Response of nutrient limitation to invasive fish suppression: How carcasses and analog pellets alter periphyton

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Abstract: The native Yellowstone Cutthroat Trout (*Oncorhynchus clarkii bouvieri* Jordan and Gilbert, 1883) population in Yellowstone Lake, Yellowstone National Park, Wyoming, USA, is in decline because of competition from the introduced, invasive Lake Trout (*Salvelinus namaycush* Walbaum in Artedi, 1792). Gillnetting is used to suppress adult Lake Trout; however, methods are being developed to suppress embryos, including adding Lake Trout carcasses and carcass-analog pellets to spawning sites. Decomposing carcasses and analog pellets cause decreased dissolved oxygen concentrations thereby leading to Lake Trout embryo mortality, but the effects of these methods on primary producers are unknown. We deployed in-situ nutrient diffusing substrates (NDS) at 3 spawning sites. The 1st site was treated with carcasses, the 2nd site was treated with analog pellets, and a 3rd lacked treatment (control). To estimate how suppression measures may alter nutrient limitation, we measured algal biomass in 6 NDS amendments at each site: nothing (control), N, P, N + P, ground carcasses, or pulverized analog pellets. We deployed 5 replicates of each amendment at each site before and after treating whole sites. N and P co-limited periphyton before carcasses or analog pellets were added to spawning sites ($p < 0.01$); however, nutrients were not limiting after the treatments were added to spawning sites ($p = 0.31–1$). Algal biomass was 4× higher after whole-site carcass treatments. In contrast, analog pellets appeared to suppress algal biomass in the amendments (20% of NDS at the control site post-treatment) and in the treatment plot (33% of pre-treatment biomass at analog pellet site). We also measured how individual ingredients in analog pellets altered periphyton biomass, which suggested that vitamin E, estrogen, and soybean oil ingredients reduced the growth of primary producers. Suppression methods may stimulate or reduce algal biomass, depending on the methods used, which could have cascading effects on food webs and potentially reduce the success of the control measures. Estimating how different Lake Trout suppression methods may alter basal resources in the littoral zone of Yellowstone Lake will help natural resource agencies develop effective plans to control invasive predators at early life stages while minimally altering ecosystems.

Key words: nutrient diffusing substrates, nitrogen, phosphorus, Yellowstone National Park, invasive species, Lake Trout, apex predator

Native freshwater fishes are declining globally, and a primary cause is the proliferation of invasive species (Duncan and Lockwood 2001, Gozlan et al. 2010, Hermoso et al. 2011). For instance, native Cutthroat Trout (*Oncorhynchus clarkii* Richardson, 1836) in the western United States have been overtaken by introduced Brook Trout (*Salvelinus fontinalis* Mitchill, 1814) populations (Benjamin and Baxter 2010), and native Bull Trout (*Salvelinus confluentus* Suckley, 1859)

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were functionally lost from food webs after Lake Trout (*Salvelinus namaycush* Walbaum in Artedi, 1792) dominated the food web in several lakes in Glacier National Park, Montana (Wainright et al. 2021). Mechanisms by which invasive species may suppress native fish include competition for resources (Sampson et al. 2009), habitat degradation (Weber and Brown 2011), predation (Vander Zanden et al. 2003), and hybridization (Mandeville et al. 2019). Additionally, introducing non-native species to new ecosystems has the potential to spread pathogens, as occurred when *Yersinia ruckeri* Ewing et al., 1978 (the bacteria that causes enteric redmouth in salmonids) was spread from the US to France by introduced minnows (Michel et al. 1986, Peeler et al. 2011).

The methods used to control invasive fish populations can have varying effects at the ecosystem scale. The most common methods to control invasive fish are chemical additions, physical techniques, and biological control (Rytwinski et al. 2019). Recent evidence suggests that using multiple methods that target different life stages of the invasive fish, in an integrated pest-management approach (Sawyer 1980), can greatly increase success of control (Buktenica et al. 2013, Koel et al. 2020a). One new technique adds organic matter in the form of fish carcasses (hereafter, carcasses) or carcass-analog pellets (hereafter, analog pellets) to spawning beds. The decomposition of carcasses or analog pellets increases biological oxygen demand, dramatically decreasing dissolved oxygen concentrations and suffocating fish embryos (Thomas et al. 2019, Koel et al. 2020c, Poole et al. 2020). Adding analog pellets to aquatic ecosystems is a technique that has previously been used to mitigate the loss of nutrients associated with fewer returning anadromous fish (Wipfli et al. 2004, Kohler et al. 2012, Marcarelli et al. 2014). In these cases, carcasses or analog pellets fertilized streams, causing bottom-up effects on primary producers. For example, adding carcasses and analog pellets increased periphyton biomass after 4 to 6 wk in a tributary of the Snake River in central Idaho, USA (Marcarelli et al. 2014). In another case, juvenile salmonid production and lipid levels increased in southeastern Alaskan streams following both treatments, but analog pellets had a larger effect (Wipfli et al. 2004). Analog pellets are composed of a variety of ingredients, such as dried hatchery salmon processed into cakes or pellets, pelletized fish meal, or soy and wheat (Wipfli et al. 2004, Kohler et al. 2012, Marcarelli et al. 2014, Koel et al. 2020c). The effects of analog pellets on primary producers likely depends on the ingredients used in the pellets.

