from mid-April through late June in 2016 and mid-July in 2017. River otters prey on YCT spawners in this stream and grizzly bears frequent the area.

The spawning reach at Clear Creek extended from the stream mouth to the upstream end of a large meadow (Reinhart 1990, Table 1). This reach was divided into two sections with a sample site near the stream mouth (site Clear1), one in the middle of the reach just upstream of the Thorofare Trail crossing (site Clear2), and one at the upstream end of the spawning distribution (site Clear3) (Figure 2). Samples were collected from two additional sites upstream of site Clear3 in 2016 and 2017 to document the presence or absence of YCT upstream of the spawning reach.

Stream discharge was measured concurrently with sample collection at site Clear1 except during high water levels. A staff gauge was installed in 2017 and estimates from the relationship of the staff gauge to measured stream discharge were used for site Clear1

on dates when no stream discharge measurement was taken. Stream discharge estimates from site Clear1 were used for all upstream sites unless stream discharge was specifically measured at those sites independently.

No samples were collected from Clear Creek in 2018 because attempts to obtain independent fish counts from a sonar unit were unsuccessful (see Independent YCT counts) and visual surveys of spawners were probably inaccurate during high water. Because of the failure to obtain independent fish counts with the sonar unit in any year, Clear Creek was ultimately excluded from the analyses.

Cub Creek. Located about 1.6 km from Clear Creek on the northeastern shore of Yellowstone Lake, Cub Creek is about 3.2 km from the Nine Mile Trailhead and is accessible by boat after ice-out (Figure 1). Cub Creek was historically a major spawning tributary with previously documented YCT spawning runs. YCT spawners were visually observed during three separate visual surveys in June of 2016 (Table 2).

The Cub Creek spawning reach extended 0.84 km upstream from the stream mouth and was determined from YNP fisheries and BMO data (Reinhart 1990; Table 1). This reach was sampled at one site near the stream mouth (site Cub1) and at one site at the upstream end of the spawning distribution, just upstream of the Thorofare Trail crossing (site Cub2) (Figure 2). Stream discharge measurements were only made twice at site Cub1, in 2016. One eDNA sample was collected at site Cub1 in April of 2017. Cub Creek was not sampled for the remainder of 2017 or in 2018 and all samples were eventually excluded from analysis because of missing stream discharge measurements.

Bridge Creek. Located at the northern end of Yellowstone Lake and adjacent to Bridge Bay Marina, Bridge Creek is accessible from the Grand Loop Road (Figure 1). Bridge Creek is one of the historically surveyed YCT spawning tributaries and an electronic fish-counting tube was used to verify survey counts on this stream from 1999 through 2004 (Koel et al. 2005). YCT spawners were observed from May through early June of 2016 and 2017 (Table 2).

The spawning reach of Bridge Creek was determined from historical spawning survey data and YNP BMO sampling protocols (Reinhart 1990, Table 1). Bridge Creek was sampled at one site directly upstream of the Grand Loop Road bridge crossing (site B2), one site at the upstream end of the spawning reach (site B3), and one site in-between (site B4) (Figure 2). Stream discharge was measured at site B2 during four sample collections in August through November of 2016. The relationship between stream depth measurements collected by the YNP BMO and actual stream discharge measurements in 2017 was used to estimate stream discharge on all sample dates in 2016 where stream depth measurements were collected. Site B2 discharge estimates were used for all upstream sample sites on a given date in 2016 if independent measurements or estimates were not available. Samples were also collected from two additional sites upstream of site B4 on three occasions in 2017 to document the presence or absence of YCT upstream of the spawning reach and one sample was collected at site B2 in January of 2017 to document the presence or absence of overwintering or resident YCT. Stream discharge was either measured or estimated using staff gauge measurements in 2017, except when the stream was snow covered. Site B2 discharge measurements or estimates were used for

all upstream sample sites on a given date if independent measurements or estimates were not available in 2017. Stream discharge measurements or estimates were not possible on three sample dates in January and early April because of snow cover. Bridge Creek was not sampled in 2018 because extremely high spring water levels made accurate discharge measurements and visual surveys impossible.

Hatchery Creek. Located in the Lake Village area at the northern end of Yellowstone Lake, Hatchery Creek is one of the historically surveyed YCT spawning tributaries (Figure 1). Hatchery Creek is a small stream with a mean wetted width of 0.73 meters (Table 2). Samples were collected at two sites on Hatchery Creek; site H1 was located immediately upstream of the Yellowstone Lake Road and site H2 immediately upstream of Lake Village Road (Figure 2). No YCT spawners were visually observed in Hatchery Creek during visual surveys in 2016 and 2017.

Arnica Creek. Arnica Creek is located at the northeast end of the West Thumb basin of Yellowstone Lake and can be accessed from the Grand Loop Road (Figure 1). An electronic fish counter was used on this stream for several years and in 2002, 455 YCT spawners were counted as they migrated upstream (Koel et al. 2003). Yellowstone Cutthroat Trout spawners were visually observed in Arnica Creek from early May through mid-June in 2017 and 2018 (Table 2).

The spawning distribution of YCT in Arnica Creek was estimated to extend about 0.59 km upstream from the mouth judging from past sampling efforts and recent observations (Reinhart 1990, Table 1). The spawning reach was sampled as one section in 2017 with the first sample site about 100 m upstream of the stream mouth (site A1)

and one at the upstream end of the spawning distribution, just downstream of the Grand Loop Road crossing (site A2) (Figure 2). A fish weir and electronic counter (Smith-Root model SR 1101) were installed in 2018 and the spawning reach was subsequently split into two sections. Sites A1 and A2 were at the downstream and upstream ends of the reach and one site was added just upstream of the fish weir in the middle of the reach (site A6). Stream discharge measurements were collected in conjunction with every sample on Arnica Creek in 2017, and in 2018 stream discharge was measured in conjunction with most sample collections, but was estimated using staff gauge measurements twice at site A6 and once at site A2.

Little Thumb Creek. Located in the West Thumb of Yellowstone Lake and accessible from the Grand Loop Road, Little Thumb Creek is one of the historically surveyed YCT spawning tributaries (Figure 1). Historically, bears often preyed on YCT spawners in this stream (Reinhart 1990). After several years with no activity, grizzly and black bears were once again observed fishing on this stream in 2016 and to a lesser extent in 2017. Spawning YCT were visually observed in Little Thumb Creek from mid-May through late June in 2016 to 2018 (Table 2).

The 0.56-km spawning reach of Little Thumb Creek was determined from historical spawning survey data and YNP BMO protocols (Reinhart 1990) (Table 1). The spawning reach was split into two sections with a sample site about 100 m upstream of the stream mouth and just upstream of the Grand Loop Road crossing (site LT2), one in the middle of the reach (site LT4), and one at the upstream end of the spawning distribution (site LT5) (Figure 2). Site LT5 is above a presumed fish barrier and in some

sample years the site was dry part way through the sampling season. One additional sample was collected from downstream of the road culvert with the intent of detecting eDNA from a grizzly bear that had been fishing in that area. Stream discharge was not measured or estimated on any dates at these sites in 2016 except August through November at site LT2. High Yellowstone Lake water levels in 2017 caused site LT2 to back up and pool whereas it flowed at the same location in 2016. Because the sample protocol (Carim et al. 2016) calls for sampling flowing water, site LT2 was not sampled after the lake backed up into it. A new site (site LT3) was established about 50 m upstream and was used for the remainder of 2017 and all of 2018. One sample was collected at site LT2 in January of 2017 to document the presence of overwintering or resident YCT. Stream discharge was measured when possible in 2017 and 2018 and was otherwise estimated from the relationship between known discharge and stream depth measurements taken by the YNP BMO. No measurement or estimate was possible when the stream was snow covered early in the season. Fewer samples were collected at sites LT4 and LT5 early in the season in all years because of significant snow cover over the stream and from LT5 later in the season because the site went dry.

Lodge Creek. Located in the Lake Village area at the north end of Yellowstone Lake, Lodge Creek is one of the historically surveyed YCT spawning tributaries (Figure 1). Single spawning-size YCT were visually observed in Lodge Creek on four separate surveys from mid-May through late June in 2016 (Table 2).

The Lodge Creek spawning reach was determined from historical spawning survey data and YNP BMO protocols (Reinhart 1990). The spawning reach was split into

two sections with a sample site about 100 m upstream of the stream mouth (site L1), one in the middle of the reach (site L2), and one at the upstream end of the spawning distribution (site L3) (Figure 2). Stream discharge was not measured except at site L1 on four occasions in August through November. One sample was collected from site L1 in April of 2017 but because of the low numbers of spawners present in 2016 no samples were collected from Lodge Creek during the remainder of 2017 or in 2018.

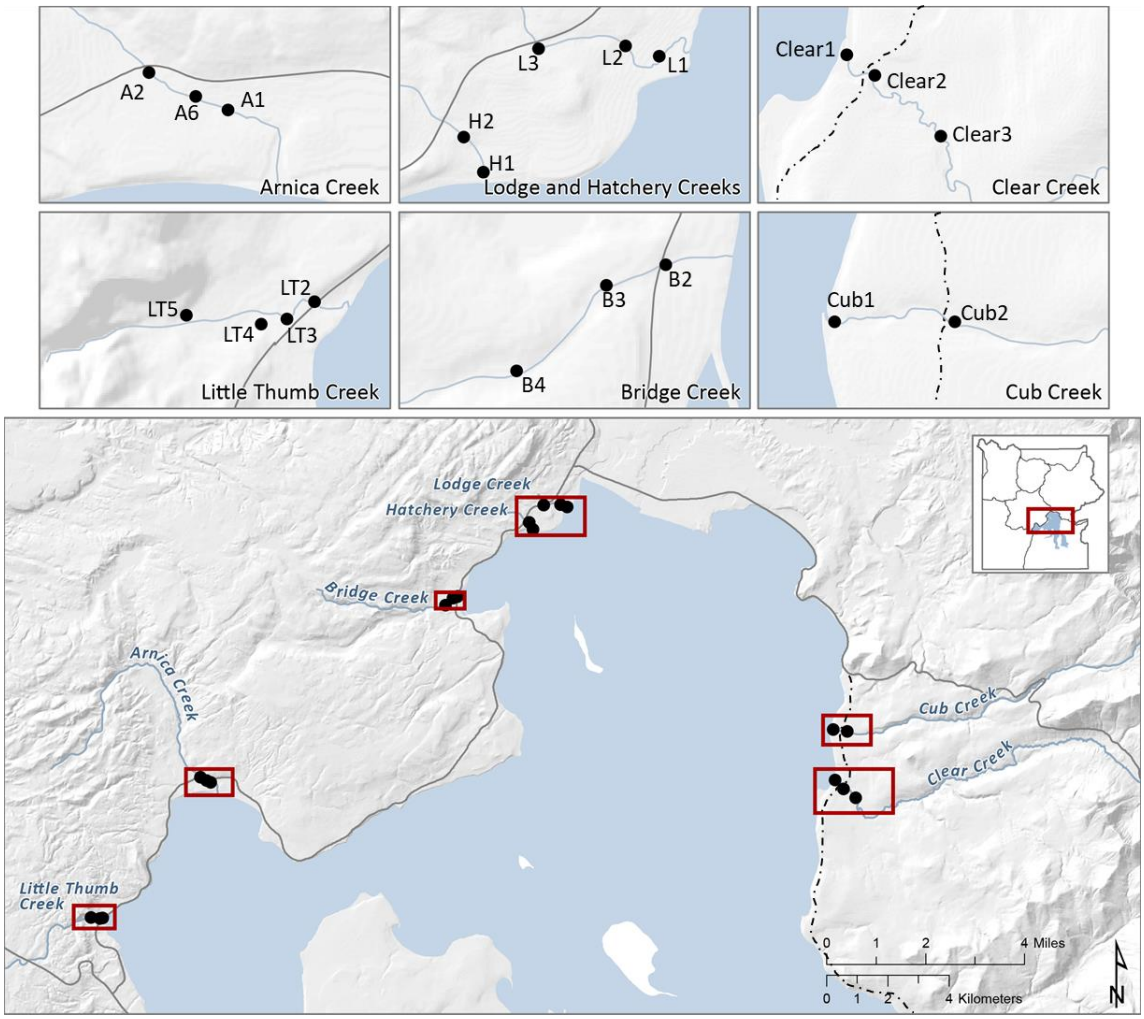


Figure 2. Sample site locations on each of the seven tributaries around Yellowstone Lake, Yellowstone National Park, where eDNA samples were collected. Roads are indicated as solid gray lines, trails by black dashed lines, and sample sites by solid black circles.