Far more studies have investigated nutrient limitation in streams than lakes, and studies measuring limitation in phytoplankton far outweigh those of periphyton (Elser et al. 2007). Freshwater ecosystems are predominantly co-limited by N and P (Francoeur 2001, Elser et al. 2007, Reisinger et al. 2016, Beck et al. 2017) or N (Reisinger et al. 2016). Periphyton in lakes can be P limited (e.g., Lake Huron, USA: Pillsbury et al. 2002, Elser et al. 2007), N limited (e.g., Lake Okeechobee, Florida, USA: Rodusky et al. 2001), or co-limited by N + P (e.g., 30 lakes in the United Kingdom: Maberly et al. 2002; Lake Baikal, Russia: Elser et al. 2007, Ozersky et al. 2018). Light and nutrient concentrations are primary environmental factors that alter nutrient limitation (Pillsbury et al. 2002, Beck et al. 2017). Adding organic matter in the form of carcasses or analog pellets may alter the availability of limiting nutrients to primary production. For example, periphyton in 7 southeastern Alaskan (USA) streams was N, P, or co-limited before a salmon run, and nutrient limitation was either alleviated or shifted from co-limited to P limited after carcasses remained post spawning (Rüegg et al. 2011). To estimate how nutrients or other amendments may alter periphyton in aquatic ecosystems, nutrient diffusing substrata (NDS) are frequently used (Tank and Dodds 2003, Rüegg et al. 2011). NDS usually consist of cups filled with agar and topped with a glass disk or glass fiber filter for biofilm to grow on. Unamended agar is used as a control, and other treatments may consist of agar amended with nutrients (Tank and Dodds 2003), fish carcass (Rüegg et al. 2011), or pharmaceuticals (Rosi-Marshall et al. 2013). After an incubation period, algal biomass on nutrient-amended agar is compared with controls to estimate the degree to which nutrients are limiting. Nutrients are considered limiting when algal biomass in the corresponding amendments is discernably higher than the control.

Although studies have demonstrated effects of organic matter additions on primary production of streams, no research has examined potential effects of fish carcasses or analog pellets on periphyton in lakes. Investigating the extent to which adding organic matter, and subsequent shifts in nutrient limitation, cause bottom-up effects in lakes is necessary to estimate how the food web may respond to these management actions. Yellowstone Lake in Wyoming, USA, provides a useful case study for investigating questions about carcass and analog-pellet addition effects on primary producers. Yellowstone Lake historically held the largest population of genetically pure Yellowstone Cutthroat Trout (*Oncorhynchus clarkii bouvieri* Jordan and Gilbert, 1883) in their native range until they precipitously declined (Koel et al. 2019) as a result of invasion by Lake Trout (Koel et al. 2020b). The United States National Park Service began gillnetting Lake Trout after they were discovered in 1994 (Kaeding et al. 1994), but additional management actions are needed to suppress early life stages of Lake Trout to improve efficiency of population suppression (Koel et al. 2020a). Experimental small-scale suppression trials, where Lake Trout carcasses were added to spawning areas in shallow water (<20 m), resulted in 99% of embryos killed (Thomas et al. 2019, Poole et al. 2020). After the carcass trial success, management strategies using carcasses and analog pellets were implemented on a larger spatial scale (Koel et al. 2020a, c).

Our objective in this study was to investigate the degree to which adding organic matter to the spawning areas of an

invasive fish may alter periphyton biomass. We assessed the response of periphyton to carcasses and analog pellet additions to estimate how the basal trophic level may respond to these management actions. We asked the following questions: 1) What nutrient(s) limit periphyton growth? 2) What is the response of periphyton to carcass and analog pellet amendments in NDS? 3) To what degree do carcasses and analog pellets alter algal biomass when added to whole spawning sites? and 4) What is the effect of the different ingredients used in analog pellets to periphyton biomass? We hypothesized that N would be limiting for periphyton growth, and that carcasses and analog pellets would stimulate periphyton by providing limiting nutrients, thereby shifting nutrient limitation from N to no limiting nutrients. We also assumed that natural preservatives in the pellets would suppress algal biomass. The results of this study will inform natural resource agencies about potential indirect effects of suppression efforts on the basal trophic level in the lake so that management actions do not unintentionally alter primary producers.

METHODS

We measured nutrient limitation using NDS at 3 Lake Trout spawning sites (control, carcass, and analog pellets) and during 2 time periods (before and after treating whole sites with carcasses or analog pellets; hereafter, treatments). At each site and time period, we measured periphytic biomass growing on unamended agar (control) and agar amended with N, P, N + P, carcasses, or analog pellets (n = 5 replicates/amendment for a total of 90 NDS per time period); Questions 1 and 2; hereafter, amendments). We measured periphyton in response to whole-site treatments of either carcasses or analog pellets and amendments in NDS (Question 3). Additionally, we estimated how the ingredients in analog pellets altered periphyton biomass by amending NDS with individual ingredients (Question 4). We used chlorophyll *a* (Chl *a*) analysis to measure periphyton biomass growing on NDS amendments. We analyzed the data with a before–after control–impact framework with analysis of variance (ANOVA) to investigate how periphyton biomass varied among treatments, amendments, and ingredients between time periods.

Study area

Yellowstone Lake, Yellowstone National Park, Wyoming, USA, is the largest high elevation (>2000 m a.s.l.) lake in North America, with a surface area of 341 km^2. The lake has an average depth of 42 m and a maximum depth of 98 m (Kaplinski 1991), and 23% of the lake is <20 m deep (Benson 1961). Yellowstone Lake is mesotrophic (Kilham et al. 1996) and dimictic, and it is covered in ice from late December through late May or early June each year. Summer stratification generally occurs from mid-July to mid-September (Koel et al. 2019, 2020a). Average monthly water temperatures vary between 9 and 18°C during the open-water season (Koel et al. 2007).

Yellowstone Lake contains 2 native fish species: Yellowstone Cutthroat Trout and a small cyprinid, the Longnose Dace (*Rhinichthys cataractae* Valenciennes in Cuvier and Valenciennes, 1842). Along with Lake Trout, the other nonnative fish established in Yellowstone Lake, presumably by introductions from anglers, include Lake Chub (*Couesius plumbeus* Agassiz, 1850), Longnose Sucker (*Catostomus catostomus* Forster, 1773), and Redside Shiner (*Richardsonius balteatus* Richardson, 1836) (Varley and Schullery 1998). Yellowstone Cutthroat Trout (hereafter, Cutthroat Trout) reside in the lake during most of the year and migrate to tributary streams where they spawn each spring (Gresswell and Varley 1988). Unlike the native Cutthroat Trout, Lake Trout complete their entire life cycle within Yellowstone Lake and spawn each autumn on cobble substrate, where they have high site fidelity (Williams 2019).

We conducted experiments at 3 spawning sites across the lake (Fig. 1). We found 14 spawning sites of varying size, depth, and substrate type by suppression gillnetting and telemetry of acoustically tagged Lake Trout, and we verified that these were spawning sites by locating live embryos (Williams 2019, Koel et al. 2020c). We then selected the 3 experimental sites based on substrate type (i.e., cobble and gravel), use, and accessibility for sampling. The control site (Elk Point) was located on the eastern shore near the mouth of Clear Creek, and experiments were set at 7-m depths in suitable spawning substrate. The carcass treatment site (Snipe Point) was in the southwestern part of the main basin near Flat Mountain Arm, and experiments were at 5-m depths. The analog pellet treatment site (Carrington Island; 3-m depths), the 1st known Lake Trout spawning location, was in the West Thumb region of the lake.