Trout Lake Inlet Spawning Stream

Trout Lake is located within the Soda Butte Creek drainage less than 1 km from the Northeast Entrance Road in the northeast corner of YNP (Figure 2). At 2,073 meters (Schreier 1997), the elevation of Trout Lake is similar to that of Yellowstone Lake, but at 5.25 ha it is much smaller in size. The water temperature of the inlet stream ranges from 2 to 12°C in summer and YCT move into the inlet to spawn in mid to late June, several weeks to a month later than in Yellowstone Lake tributaries (Table 2). The inlet stream is rarely turbid and therefore facilitates accurate visual surveys. Moreover, the number of fish that spawn each year is consistently higher than the numbers that currently spawn in many Yellowstone Lake tributaries. Trout Lake was stocked with native YCT from 1881 until 1931 and Rainbow Trout *Oncorhynchus mykiss* from 1931 until 1955 and both species are now well established in the lake (Varley 1981). The YCT population in Trout Lake was suspected to be hybridized because of the presence of Rainbow Trout, but genetic analyses in 2007 indicated that both pure Rainbow Trout and pure YCT were present in the lake (Koel et al. 2010). Trout Lake was sampled in 2018 to address the same objectives as the Yellowstone Lake tributaries, but in a system that maintains more consistent flows and numbers of spawners, regardless of snow and rain levels. It therefore offered a best-case scenario for assessing the relationship between spawner abundances and eDNA concentrations. Trout Lake is also frequented by otters and provided the opportunity to collect otter eDNA samples.

The spawning reach at Trout Lake was determined by habitat as a probable barrier to upstream fish movement is present 0.15 km from the mouth of the inlet. The spawning

reach was split into two sections with one sample site located about 20 m upstream of the lake and upstream of a small footbridge (site TL1), one site in the middle of the reach (site TL2), and one site above the upper end of the spawning reach and upstream of a steep cascade and probable fish barrier (site TL3). One additional sample was collected at a site upstream of TL3 to document the presence or absence of resident YCT and one from the outlet of the lake to document the presence of North American river otter eDNA. Stream discharge was measured concurrently with all samples.

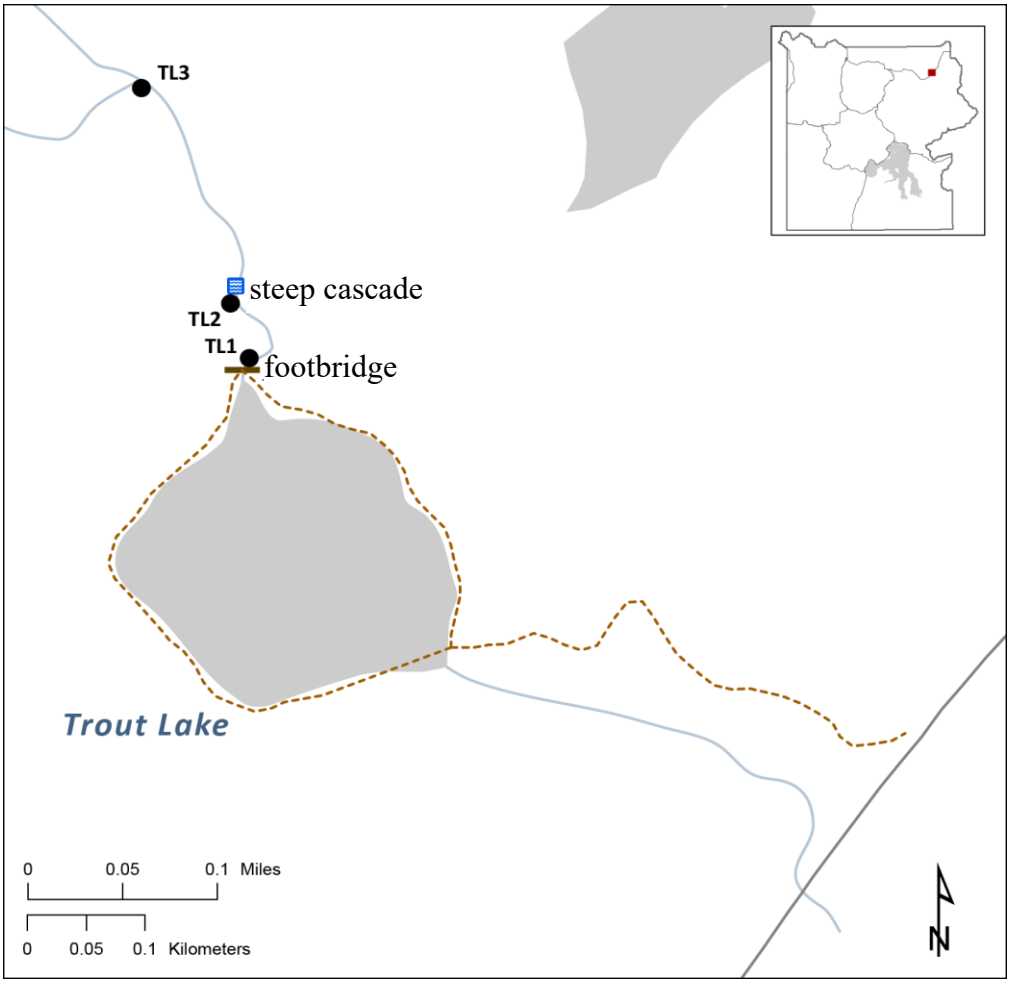


Figure 3. The locations of the three eDNA sample sites on the inlet to Trout Lake, Yellowstone National Park. The footbridge downstream of the first sample site and the steep cascade downstream of the third site are also denoted. The hashed brown line represents an established hiking trail.

Table 1. Streams, sites, and years sampled. Total visual survey distance upstream from each site is in parentheses. Samples collected refers to eDNA samples; the number of visual surveys conducted in conjunction with eDNA sample collection is in parentheses. Stream, site, and year combinations included in final analyses are highlighted in gray.

| | | UTM | | | | | |
|---|---|---|---|---|---|---|---|
| Stream | Site | Easting | Northing | Visual survey (km) | Year sampled | Months sampled | Samples collected |
| Arnica | A1 | 536765 | 4924827 | 0.59 | 2017 | Apr. – Oct. | 24 |
| (0.59 km) | | | | 0.24 | 2018 | May – Oct. | 19 |
| | A6 | 536623 | 4924886 | 0.35 | 2018 | May – Oct. | 19 |
| | A2 | 536420 | 4924991 | 0.14 | 2017 | May – Jun. | 8 |
| | | | | – | 2018 | May – Oct. | 16(0) |
| Bridge | B2 | 544780 | 4930874 | 0.22 | 2016 | Apr. – Nov. | 14(11) |
| (0.88 km) | | | | 0.22 | 2017 | Apr. – Oct. | 48(44) |
| | B3 | 544648 | 4930837 | 0.66 | 2016 | May – Jun. | 7 |
| | | | | 0.66 | 2017 | May – Jun. | 6 |
| | B4 | 544404 | 4930604 | 0.18 | 2016 | May – Jun. | 4 |
| | | | | 0.18 | 2017 | Jun. | 4 |
| Clear | Clear1 | 557090 | 4924912 | 0.33 | 2016 | Apr. – Oct. | 15(11) |
| (1.55 km) | | | | 0.33 | 2017 | Apr. – Oct. | 48(43) |
| | Clear2 | 557290 | 4924766 | 1.23 | 2016 | Apr. – Jul. | 11(8) |
| | | | | 1.23 | 2017 | Apr. – Jul. | 12(11) |
| | Clear3 | 557770 | 4924326 | 0.10 | 2016 | May – Jun. | 5(0) |
| | | | | 0.10 | 2017 | May – Jul. | 8(7) |
| Cub | Cub1 | 556963 | 4926490 | 0.84 | 2016 | Apr. – Oct. | 12(11) |
| (0.84 km) | Cub2 | 557496 | 4926491 | 0.20 | 2016 | May – Jul. | 6(4) |
| Hatchery | H1 | 547254 | 4933075 | 0.32 | 2016 | Apr. – Nov. | 12(7) |
| (0.16 km) | | | | 0.16 | 2017 | Apr. – Nov. | 18(15) |
| | H2 | 547129 | 4933296 | 0.23 | 2016 | May – Jun. | 4 |
| Little | LT2 | 533344 | 4920482 | 0.39 | 2016 | Apr. – Nov. | 15(11) |
| Thumb | LT2/LT3 | | | 0.39 | 2017 | Apr. – Nov. | 20(15) |
| (0.56 km) | LT3 | 533238 | 4920416 | 0.22 | 2018 | May – Oct. | 18 |
| | LT4 | 533140 | 4920395 | 0.34 | 2016 | Apr. – Jun. | 7(6) |
| | | | | 0.34 | 2017 | May – Jul. | 9 |
| | | | | 0.34 | 2018 | May – Oct. | 17 |
| | LT5 | 532854 | 4920430 | – | 2016 | May | 1(0) |
| | | | | – | 2017 | Jun. | 3(2) |
| | | | | – | 2018 | May – Jul. | 5(0) |
| Lodge | L1 | 548369 | 4933809 | 0.54 | 2016 | May – Nov. | 11(8) |
| (0.67 km) | L2 | 548155 | 4933874 | 0.67 | 2016 | Apr. – Jun. | 8(7) |
| | L3 | 547603 | 4933857 | – | 2016 | May – Jun. | 4(1) |
| Trout | | | | | | | |
| Lake | TL1 | 568674 | 4972448 | 0.095 | 2018 | Jun. – Oct. | 17 |
| (.15 km) | TL2 | 568658 | 4972496 | 0.057 | 2018 | Jun. – Oct. | 17 |
| | TL3 | 568673 | 4972543 | – | 2018 | Jun. – Oct. | 14(1) |

Table 2. Sampled mean and range of wetted width, stream discharge, and number of YCT spawners visually observed at each stream within the study area.

| | Wetted width (m) | | Discharge (CMS) | | YCT spawners | |
|---|---|---|---|---|---|---|
| Stream | Mean | Range | Mean | Range | Mean | Range |
| Arnica Creek | 2.88 | 2.00 – 4.60 | 0.33 | 0.08 – 1.06 | 10 | 0 – 27 |
| Clear Creek | 10.91 | 6.80 – 12.50 | 5.90 | 0.11 – 13.50 | 11 | 0 – 129 |
| Cub Creek | 3.82 | 3.40 – 4.23 | 0.12 | 0.05 – 0.17 | 3 | 0 – 9 |
| Bridge Creek | 1.24 | 0.42 – 1.50 | 0.11 | 0.001 – 0.56 | 4 | 0 – 20 |
| Hatchery Creek | 0.73 | 0.60 – 0.95 | 0.02 | 0.002 – 0.06 | 0 | 0 |
| Little Thumb Cr. | 1.80 | 0.40 – 4.30 | 0.15 | 0.0004 – 1.04 | 16 | 0 – 47 |
| Lodge Creek | 1.45 | 1.30 – 1.50 | 0.15 | 0.02 – 0.24 | 1 | 0 – 1 |
| Trout Lake inlet | 1.81 | 1.20 – 2.70 | 0.18 | 0.07 – 0.30 | 35 | 0 – 90 |

## Methods

### Visual surveys

Visual survey counts of YCT spawners were completed in conjunction with eDNA sample collections. Surveys were completed by 2 to 4 people walking slowly upstream along each bank and visually counting YCT spawners. Visual survey data were recorded separately by stream section: sample site 1 to site 2, and sample site 2 to site 3. Fish numbers were recorded separately by size classes estimated by observers: age 0, 50-100 mm, 101-150 mm, 151-200 mm, and spawning-sized adults. The accuracy of visual surveys depended on water clarity and the likelihood of being able to see the fish and was not assessed in this study because of the failure of independent counting methods (i.e., electronic counters and sonar unit). Because of their large size and bright coloration, counts of spawners were most accurate. Visual survey completion was contingent upon the absence of bears and associated safety concerns. Visual surveys were never conducted without a corresponding eDNA sample, but eDNA samples were collected

without a corresponding visual survey in some circumstances (i.e., at the upstream end of some survey reaches, in the presence of bears, or when snow completely covered the stream; Table 1). Signs or sightings of bear and otter, including tracks, scat, hair, or partially consumed fish, were recorded whenever visual surveys for fish were conducted.