Field measurements

We measured water conditions at each site at the time experiments were deployed and again when they were retrieved. We used a YSI Professional Plus multiprobe (Yellow Springs Instruments, Yellow Springs, Ohio), which was calibrated weekly, to measure specific conductivity (μS/cm) and pH above the substrate. We recorded hourly dissolved oxygen (% saturation and mg/L) and water temperature (°C) measurements with a miniDOT® logger (Precision Measurement Engineering, Vista, California) placed on the substrate at each site for the duration of the experiment. We collected water just above the substrate at each site in a beta bottle and measured ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{3-}) concentrations. NH_4^+ concentrations were measured within 24 h of collection on a fluorometer (model TD-700; Turner Designs, San Jose, California), according to Taylor et al. (2007). We froze samples at –18°C until NO_3^- and PO_4^{3-} analyses, which were estimated using

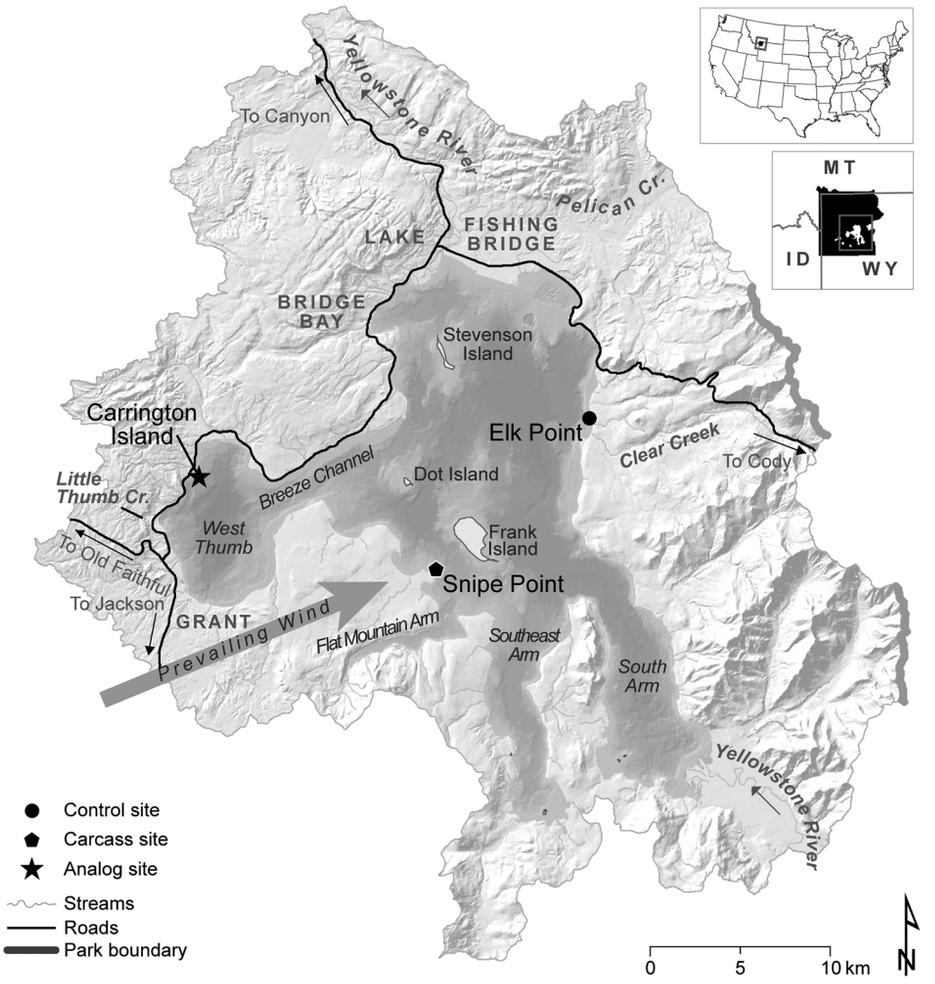

Figure 1. We measured nutrient limitation at 3 sites in Yellowstone Lake, Wyoming, USA. Elk Point was the control site, Lake Trout carcasses were added to Snipe Point (2.0 ha) from mid-August to early October 2019, and analog pellets were added to Carrington Island (0.5 ha) in early October 2019.

a Dionex™ ICS-5000 ion chromatograph (Thermo Fisher Scientific, Waltham, Massachusetts) equipped with a Dionex IonPac® AS23 anion separation column and suppressed conductivity detection. We used a Secchi disk to measure water clarity and a Lowrance® depth finder (model HDS9; Lowrance Electronics, Tulsa, Oklahoma) mounted on the boat to measure site depth. We estimated the wet mass (kg) of carcass material deposited at the carcass site from the number of bins containing Lake Trout carcasses and the mass of bins full of carcasses filled with each gillnet mesh size (n = 5/mesh size; stretch size: 5.08, 6.35, 7.62, 8.89, 10.16, and 11.43 cm). Whole fish carcasses (5937 kg) were deposited opportunistically from gillnetting boats at the site starting on 10 August 2019 to early October. Analog pellets were weighed before being transported, and 17,821 kg of pellets were deposited on the site via helicopter on 3 October 2019.

We used NDS at 3 sites to assess our question about nutrient limitation of periphyton in Yellowstone Lake. In 2019, we compared nutrient limitation in the lake before (29 July–19 August; hereafter, pre-treatment) and after the carcass (5 September–25 September) or analog pellet (30 September–15 October) treatments (hereafter, post-treatment) of the respective sites. We added NDS a few days before analog pellets were added to the entire site to more closely simulate the effect of adding analog pellets to periphyton growing on cobble. NDS at the control site were deployed at the same times as at the carcass treatment site. We deployed 6 amendments of NDS, with 5 replicates of each amendment per site: control (agar only), N (NH_4^+), P (PO_4^{3-}), N + P, ground carcass, and analog pellets. To make each amendment, we combined 20 g of agar with either nothing (control), 27 g NH_4Cl for the N amendment, 45 g of KH_2PO_4 and 23g K_2HPO_4 for the P amendment (Tank and Dodds 2003, Beck and Hall 2018), 200 g of ground Lake Trout filet for the carcass amendment (containing ~6.0 g N and ~0.65 g P; Stansby and Hall 1965, Rüegg et al. 2011), and 30 g pulverized analog pellets

(1.55 g N and 0.018 g P) for the pellet amendment. We added 27 g NH_4Cl, 45 g KH_2PO_4, and 23g K_2HPO_4 to 30 g of agar for the N + P amendment (Tank and Dodds 2003, Beck and Hall 2018).

We attached NDS containers to L-bars (10 containers/L-bar) using cable ties and silicone arranged by amendment and stored them in a refrigerator overnight before deploying the next day. We deployed 3 L-bars, each with 10 NDS containers, at each site and date. Each L-bar was secured with two 2.3-kg dumbbells attached to each end. We lowered the L-bars from a boat where SCUBA divers placed them securely on the lake bottom ranging in depth from 3 to 7 m. After 21 d, we retrieved the NDS containers and transported them to the laboratory in a cooler. We used the acid method to analyze filters for Chl *a* concentration by incubating filters in 15 mL of ethanol buffered with $MgCO_3$ for 10 to 12 h and measuring fluorescence on a TD-700 fluorometer (Turner Designs) before and after adding 0.1 N HCl for a phaeopigment correction (Nusch 1980). We used a secondary solid standard calibrated with a commercial Chl *a* standard of *Anacystis nidulans* (MilliporeSigma, St. Louis, Missouri).

We used a before–after control–impact framework (Underwood 1991) to estimate the degree to which adding carcasses or analog pellets altered periphytic biomass. We analyzed algal biomass with ANOVA in R (version 4.0.3; R Project for Statistical Computing, Vienna, Austria). For the initial experiments in 2019, we included amendment (control, N, P, N + P, carcass, or analog pellet; $n = 5$ replicates for each of the 6 amendments/site), period (before or after carcasses or analog pellets were added), site (control, carcass, and analog pellet sites), and an interaction term between site and period in our model to estimate how algal biomass varied. Additionally, we analyzed the pre- and post-treatment data separately using amendment, site, and an interaction term between them to further estimate differences. We considered an interaction term with $p \leq 0.05$ to indicate that treatments or amendments differentially affected algal biomass among sites.

To answer our 4th research question and estimate how the ingredients in analog pellets may alter periphyton biomass (without additional carcasses or analog pellets added to the site), we deployed experiments at the same 3 sites 1 y after whole-site treatments (10 August–3 September 2020). We combined 20 g of agar with either nothing (control), 35 g of soybean meal, 37 g of soy protein isolate, 15 mL of soybean oil, 15 mL of vitamin E oil, or 1.5 g estrogen (1 pulverized pack of Nortrel). Soy products contain phytoestrogens; however, we used ethinyl estradiol, which is 1000× more potent than soy sources (Kuiper et al. 1998). We poured amendments into 15 polyethylene plastic containers (30 mL/container). Once the agar cooled, we placed a 47-mm type-A/E glass-fiber filter (Pall® Corporation, Port Washington, New York) on top of the agar, trimmed excess filter from the edges, and closed the lid. We cut a 22-mm diameter hole in the lid to allow algae to grow on the filter. L-bars and NDS were placed as described above. We did not measure water quality or nutrient concentrations in 2020.

For the pellet ingredient experiments in 2020, we included amendment (control, soybean meal, soy protein isolate, soybean oil, vitamin E, or estrogen; $n = 5$ replicates for each of 6 amendments/site) and site (control, carcass, and analog pellet sites). When a factor had a p-value ≤0.05 and had ≥3 levels, we used Tukey's honestly significant difference (HSD) pairwise test to estimate differences among them. Data were natural log transformed to normalize the distribution and non-constant variance.