Independent YCT Counts

Independent counts of YCT spawners using a DIDSON sonar unit were unsuccessful in all years of this study and were therefore excluded from the analyses. The Yellowstone National Park Native Fish Conservation program has attempted to count upstream migrating YCT in Clear Creek with a sonar unit since 2013 (Koel et al. 2015). Volunteers were stationed at the Clear Creek cabin to monitor the sonar unit and troubleshoot ongoing problems in 2016 and 2017. These volunteers assisted with visual surveys and daily eDNA sample collection on Clear Creek in 2017. However, because of consistent power failures and software malfunctions, the DIDSON sonar unit in Clear Creek was unsuccessful at providing independent counts of YCT spawners in any year of operation, including study years 2016 and 2017. Clear Creek was the largest stream in this study and the water is often turbid and the stream discharge is high during the YCT spawning season, making accurate visual surveys of YCT spawners difficult without a method for independent verification. Therefore, samples from Clear Creek were not included in the analyses.

Electronic fish counters were installed on Bridge and Arnica creeks in 2017 and on Arnica Creek in 2018, but complete counts were not obtained on either stream or in either year. Fluctuating water levels repeatedly rendered the counter in Bridge Creek

inoperable in 2017. Because of similar water level predictions, the counter was not installed on Bridge Creek in 2018. A second counter was installed on Arnica Creek in 2017 and 2018, but because of difficulties calibrating the unit and fluctuating water levels, counts of YCT spawners were not obtained in either year.

Sample Collection

We used established eDNA sample collection methods (Carim et al. 2016). A single-use sample kit contained within an individual sterile plastic bag and consisting of a pair of latex gloves, tweezers, filter cup, filter, and silica bag was used at each sample site. All filters and associated material were sterilized, packaged, and distributed by the United States Forest Service Rocky Mountain Research Station in Missoula, Montana. We discarded any kit that was suspected of contamination for any reason before, during, or after sample collection (e.g., collector touched gloves to waders, filter was dropped, bag was opened before reaching the sample site).

Each sample was collected from a well-mixed portion of the stream with flowing water using a peristaltic pump (GeoTech, Denver, Colorado) to draw water through a 1.5-µm glass microfiber filter (Whatman® 1827-047 Glass Microfiber Binder-Free Filter). The filter was held in place by a single-use plastic filter-holder cup that was used to scoop water from the creek or was held in the stream until the appropriate volume of water had been filtered. The amount of water filtered in each sample was dependent on the turbidity of the water and the frequency with which the filter became clogged. If one filter became clogged, a second filter was used. No more than 6 L of water were filtered and no more than two filters were used per sample. The water was filtered on site; the

only material removed from each site was that which ended up on the filter. After each sample was collected, tweezers were used to remove and fold the filter in half. The filter was then placed in a Ziplock plastic bag with silica, the bag was placed in an envelope, and all envelopes were placed in a -80°C freezer until analysis.

Stream discharge ($m^3/s$, CMS) were measured concurrently with eDNA sampling when possible. Stream discharge was calculated based on stream velocities measured with a Marsh McBirney Flow-Mate (Model 2000 portable flowmeter), stream depths measured with a staff rod, and wetted stream widths measured with a measuring tape. Stream discharge measurements were taken at a consistent location at each site, staff gauges were installed on two streams (Bridge and Clear Creeks), and an existing staff gauge was used on Arnica Creek. Stream discharge at sample site 1 on each tributary and at each individual site was calculated when possible in 2017 and 2018. Rating curves were developed from staff gauge heights (Arnica, Bridge, and Clear creeks) or BMO stream depth measurements and concurrent stream discharge calculations when possible and used to estimate stream discharge in the absence of velocity measurements.

Data Analyses

All samples were sent to the United States Forest Service Rocky Mountain Research Station, in Missoula, Montana, for analysis. Samples were analyzed using quantitative polymerase chain reaction (qPCR) and a standard curve (Wilcox et al. 2013). Some samples were depleted during analysis; any samples with usable material remaining were frozen and stored for future analyses. All samples were analyzed for YCT eDNA. Selected samples were analyzed for DNA from grizzly bear or river otter

because the abundance of these species in the Yellowstone Lake basin has been associated with trends in YCT abundance (Teisberg et al. 2014; Crait et al. 2015). Each eDNA sample was analyzed according to the detailed protocol prescribed by the United States Forest Service Rocky Mountain Research Station, Missoula, Montana (Wilcox et al. 2015; McKelvey et al. 2016).

Standardized eDNA concentrations (copies/L) were calculated by dividing the qPCR estimated number of YCT DNA copies per filter by the amount of water filtered in the field. This concentration was then multiplied by 1,000 to calculate copies/cubic meter (copies/$m^3$). Stream discharges probably affect eDNA concentrations in stream environments by way of dilution and estimates of fish abundance from eDNA are more accurate when eDNA concentrations are adjusted for discharge (Levi et al. 2019; Tillotson et al. 2018). Therefore, we calculated the number of eDNA copies passing the sampling location in one second by multiplying copies/$m^3$ by stream discharge ($m^3$/second), resulting in copies/s, hereafter "eDNA rate."

Environmental DNA rates (copies/second) and numbers of YCT spawners were ln-transformed to meet model assumptions of normality in the data and equal variances among groups (i.e. streams, sites, and presence of spawners). Two-sample t-tests were used to compare mean ln-transformed eDNA rates when spawning fish were and were not visually observed at each site within a year to determine if eDNA rates could be used to detect the presence of spawning YCT. One-way ANOVA was used to compare means in groups (spawner present or absent) from each stream at different site and year combinations. If a difference in mean eDNA rates among sites was detected, a Tukey

multiple-comparison post-hoc analysis was used to determine which mean or means were different from one another. Logistic regression was used to estimate the probability of YCT spawner presence across the range of observed eDNA rates. Environmental DNA rates and numbers of YCT observed were plotted at broad and fine temporal scales. Linear regression was used to model the relationships between eDNA rates and numbers of YCT spawners present and to predict numbers of YCT spawners from eDNA rates. Confidence intervals were used to evaluate whether slope and *y*-intercept estimates differed among stream, site, and year combinations and a one-way ANCOVA was used to compare slope and *y*-axis intercept estimates among years at a single stream and site combination. Data were formatted using the "dplyr" package (Wickham et al. 2020) in Program R and analyzed using Program R (R Core Team 2020).

## Results

### YCT Spawner Presence vs. Absence

Environmental DNA rates were higher when YCT spawners were present than when spawners were absent in all but one combination of stream, site, and year in Yellowstone Lake tributaries (Figure 4). Environmental DNA rates differed the most at Little Thumb site LT3 in 2017, where the median eDNA rate was 331 times higher when YCT spawners were visually observed than when they were absent (Table 3). Mean eDNA rates did not differ between samples when spawners were present and when they were absent at Arnica Creek site A6 in 2018 (Table 3) because the eDNA rate when only one spawner was present above site A6 was more consistent with the eDNA rates from

samples when spawners were absent than with other samples where spawners were present.

Environmental DNA rates differed between years, but not between sites in the same year, when YCT spawners were absent and did not differ among sites or years on the same stream when YCT spawners were present. Among the three Arnica Creek site and year combinations (A1 in 2017, A1 in 2018, and A6 in 2018), significant differences existed between mean eDNA rates at site A1 in 2017 versus sites A1 and A6 in 2018, but not between sites A1 and A6 in 2018 (Table 4). The mean eDNA rates did not differ among the three Arnica Creek sites when YCT spawners were present (Table 5). Mean eDNA rates did not differ between sites in Little Thumb Creek in 2017 when YCT spawners were either present or absent (Tables 4 and 5).

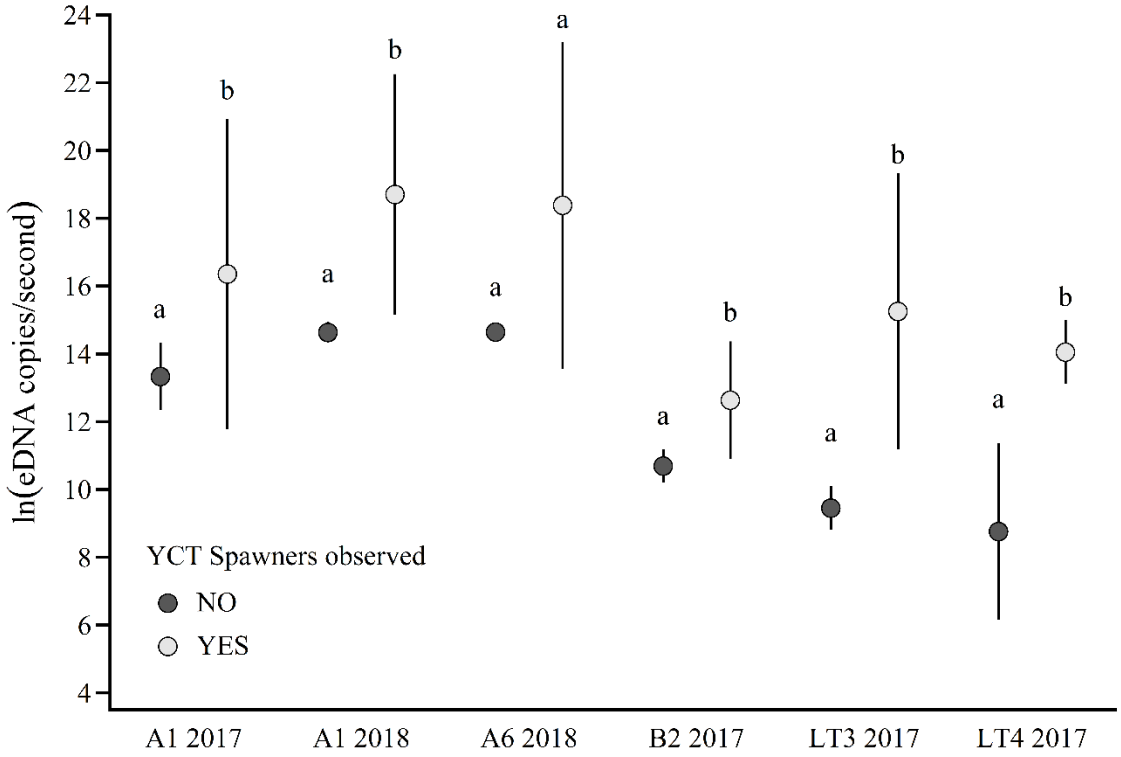


Figure 4. Mean ln-transformed eDNA rates when YCT spawners were not (dark gray circles) and were visually observed (light gray circles) at Yellowstone Lake sites, 2017-2018. The vertical lines represent 95% confidence intervals. Site locations (A1, A6, B2, LT3, LT4) refer to Figure 2 and Table 3. Different letters (a-b) indicate a significant difference in mean ln-transformed eDNA rate between groups (spawners observed or not) at each combination of stream, site, and year.

Table 3. Median differences in eDNA rates when spawners were present versus absent at each combination of stream, site, and year.

| Location | Median eDNA rate difference | df | *t* | *P* |
|---|---|---|---|---|
| Arnica Cr. A1 2017 | 19.60 | 14 | -2.8200 | 0.0136 |
| Arnica Cr. A1 2018 | 57.50 | 17 | -8.8293 | < 0.0001 |
| Arnica Cr. A6 2018 | 41.04 | 3 | -2.4635 | 0.0902 |
| Bridge Cr. B2 2017 | 5.96 | 15 | -3.7412 | 0.0020 |
| Little Thumb Cr. LT3 2017 | 330.61 | 13 | -7.9278 | < 0.0001 |
| Little Thumb Cr. LT4 2017 | 197.31 | 7 | -3.5538 | 0.0093 |
| Trout Lake TL1 2018 | 323.40 | 15 | -4.4742 | 0.0005 |
| Trout Lake TL2 2018 | 788.52 | 15 | -2.9647 | 0.0096 |

Table 4. Median differences in eDNA rates between sites when no spawners were visually observed. Environmental DNA rates from both pre- and post-spawn were included.

| Location | Comparison | Difference in median eDNA rate | df | *t* | *P* |
|---|---|---|---|---|---|
| Arnica Creek | A1 2018 – A1 2017 | 2.6758 | 27 | -2.9580 | 0.0064 |
| Arnica Creek | A6 2018 – A1 2017 | 2.7107 | 13 | -2.8347 | 0.0144 |
| Arnica Creek | A6 2018 – A1 2018 | 0.0095 | 29 | -0.0566 | 0.9553 |
| Little Thumb Cr. | LT3 2017 – LT4 2017 | -0.4976 | 16 | 0.8489 | 0.4085 |
| Trout Lake | TL2 2018 – TL1 2018 | -0.9375 | 22 | 1.7692 | 0.0907 |

Table 5. Median differences in eDNA rates between sites when spawners were visually observed.

| Location | Comparison | Difference in median eDNA rate | df | *t* | *P* |
|---|---|---|---|---|---|
| Arnica Creek | A1 2018 – A1 2017 | 9.4362 | 4 | -1.7402 | 0.1568 |
| Arnica Creek | A6 2018 – A1 2017 | 6.5712 | 5 | -1.0109 | 0.3585 |
| Arnica Creek | A6 2018 – A1 2018 | -0.2745 | 5 | 0.1670 | 0.8739 |
| Little Thumb Cr. | LT4 2017 – LT3 2017 | -0.6996 | 4 | 1.2382 | 0.2833 |
| Trout Lake | TL2 2018 – TL1 2018 | -0.8478 | 8 | 2.9589 | 0.0182 |

The mean ln-transformed eDNA rates present in the Trout Lake inlet differed when spawners were present and absent at each site (Table 4, Figure 5). Mean ln-transformed eDNA rates did not differ between sites when YCT spawners were either present or absent (Tables 4 and 5).

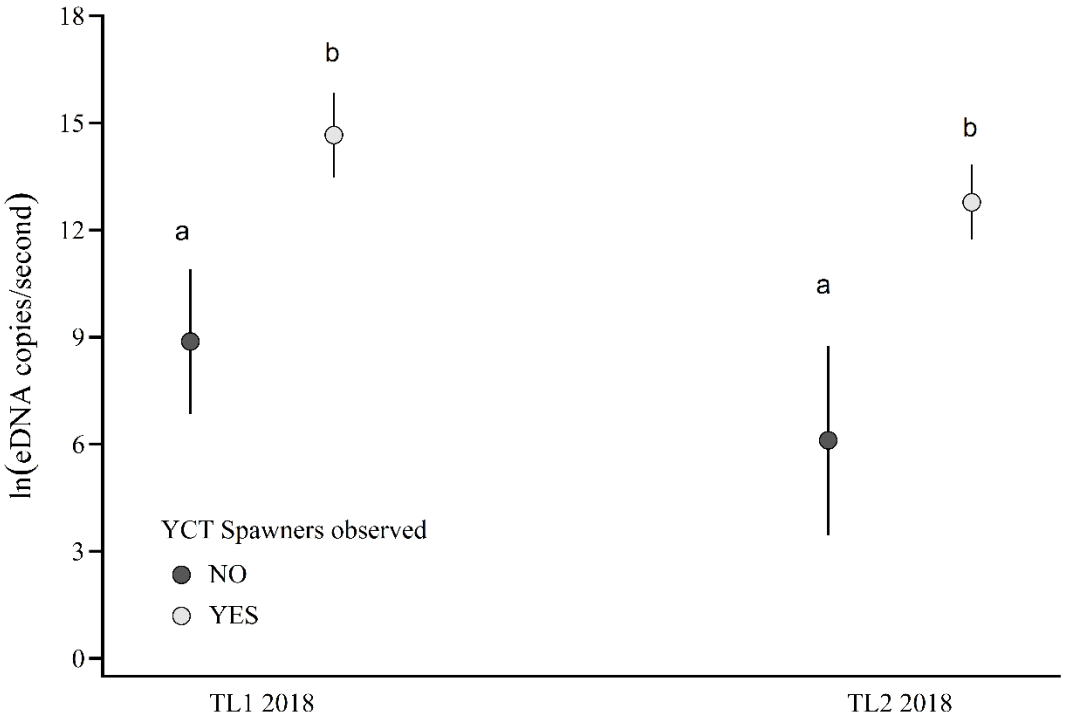


Figure 5. Mean ln-transformed eDNA rates when YCT spawners were not visually observed (dark gray circles) and when they were visually observed (light gray circles) at Trout Lake sites TL1 and TL2 in 2018. The vertical lines represent the 95% confidence intervals around the mean. Different letters (a-b) indicate a significant difference in mean ln-transformed eDNA rate between groups at each site. Site locations TL1 and TL2 refer to Figure 3 and Table 3.

The mean ln-transformed eDNA rates when spawners were present differed from those when spawners were absent when data from all combinations of stream, site, and year were combined (*t*-test: $t = -5.5129$; df = 127; $P < 0.0001$) (Figure 6). Median eDNA rates were 32 times higher when spawners were present. The logistic regression model of these combined data indicated that the probability of YCT spawner presence exceeded 0.50 when the ln-transformed eDNA rate was 9.1 copies/s, 0.80 at 13.4 copies/s, and 0.90 at 15.9 copies/s (Figure 7, $z = 4.43$, $P < 0.0001$).

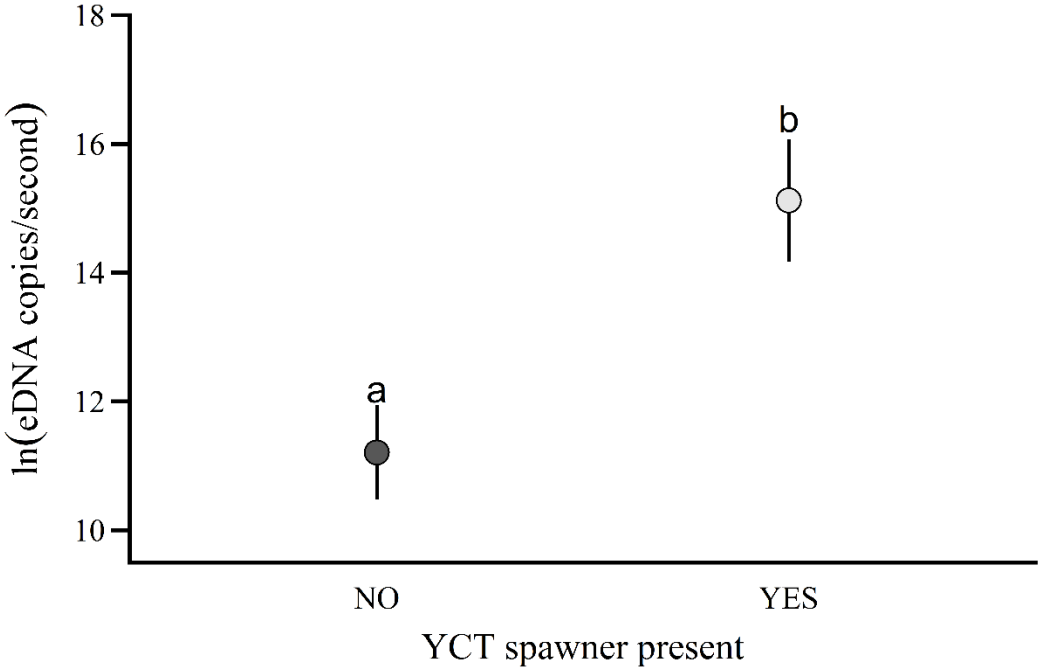


Figure 6. Mean ln-transformed eDNA rates when YCT spawners were not visually observed (dark gray circle) and when they were visually observed (light gray circle) with all combinations of stream, site, and year combined. The horizontal lines represent the 95% confidence intervals around the mean. Different letters (a-b) indicate a significant difference in mean ln-transformed eDNA rate between groups.

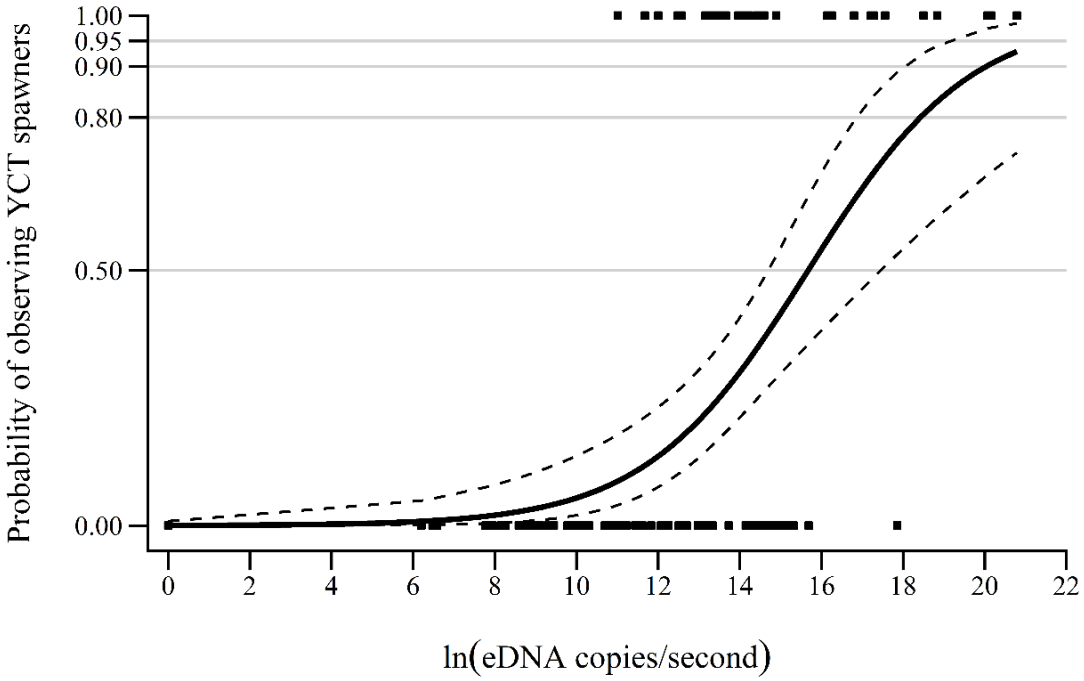


Figure 7. Probability of presence of YCT spawners (solid black line) in relation to ln(eDNA copies/s) with all combinations of stream, site, and year combined. The dashed lines represent the 95% confidence interval around the regression.

Temporal Trends

Temporal trends in eDNA rates generally followed the temporal trends in abundances of YCT spawners and age-0 fish. Environmental DNA rates at all combinations of stream, site, and year among Yellowstone Lake tributaries were higher when YCT spawners were present than when they were not and decreased after the spawning season, with subsequent, slight, increases coinciding with the presence of age-0 YCT at some sites (Figures 8 and 9). The highest eDNA rates at Little Thumb Creek site LT3 and Bridge Creek site B2 in 2017 corresponded to the highest numbers of YCT spawners present during the spawning season (Figure 9) and the highest numbers of age-0 YCT corresponded to maximum post-spawning season eDNA rates at Arnica Creek site

A1 and Bridge Creek site B2 in 2017 (Figures 8 and 9). However, among other stream, site, and year combinations, the highest eDNA rates did not coincide with the highest numbers of YCT spawners (Figures 8 and 9). Environmental DNA rates at all sites on Arnica and Bridge creeks and at site LT3 on Little Thumb Creek in 2017 never decreased to 0 copies/s, suggesting the contribution of eDNA from overwintering, resident, or age-0 YCT (or some combination thereof; Figures 8 and 9). Overall, eDNA rates at all Yellowstone Lake tributary sites and in all years reached highest values during the spawning season, whereas values pre- and post-spawning remained relatively constant.