RESULTS

General measurements

Water chemistry differed among sites (control, carcass, and analog pellet treatments) and the 2 periods in 2019 (before and after treatments). Water temperature differed between the pre- and post-treatment periods. Carcass and control sites were 2°C warmer post-treatment, and the analog pellet site was 4.6°C cooler post-treatment compared with pre-treatment (Table 1). Specific conductivity was 29, 34, and 20 µS/cm higher at the control, carcass, and analog site pre-treatment vs post-treatment, respectively, but

Table 1. Water temperature (°C), specific conductivity (SC), pH, dissolved oxygen (DO), water depth, Secchi disk depth, ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{3-}) concentrations, and the amount of carcass or analog pellet material added and the treated area at each site before (pre) and after (post) treatment.

Site	Treatment	Temperature (°C)	SC (µS/cm)	pH	DO (mg/L)	DO (% saturation)	Depth (m)	Secchi (m)	NH_4^+ (µg N/L)	NO_3^- (µg N/L)	PO_4^{3-} (µg P/L)	Material added (kg/ha)	Area treated (ha)
Control	Pre	11.5	120.8	7.25	8.85	108.5	7	7.50	4.5	<50	<100	0	0
	Post	13.2	92.0	7.13	8.07	102.9	7	11.25	6.4	<50	<100	0	0
Carcass	Pre	10.5	126.1	6.82	9.23	110.2	5	8.75	3.6	<50	<100	0	0
	Post	13.8	91.9	7.70	6.62	86.4	5	10.00	6.5	<50	<100	2968	2.0
Analog	Pre	13.4	120.6	7.50	8.60	110.0	3	6.40	2.5	<50	<100	0	0
	Post	8.8	100.6	8.14	8.20	94.0	3	9.00	4.5	<50	<100	35,642	0.5

measurements were low overall. The pH at the control site varied little pre- to post-treatment and was near neutral; however, pH was higher at the carcass and analog pellet sites during post-treatment (Table 1). NH_4^+ concentrations were 2.3 μg/L higher post-treatment at all sites compared with the pre-treatment period. Dissolved oxygen concentrations at the carcass site decreased the most post-treatment (by 2.6 mg/L). Secchi disk depth increased at all sites post-treatment as typically occurs through the open-water season.

Limiting nutrients to periphyton

Periphyton in Yellowstone Lake were limited by both N and P. Periphytic algal biomass in the control amendments prior to treatments averaged 3.2 mg/m^2 (0.25–5.4 mg/m^2). Algal biomass, as indicated by Chl *a* concentrations, before treatments differed by amendment (ANOVA, $F_{5,84} = 7.7$, $p < 0.0001$; Tables S1, S2, Fig. 2A–F) and site ($F_{2,87} = 4.3$, $p = 0.02$), but an interaction term between amendment and site did not explain variation in Chl *a* ($F_{10,72} = 1.4$, $p = 0.18$). The N + P amendments had the highest algal biomass, indicating that periphyton in Yellowstone Lake was co-limited by N and P (HSD, $p < 0.001$).

Whole-site additions of carcasses and analog pellets

As we predicted, adding carcasses to a site increased algal biomass, and analog pellet additions decreased biomass. The highest periphytic biomass before whole-site treatments was at the carcass site, the lowest biomass was measured at the control site (ANOVA, $F_{2,87} = 4.3$, $p = 0.02$, HSD, $p = 0.013$), and the analog pellet site had intermediate biomass. The effect of whole-site treatments on algal biomass differed by period (pre- vs post-treatment; ANOVA, $F_{2,178} = 28.3$, $p < 0.001$), as indicated by an interaction term (Tables S1, S2, Fig. 2A–F). Algal biomass at the control site did not differ pre- vs post-treatment (HSD, $p = 0.24$); however, carcasses increased algal biomass and analog pellets suppressed algal growth post-treatment. Algal biomass did not differ between the analog pellet and control sites pre-treatment (HSD, $p = 0.79$); however, post-treatment algal biomass at the analog pellet site was 20% of the biomass at the control site (HSD, $p < 0.0001$). At the analog pellet site, algal biomass post-treatment was 33% of pre-treatment biomass (HSD, $p = 0.0002$). Conversely, the carcass site had 2.4× more algal biomass post-treatment compared with pre-treatment (HSD, $p < 0.0001$) and >4× as much biomass as the control site post-treatment (HSD, $p < 0.0001$). Nutrients were not limiting post-treatment at the carcass and analog pellet sites; however, algal biomass was 13× higher at the carcass site than the analog pellet site (HSD, $p < 0.0001$).