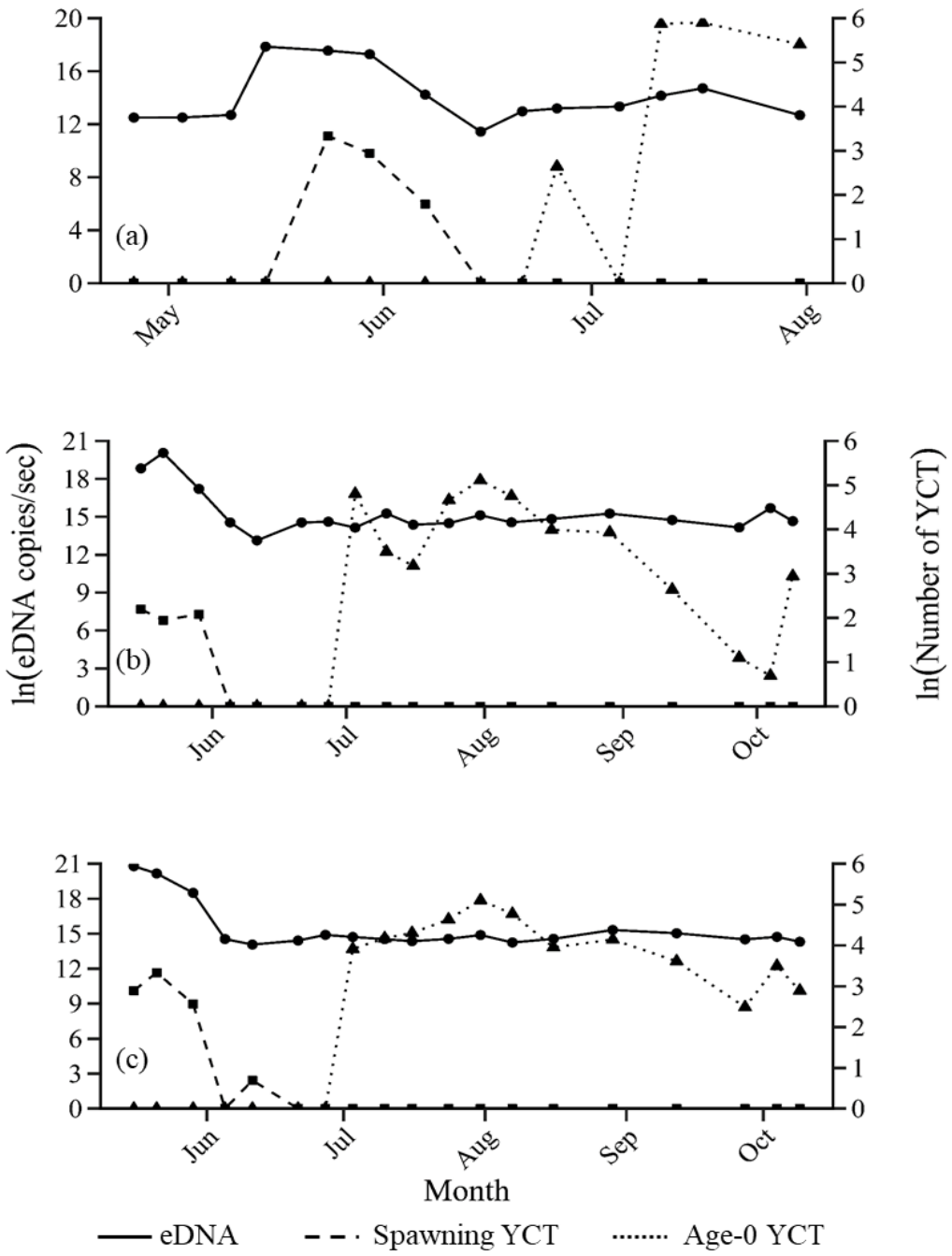


Figure 8. Ln-transformed YCT eDNA rates (copies/s) and ln-transformed numbers of YCT visually observed at Arnica Creek sites (a) A1 in 2017 and (b) 2018 and (c) A6 in 2018. Environmental DNA rates are on the left axis and represented by the solid line. Numbers of YCT observed are on the right *y*-axis. The dashed line and squares represent numbers of spawning-sized YCT; the dotted line and triangles represent the numbers of age-0 YCT observed.

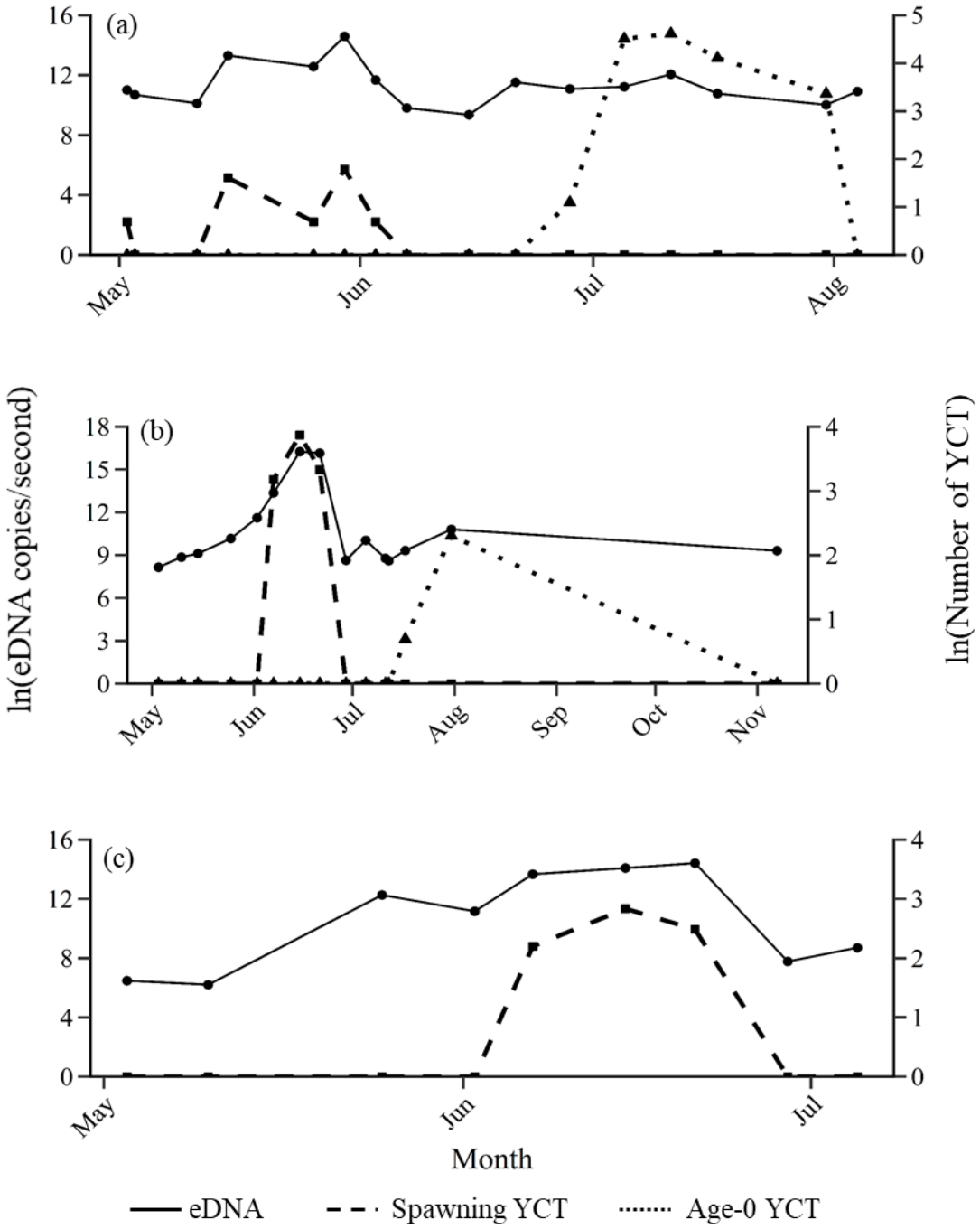


Figure 9. Ln-transformed YCT eDNA rates (copies/s) and ln-transformed numbers of YCT visually observed at (a) Bridge and (b) Little Thumb creeks sites LT3 and (c) LT4 in 2017. Environmental DNA rates are on the left axis and represented by the solid line circles. Numbers of YCT observed are on the right *y*-axis. The dashed line and squares represent numbers of spawning-sized YCT; the dotted line and triangles represent the number of age-0 YCT observed.

Environmental DNA rates increased when YCT spawners were present and decreased after the spawning season at both Trout Lake sites in 2018 (Figure 10). Ln-transformed eDNA rates of 0 copies/s were recorded at both sites before spawners were visually observed, suggesting no overwintering or resident fish were present at the time of sampling. Ln-transformed environmental DNA rates increased to a maximum of 16.80 and 13.47 copies/s at Trout Lake sites TL1 and TL2, respectively, during the spawning period. Environmental DNA rates subsequently decreased to a mean of 8.88 copies/s for the remainder of the sampling period at Trout Lake site TL1. The eDNA rate and numbers of YCT spawners concurrently decreased to 0 post-spawn at Trout Lake site TL2. Environmental DNA rates subsequently increased slightly, probably as a result of age-0 YCT present in the stream (although none were ever visually observed), before decreasing slightly in October.

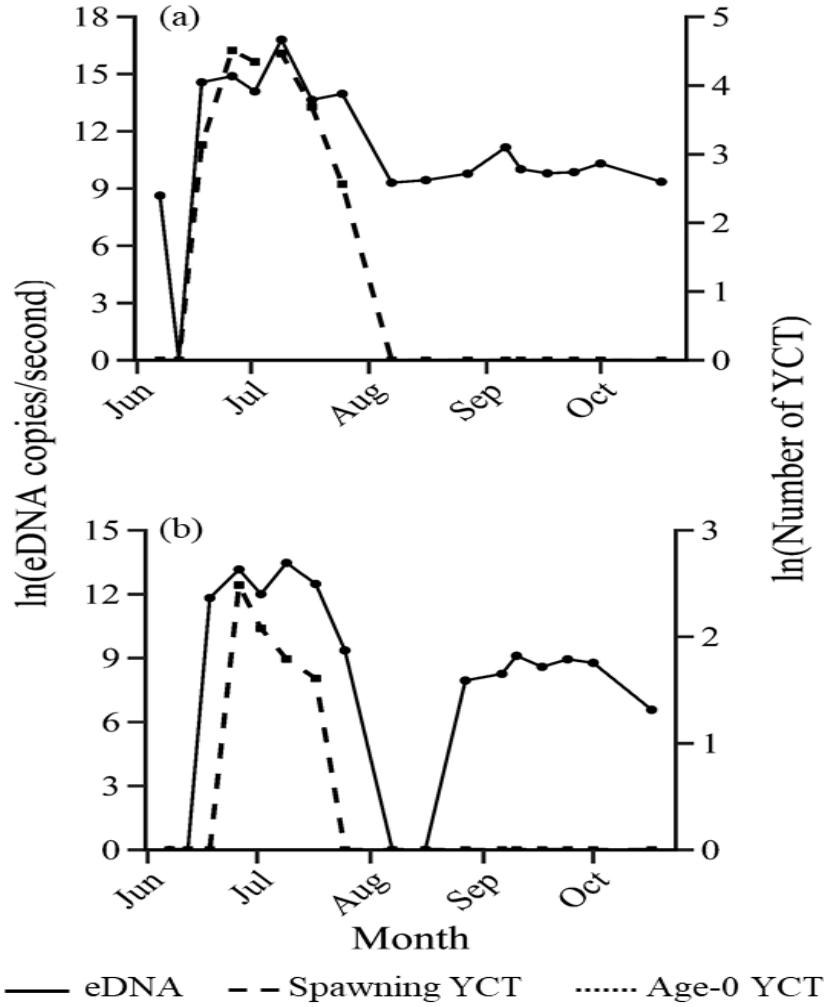


Figure 10. Ln-transformed YCT eDNA rates (copies/s) and ln-transformed numbers of YCT visually observed at Trout Lake sites (a) TL1 and (b) TL2 in 2018. Environmental DNA rates are on the left axis and represented by the solid line. Numbers of YCT spawners observed are on the right *y*-axis and represented by the dashed line and squares.

Ln-transformed eDNA rates did not vary significantly when sampled over a 24-h period or across consecutive days of sampling, regardless of the presence of spawner or age-0 YCT. Numbers of spawning size YCT visually observed ranged from 0 to 9 over one 24-h period on Bridge Creek and from 0 to 9 and 0 to 18 over 5 consecutive days at Bridge and Arnica creeks, respectively (Figures 11 and 12). Numbers of age-0 YCT

visually observed ranged from 20 to 81 over one 24-h period on Bridge Creek and from 0 to 80 and 30 to 222 over 5 consecutive days on Bridge and Arnica creeks, respectively (Figures 11 and 12). However, concurrent eDNA rates were stable in all of these situations (Figures 11 and 12). Significant differences in eDNA rates may become apparent when numbers of visually observed YCT present during eDNA sampling vary more.

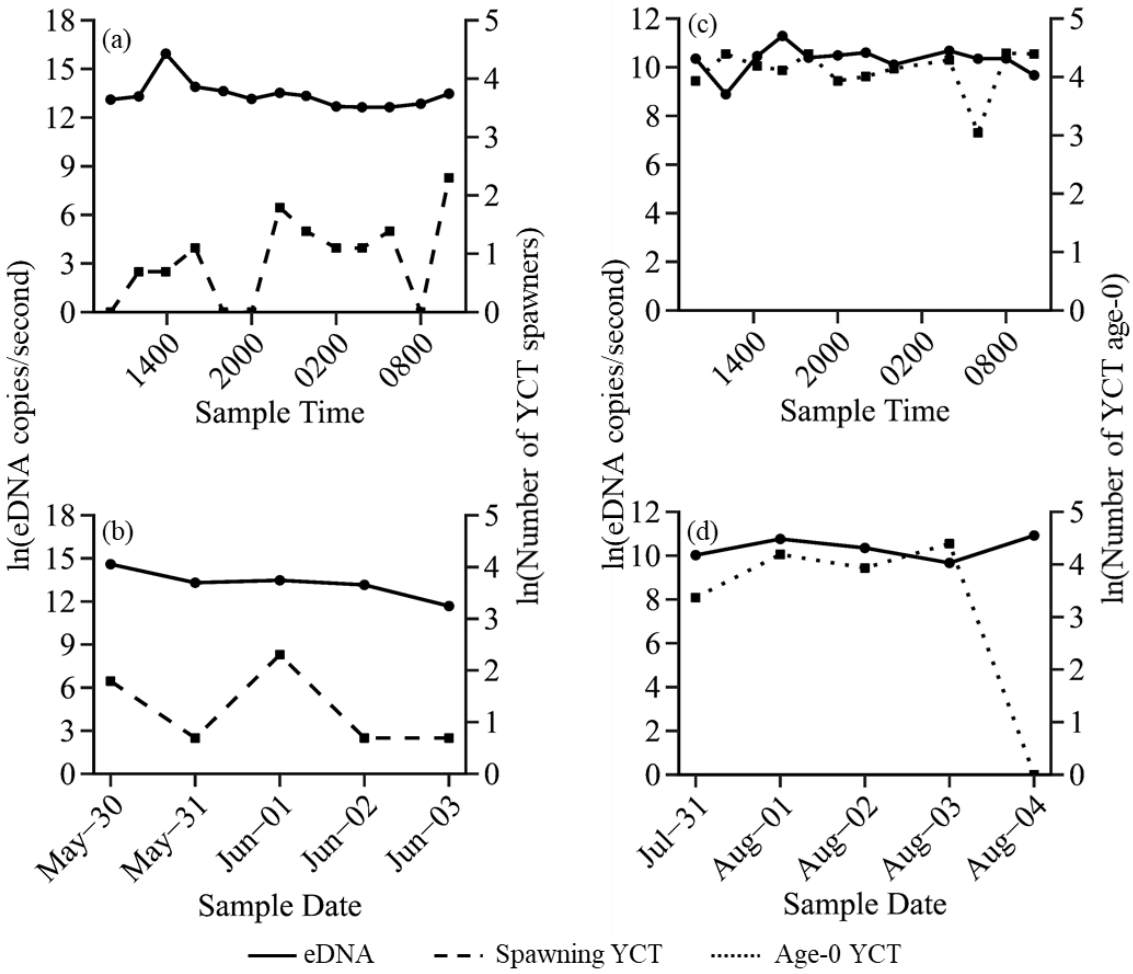


Figure 11. Ln-transformed YCT eDNA rates (copies/s) and ln-transformed numbers of spawning (a, b) and age-0 (c, d) YCT visually observed at Bridge Creek site B2 at 2-h intervals throughout one 24-h period (upper panels, a and c) and across five consecutive days (lower panels, b and d) in 2017.

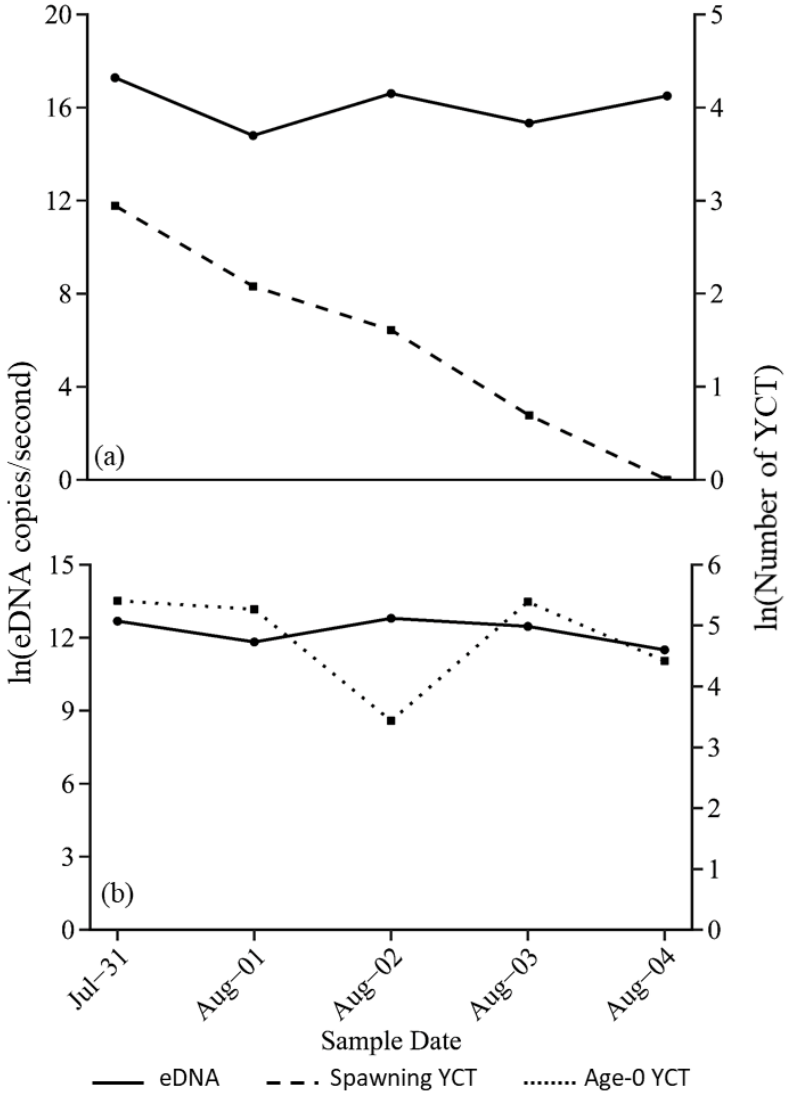


Figure 12. Ln-transformed YCT eDNA rates (copies/s) and ln-transformed numbers of (a) spawning and (b) age-0 YCT visually observed at Arnica Creek site A1 across five consecutive days in 2017.

Relationships between eDNA Rates and YCT Spawner Abundances

The slope of the positive relationships between eDNA rates and numbers of YCT spawners were consistent across sites, but background levels of eDNA varied (Figure 13). The 95% confidence intervals of the slope estimates at all sites overlapped, indicating

that the slopes of the relationships at all sites were not significantly different (Figures 13 and 14). However, the large confidence intervals around the slope estimates at most sites were probably the result of small sample sizes and few sample dates when spawners were visually observed (i.e., many zeroes) (Figures 13 and 14). Significantly different slopes among sites might have become apparent with larger non-zero sample sizes. The *y*-intercept estimates of the two Arnica Creek relationships (2017 and 2018) were not significantly different from one another, but were significantly different from those of other stream, site, and year combinations, suggesting that more eDNA was present in Arnica Creek when no YCT spawners were visible than in the other streams. No significant differences existed among the *y*-intercept estimates of all other relationships. The presence and abundance of overwintering or resident fish probably contributed eDNA that was not accounted for by the number of spawners counted and caused the differences in *y*-intercept estimates. When data from all combinations of stream, site, and year were combined, a positive relationship existed between ln-transformed eDNA rates and ln-transformed numbers of YCT spawners visually observed ($P < 0.0001$; Figure 14).

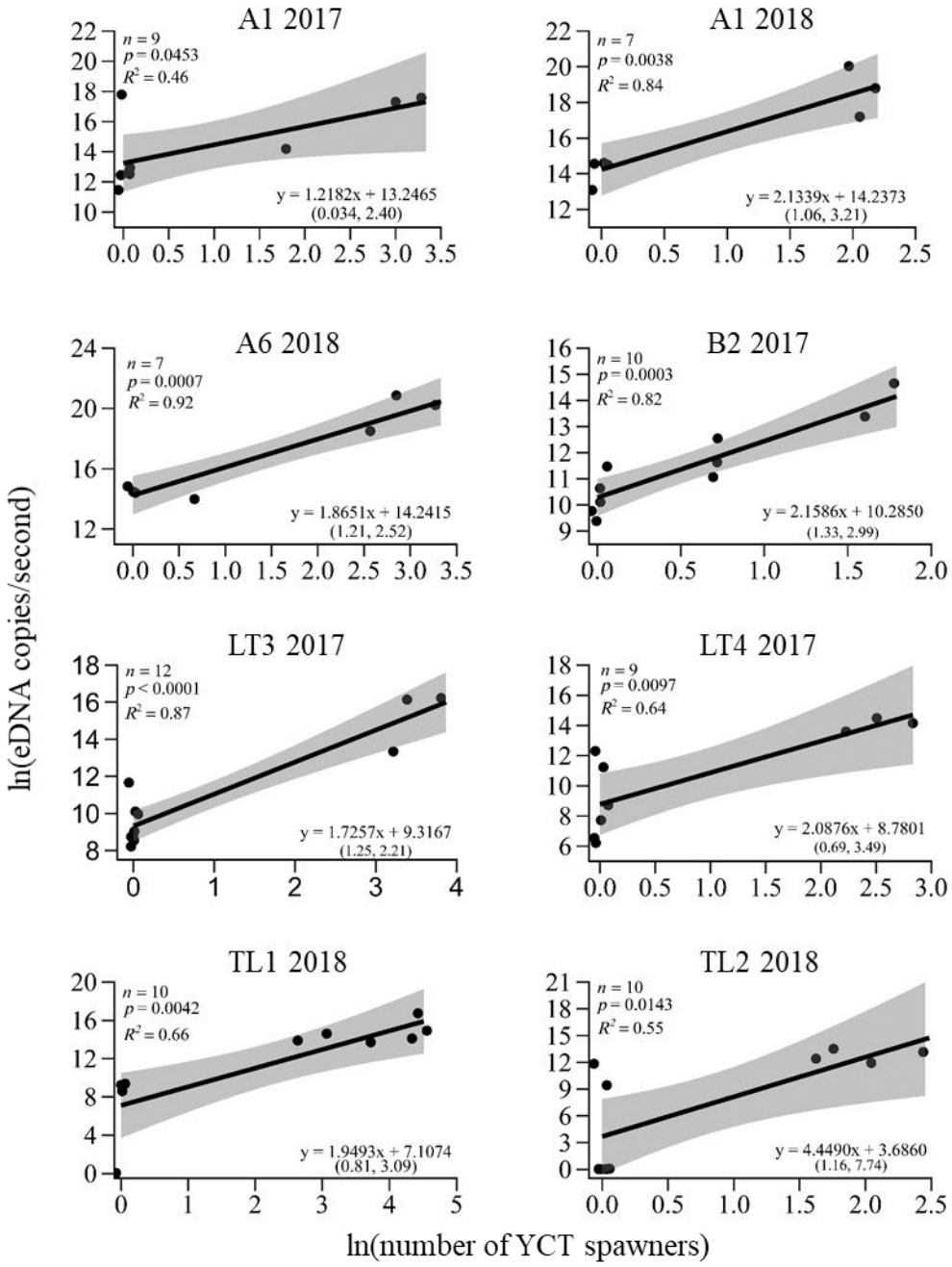


Figure 13. Relationships between ln-transformed eDNA rates (copies/s) and the ln-transformed numbers of YCT spawners visually observed at all site and year combinations, with 95% confidence intervals of the relationships in gray. Site locations refer to Figure 2. The 95% confidence limits of the slope estimates are below the equations in parentheses. Each plot represents a different combination of stream, site, and year.