NDS amendments

The analog pellet NDS amendment had lower algal biomass compared with the other amendments, but the algal biomass growing on the carcass amendment varied. Pre-treatment, the analog pellets in NDS had the lowest biomass (ANOVA, $F_{5,84} = 7.7$), which differed from the carcass

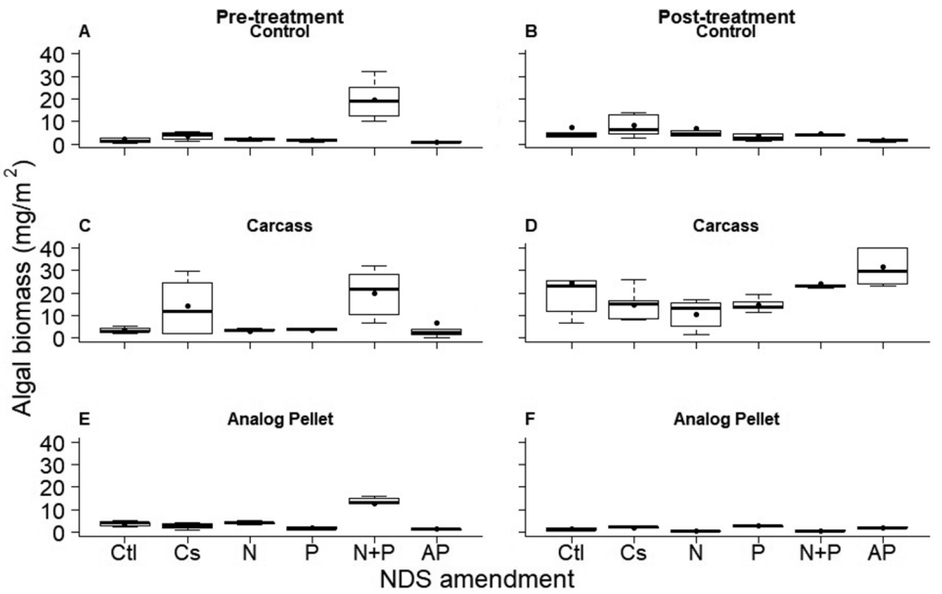

Figure 2. Chlorophyll *a* concentration as an indicator of algal biomass at the control site (A, B), the carcass site (C, D), and the analog pellet site (E, F). Limiting nutrients were assessed before (A, C, E) and after (B, D, F) whole-site additions of carcasses or analog pellets in 2019 at Yellowstone Lake, Wyoming, USA. Amendments were control (Ctl), carcasses (Cs), nitrogen (N), phosphorus (P), N + P, and analog pellets (AP). Black lines are medians, black circles are means, bottom and top of the boxes are the 25th and 75th percentiles, and the whiskers are minimum and maximum values excluding outliers.

(HSD, $p = 0.016$) and N + P amendments (HSD, $p < 0.0001$). Algal biomass post-treatment differed by NDS amendment (ANOVA, $F_{5,84} = 3.3$, $p = 0.01$; Tables S1, S2, Fig. 2A–F) and site ($F_{2,87} = 181.9$, $p < 0.0001$), and there was an interaction between amendment and site ($F_{10,72} = 7.5$, $p < 0.0001$). Overall, periphytic biomass was higher at the carcass site (mean = 20 mg/m^2), much lower at the analog pellet site (1.5 mg/m^2), and intermediate at the control site (5.4 mg/m^2). All NDS amendments displayed the same pattern, wherein the carcass site had the highest periphytic biomass and the analog pellet site had lowest biomass (HSD, $p < 0.01$). Biomass was lower at the analog pellet site compared with the control site for the control, N, N + P, and carcass amendments (HSD, $p < 0.03$).

Analog pellet ingredients

Ingredients of analog pellets both increased and decreased periphyton biomass. The ingredients in pellets altered algal biomass by site (ANOVA, $F_{2,87} = 11$, $p < 0.0001$) and amendment (ANOVA, $F_{5,84} = 19$, $p < 0.0001$; Tables S1, S3, Fig. 3A–C). The control site had higher algal biomass compared with the carcass (HSD, $p = 0.027$) and analog pellet sites (HSD, $p \leq 0.0001$). Algal biomass growing on soy protein isolate (HSD, $p = 0.56$) and soybean meal (HSD, $p = 0.99$) amendments did not differ from the control amendment, whereas amendments of soybean oil (HSD, $p < 0.0001$), vitamin E (HSD, $p < 0.002$), and estrogen (HSD, $p = 0.03$) suppressed algal biomass compared with the control amendment. The amendments with soy protein isolate had higher algal biomass than soybean oil, vitamin E, and estrogen (HSD, $p < 0.0001$). Soybean meal had higher algal biomass than soybean oil, vitamin E, and estrogen (HSD, $p < 0.002$).