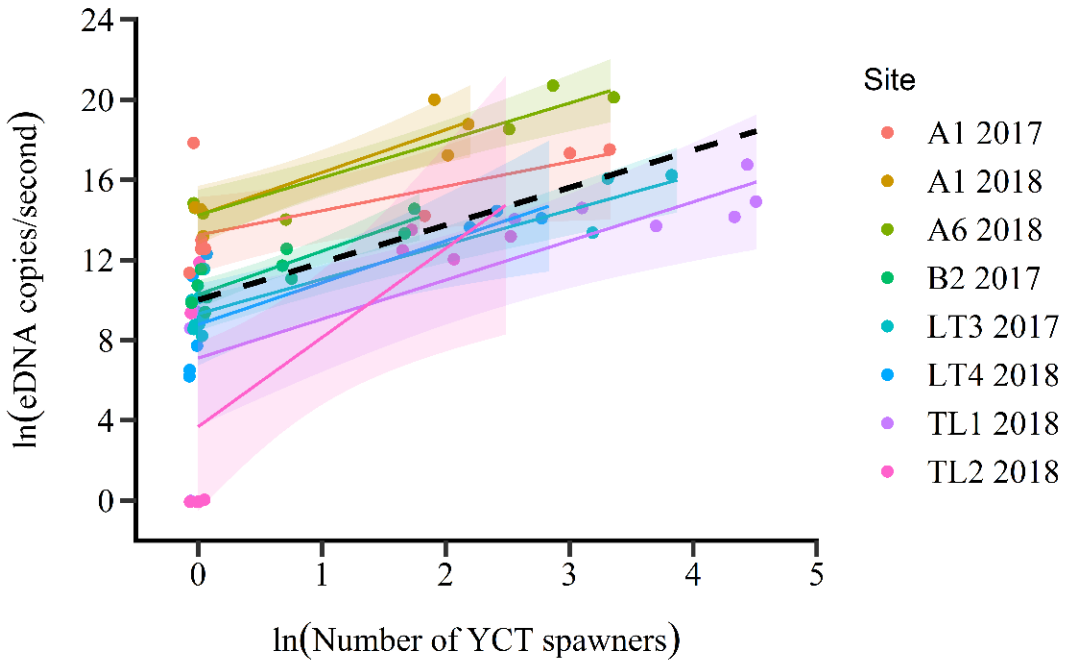


Figure 14. Relationships between ln-transformed numbers of spawners observed and ln-transformed eDNA rates (copies/s). Each color represents a different combination of stream, site, and year. The shading represents the 95% confidence intervals around the slope estimates. Site refers to Figures 2 and 3 and Table 4. The relationship for all combinations of stream, site, and year combined is represented by the dashed, black line.

The relationships between eDNA and numbers of YCT spawners at Arnica Creek site A1 were not significantly different between years (ANCOVA: F = 1.43; df = 1, 13; $P$ = 0.2532). The 95% confidence intervals of both the slope and $y$-intercept estimates of the 2017 and 2018 samples overlapped (Figure 15). Sample sizes were small ($n$ < 10) and YCT spawners were visually observed on less than five sample dates in each year. Significant differences in these estimates across multiple years may become apparent with larger sample sizes.

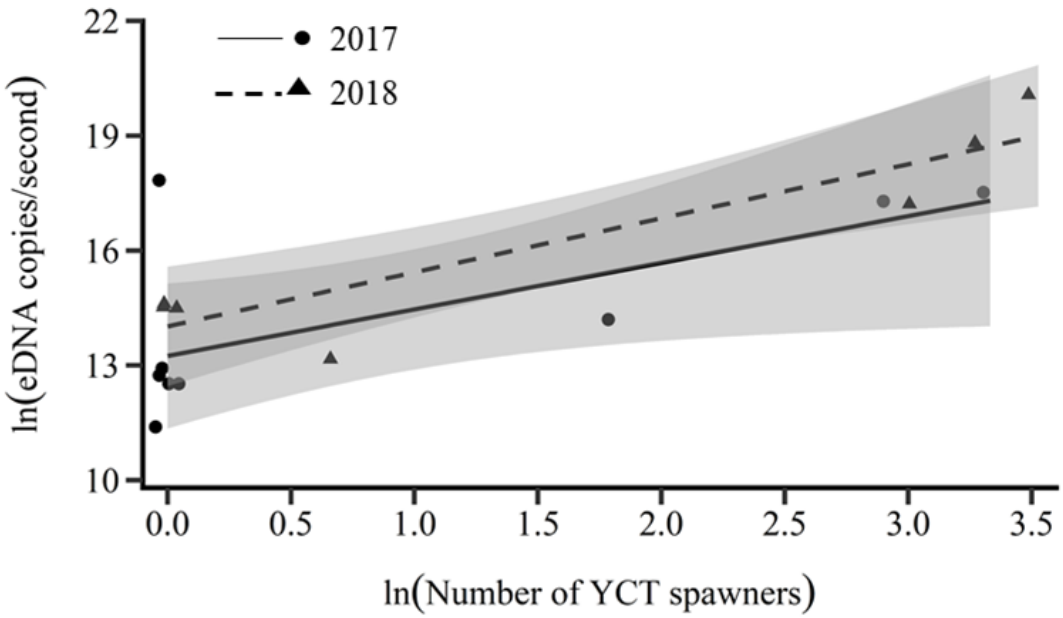


Figure 15. Relationships between ln-transformed numbers of YCT spawners visually observed and ln-transformed YCT eDNA rate (copies/s) at Arnica Creek site A1 in 2017 and 2018.

The ranges in the numbers of fish predicted by eDNA rates were wide and varied by site (Figure 16). For example, the 95% prediction interval at Bridge Creek site B2 ranged from 1 to 13, with a predicted number of 5 spawners present, but the 95% prediction interval at Trout Lake site TL1 ranged from 1 to 1451 spawners with a prediction of 57 (Figure 16 and Table 6). Small sample sizes and low numbers of fish visually observed at the time of sampling affected the amount of variance at all sites. Furthermore, maximum eDNA rates did not correspond with maximum numbers of visually observed YCT spawners at six of the eight sites. Observed numbers of YCT spawners were within the 80% prediction intervals of the maximum eDNA values at seven of the eight sites.

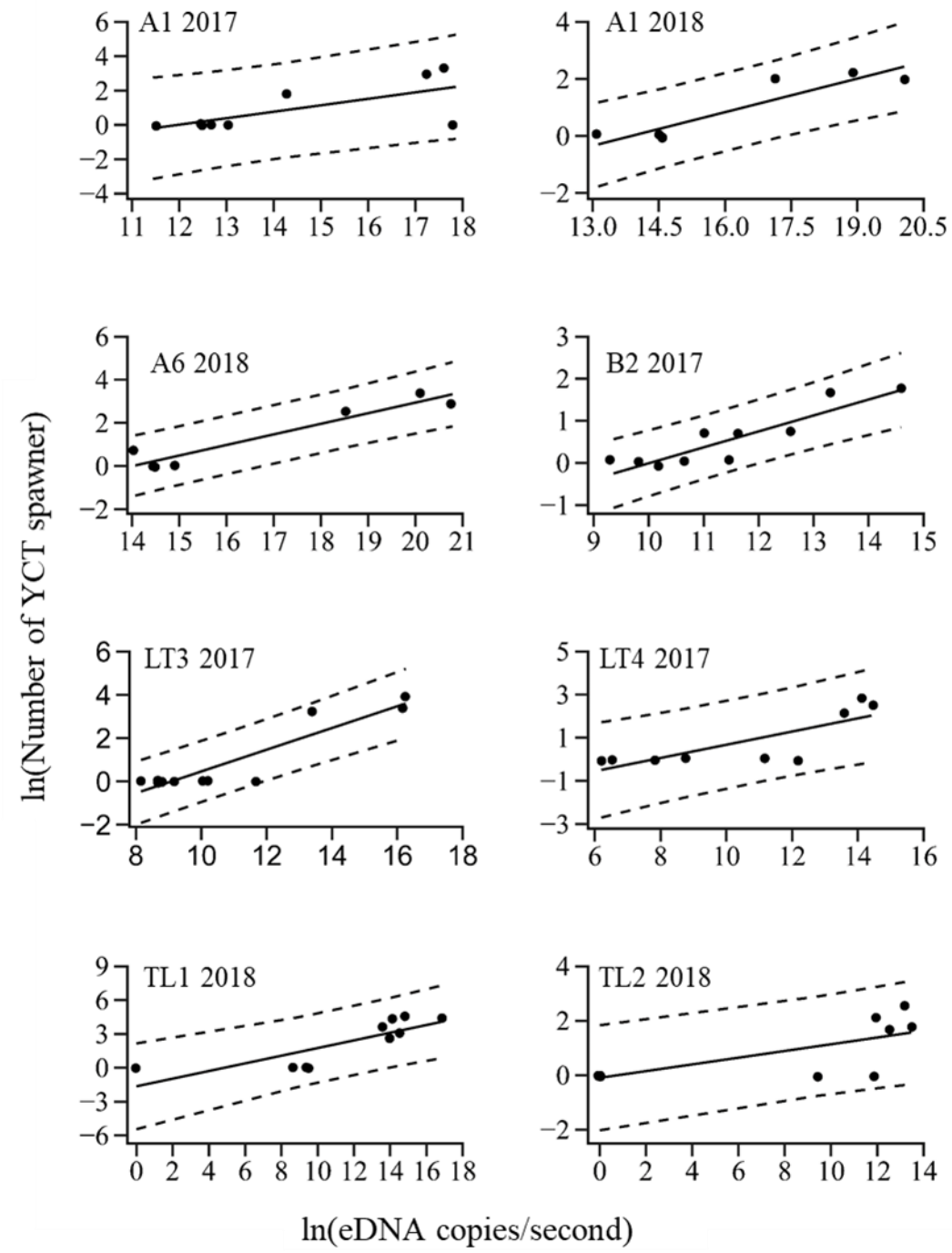


Figure 16. Relationships between ln-transformed YCT eDNA rate (copies/s) and ln-transformed numbers of YCT spawners visually observed at all site and year combinations, with 95% prediction intervals (dashed lines). Site locations refer to Figure 2.

Table 6. Observed and predicted number of YCT spawners at the maximum observed eDNA rate at each site, and associated 95%, 90%, and 80% prediction intervals.

| | eDNA copies/s | Number of YCT spawners | | Prediction Interval | | |
|---|---|---|---|---|---|---|
| Location | Observed | Observed | Predicted | 95% | 90% | 80% |
| Arnica A1 2017 | 56823105 | 0 | 8 | 0, 190 | 0, 103 | 1, 55 |
| Arnica A1 2018 | 515807961 | 6 | 10 | 1, 53 | 2, 38 | 4, 27 |
| Arnica A6 2018 | 1065322580 | 17 | 27 | 5, 123 | 8, 89 | 11, 65 |
| Bridge B2 2017 | 2169426 | 5 | 5 | 1, 13 | 2, 11 | 2, 9 |
| Little Thumb LT3 2017 | 11462393 | 47 | 35 | 6, 179 | 9, 133 | 13, 96 |
| Little Thumb LT4 2017 | 1818360 | 11 | 7 | 0, 66 | 0, 42 | 1, 27 |
| Trout Lake TL1 2018 | 19757759 | 86 | 57 | 1, 1451 | 3, 777 | 7, 407 |
| Trout Lake TL2 2018 | 706245 | 5 | 4 | 0, 31 | 0, 21 | 1, 14 |

eDNA Detection of Semi-Terrestrial Predators

Environmental DNA analysis of water samples from YCT spawning tributaries detected grizzly bear but not river otter. Grizzly bear eDNA was positively identified in 3 of the 16 samples analyzed for Little Thumb Creek; sightings or sign (i.e., tracks or scat) were associated with all 3 of the positive samples but not with any of the 13 negative samples. Grizzly bear eDNA was not detected in a sample taken at Clear Creek in association with a bear sighting (the night before the sample was collected) and tracks. No river otter eDNA was detected in 7 samples from the Trout Lake inlet, but river otters or their sign were never observed at the Trout Lake inlet during my study.

## Discussion

Environmental DNA rates were good indicators of YCT spawner presence, especially for individual streams, but with larger sample sizes a single eDNA rate indicative of spawner presence in any stream could probably be established. Collection of samples before and after the YCT spawning season to define a baseline level of eDNA in each stream of interest would probably allow for the most accurate determination of the eDNA rate indicative of the presence of YCT spawners there. Baseline eDNA rates varied across all combinations of stream, site, and year, but were most similar among sites on the same stream, indicating that this variation was probably caused by variation in the numbers of overwintering or resident YCT, or both, present in each stream. Although adult fish probably shed more eDNA per fish than juveniles, juvenile fish may shed eDNA at a higher rate per body weight than adult fish (Maruyama et al. 2014). Therefore, eDNA contributions from life stages other than spawners cannot be ignored. Because these smaller fish were difficult to accurately count during visual surveys, their presence was impossible to quantify during each sample visit without the aid of additional sampling methods such as electrofishing, which would have been time prohibitive in my study. Other factors that probably affected eDNA rates in each stream, but that were not accounted for in my study, include the proximity of fish to the sample site (Jane et al. 2015), the amount of time fish had been upstream of the site before a sample was collected, the number of fish that had been within the visual survey reach prior to sample collection but moved outside of the reach before being visually counted (leaving behind residual eDNA), and the shedding rate of spawning and other life stages

of fish (Klymus et al. 2015; Bracken et al. 2019). Although most of my streams were small and spawners were often easy to see, observer error (not counting a fish that was present or counting a single fish more than once) was possible and was probably affected by water and weather conditions (i.e., turbidity and insolation). With additional eDNA sample collection from streams throughout the Yellowstone Lake basin, before, during, and after the spawning season, an average baseline could be established for all Yellowstone tributaries and may allow eDNA sampling to become an effective method of detecting the presence of YCT spawning activity in Yellowstone Lake tributaries. After establishing individual or average baselines, streams could then be sampled routinely throughout the spawning season to detect the presence of YCT spawners without the time and safety concerns of visual surveys. Multiple visits to each stream would be necessary to ensure that any lack of effect in changes to eDNA rates (i.e. significant departures from the established baseline eDNA rate) was due to the absence of YCT spawners rather sample collection that occurred before or after YCT spawners were present in a particular stream.

Additional research is needed to determine how many samples with spawners visually present are needed to develop a sufficiently precise relationship. Few samples where spawners were visually observed were collected from any stream, site, and year combinations in each field season (i.e., many zero fish counts populated my data set) because each stream was typically sampled only once per week and stream occupation by adult YCT for spawning was brief (Gresswell et al. 1997). More frequent sampling within a spawning season (i.e., daily), more years of sampling, or sampling at more sites,

of both eDNA rates and spawner abundances simultaneously are needed to develop a more precise relationship (Tillotson et al. 2018; Levi et al. 2019). Less frequent sampling (i.e., 1–2 samples per week) before and after the spawning season (i.e., when no spawners are present) would be sufficient for determining background levels of eDNA (Levi et al. 2019). The number of samples needed may also depend on the specific stream or the level of precision needed for a specific research question. Overall, collection of more samples throughout the spawning season at each stream in multiple years may mitigate variation in eDNA rates and enhance precision of the relationships between eDNA rates and numbers of YCT spawners.

Environmental DNA rates were not a good indicator of age-0 YCT abundances, as the rates were similar both pre- and post-spawning when age-0 abundances would be expected to differ markedly (if reproduction occurred). Although eDNA rates did appear to increase in some instances as visually observed numbers of age-0 YCT increased, no significant relationship existed between these variables. For example, at site TL2 in the Trout Lake inlet, I detected no eDNA before spawning, an increase when spawners were present, and none again post-spawn before increasing, probably in response to the presence of hatched age-0 YCT, which however were never observed. The inefficiency and inaccuracy of visual surveys for age-0 YCT, because of their low visibility, probably influenced this finding. Collection of more samples prior to the spawning season to account for eDNA contributions from overwintering or resident YCT, or both, and the use of more accurate methods of counting age-0 YCT may provide evidence of a relationship between eDNA rates and age-0 YCT. Use of summer age-0 YCT eDNA as a

metric of YCT spawner relative abundance would be advantageous because some YCT spawning tributaries are difficult to access in April and May when fish begin spawning and because the spawning period is limited. However, existence of a relationship between spawner abundance and subsequent offspring abundance would first need to be assessed.

The lack of significant variation in eDNA rates over 24-h and 5-d periods suggests that adherence to a strict sampling schedule (i.e., sampling each stream at a consistent time and day of week) may not be necessary in this study area. The ability to collect eDNA samples at varying times throughout the day and varying days within a week is useful because field work is often delayed or interrupted by weather, equipment failures, wildlife interactions, and other unforeseen circumstances. However, the variation in the numbers of fish during these sample periods was small, and greater temporal changes in eDNA rates may occur when YCT become more abundant. The variation in eDNA rates over these time periods was slightly higher when YCT spawners were present than when age-0 YCT occupied a stream, probably because YCT spawners were shedding a larger quantity of eDNA, especially when actively spawning (Bracken et al. 2019). The lack of effect over these time periods may also be indicative of the ability of eDNA to remain in a system after the target fish is no longer present (Dejean et al. 2011) and may be confounded by eDNA from fish that were present but not observed or from fish that were within the survey reach during sample collection but left the reach before being visually counted.

The relationships (i.e., slopes) between ln-transformed eDNA rates and ln-transformed numbers of YCT spawners were consistent across streams within and outside

of the Yellowstone Lake drainage, but background eDNA rates (i.e., *y*-axis intercepts) varied. Relationships among all streams in this study were probably similar because of their close proximity, similarities in size and discharge, and because eDNA from one target species (YCT) of a similar size and the same life history stage (spawners) was compared across all streams. Because of these similarities, use of a single, combined slope relationship for all Yellowstone Lake tributaries may eventually be useful to detect changes in the number of YCT spawners and achieve management objectives. This relationship may not apply if more spatially distant streams of different drainage sizes were included, but inclusion of data from many streams might also provide a more robust assessment of the number of YCT spawners present throughout the lake basin (Levi et al. 2019).

Similarity in the slopes of the relationships between eDNA rates and spawners between two years of sampling Arnica Creek, despite differing levels of eDNA contribution from YCT other than spawners, highlights the management potential of this sampling technique; my data support the conclusion that eDNA rate is an indicator of spawning YCT abundance in tributaries to Yellowstone Lake. Although the relationships between eDNA rates and spawners were similar between years at the same site in Arnica Creek, the confidence intervals around the relationships were large and spawning size YCT were only visually observed in conjunction with three sample dates in 2017 and four in 2018. The eDNA rates when spawners were present versus absent and the *y*-intercepts of the relationships between eDNA rate and spawners were different between years, probably as a result of eDNA contributions from changing abundances of juvenile,

overwintering, or resident fish (Maruyama et al. 2014), although I did not quantify these population segments.

The positive relationships between eDNA rates and YCT spawner counts were stronger when eDNA concentrations were adjusted for variability in stream discharge than when they were not and should be included in future analyses. Stream discharge rates affect the transport distance and downstream persistence of eDNA and high stream flows may dilute eDNA concentrations (Jane et al. 2015; Wilcox et al. 2016), thereby affecting the relationship between eDNA and spawner abundance. For example, the consistency in the relationship between eDNA and Pacific salmon counts across years was determined to be dependent on precise stream discharge measurements (Levi et al. 2019). Collection of stream discharge measurements at each site and in conjunction with each sample in my study was often time consuming and complicated by the difficulty of measuring or precisely estimating (using staff gauges) stream discharge at high and low flow rate extremes. Precise and efficient stream discharge measurements are critical to application of this method to large streams within the Yellowstone Lake basin.

Environmental DNA rates predicted numbers and associated prediction intervals of YCT spawners present with large prediction intervals at high confidence levels, but lower confidence levels may be sufficient for some management goals. Because eDNA degrades over time and distance travelled (Denier and Altermatt 2014; Tsuji et al. 2017), more research is needed to determine how many sample sites may be necessary to obtain suitable prediction precision on some streams (i.e., those with longer spawning reaches). The uncertainty and variability associated with the distance from YCT spawners to the

downstream sample site and the location of spawners within a reach further complicates predictions from eDNA (Jane et al., 2015). I used maximum eDNA rates to predict abundances and calculate prediction intervals because of the dearth of sample events when spawners were visually observed (i.e., many zeroes), but this metric may be problematic because of the potential for sampling large pieces of DNA during spawning activity (Bylemans et al. 2017; Bracken et al. 2019), which could result in an unusually large quantity of eDNA in a single sample and artificially inflate prediction values. Outlying maximum eDNA rates may be more probable in streams with high concentrations of spawners and may be mitigated by collecting multiple samples at each sampling event and evaluation of whether more precise prediction intervals with higher confidence levels may be established with more frequent sampling throughout the spawning season. Furthermore, ln-transformation of the eDNA rate minimizes the effect of these outliers. Precise prediction intervals may also become apparent when more frequent sampling is combined with independent counts of YCT spawners from an electronic counter or sonar unit (Levi et al. 2019).

The relationships between eDNA rates and spawner numbers may be unreliable at fish count extremes. The numbers of YCT spawners that migrate into tributaries around Yellowstone Lake vary among streams and the relationships between eDNA rates and YCT spawner abundances may be different when few (< 3) or many (> 200) spawners are present in a survey reach and may be affected by the lengths of survey reaches (Levi et al. 2019; Tillotson et al. 2018; Wilcox et al. 2016), which were not standardized in this study. For example, the eDNA rate in one Arnica Creek sample when only one spawner

was present (site A6 in 2018) was more consistent with the eDNA rate of samples when no spawners were present than with those when multiple spawners were counted.

Environmental DNA sampling of YCT spawning tributaries successfully detected grizzly bear eDNA in this study, but not that of North American river otter. Because grizzly bear use of YCT spawning streams is dependent on the abundance of fish present (Robbins and Fortin-Noreus 2017), the ability to detect grizzly bear DNA from the same water sample used to evaluate the relative abundance or presence of YCT spawners would provide managers with another indicator of YCT recovery (i.e., fishing activity by bears). Grizzly bear eDNA was detected in the majority of samples taken on dates when evidence of grizzly bear activity (i.e., scat or tracks) was also noted. Detection of grizzly bear activity on spawning streams from eDNA samples would be less invasive, less time intensive, and pose fewer safety concerns (i.e., negative bear encounters) than conducting weekly visual surveys. North American river otter eDNA was not detected in any of the samples selected for analysis of that species, nor was any sign evident (i.e., scat or tracks), but eDNA from this species has been successfully detected in a controlled study (Padgett-Stewart et al. 2015). Environmental DNA has been used to detect terrestrial and semi-terrestrial species in experimental and natural ponds as well as drinking water samples (Rodgers and Mock 2015; Harper et al. 2019), but the reliability of detecting these species in flowing water requires further study before implementation as a management strategy is possible. Absence of eDNA from these terrestrial and semi-terrestrial species in a water sample cannot be equated with absence of the species from the area entirely, but because eDNA degrades over time and is eventually washed out of

the system, detection in a water sample is a reliable indicator of recent stream use by these species.

## Conclusions

Environmental DNA sampling is unlikely to entirely replace traditional methods for monitoring YCT spawners on all streams within the Yellowstone Lake basin. However, I demonstrated that eDNA is effective for detecting the presence of YCT spawners and shows potential for estimating their abundance. Future research should examine the effects of additional factors such as visual survey length (i.e., eDNA degradation and transport distance), water temperature, and stream habitat characteristics on the relationship between eDNA rate and numbers of YCT (Sepulveda et al., in press). A single model that incorporates these effects (water temperature, survey length, etc.) may provide an accurate method for monitoring YCT abundance across the entire lake basin. Incorporation of eDNA sampling with current methods, such as BMO spawning stream surveys and the future Clear Creek weir and trap, will provide managers with an additional, reliable method for monitoring long term trends in YCT abundance. The ability to analyze a single sample for multiple species and to analyze the remainder of that same sample for different species in the future, may also prove to be a valuable tool for detecting and tracking both species of interest (i.e., grizzly bears and river otters) and invasive species. Environmental DNA sampling is a simple, efficient method that complements traditional methods for monitoring YCT abundance, while allowing for detection and monitoring of stream use by other species and increasing the spatial scale of current monitoring efforts to include all tributaries within the Yellowstone Lake basin.

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