DISCUSSION

Periphyton in Yellowstone Lake was co-limited by N and P before treating sites, which differed from the N limitation measured previously for phytoplankton (Interlandi et al. 1999). Adding carcasses or analog pellets to entire sites had opposing effects on periphyton. Carcasses increased periphyton biomass >2× compared with pretreatment estimates, and analog pellets decreased algal biomass to ≤33% of estimated biomass prior to whole-site treatment. Carcasses as NDS amendments sometimes increased algal biomass, but this was likely because only a small amount of carcass material was incorporated with the agar. NDS amendments containing analog pellets decreased algal biomass across sites. The ingredients soybean oil, vitamin E, and estrogen in analog pellets decreased algal biomass and had a stronger effect compared with soy protein isolate and soybean meal, which increased periphyton biomass in isolation. Effects from carcass and analog pellet treatments appeared to be restricted to the site where they were applied, and we did not detect changes at the control site after treatments. Adding carcasses or analog pellets to suppress invasive Lake Trout is a viable option that may only have localized effects on treated sites. Spawning sites compose a small area within Yellowstone Lake, and we surmise the impacts would be minimal. More investigation is needed to estimate the degree to which treating spawning sites may affect lake water quality and the food web at the ecosystem level.

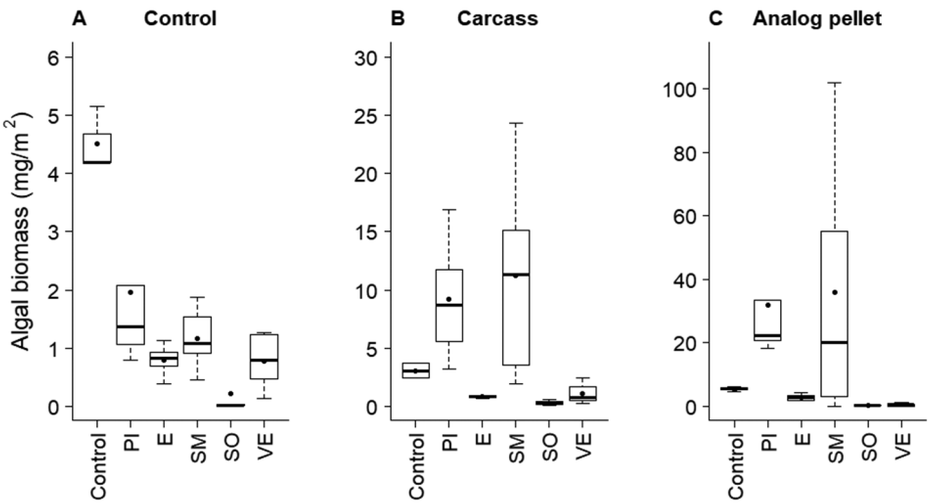

Figure 3. Effects of different analog pellet ingredients on chlorophyll *a* at the control site (A), carcass site (B), and analog pellet site (C) at Yellowstone Lake, Wyoming, USA. Ingredients tested were soy protein isolate (PI), estrogen (E), soybean meal (SM), soybean oil (SO), and vitamin E (VE). No carcasses or analog pellets were added while the experiment incubated. Note the different scales of the *y*-axis. Black lines are medians, black circles are means, bottom and top of the boxes are the 25th and 75th percentiles, and the whiskers are minimum and maximum values excluding outliers.

Carcass additions

Adding Lake Trout carcasses to spawning areas in Yellowstone Lake alleviated nutrient limitation in periphyton and increased algal biomass, which may have overshadowed the effects of individual NDS amendments; however, NDS carcass amendments did not increase biomass in all trials. Salmonids are composed of 3.0% N and 0.33% P (wet mass; Stansby and Hall 1965) that is returned to the ecosystem during decomposition (Parmenter and Lamarra 1991). Therefore, if 100 kg wet mass of Lake Trout are deposited in Yellowstone Lake, 3000 g N and 330 g P are added to the lake. These amounts result in a molar N:P ratio of ~20, indicating, according to the Redfield ratio (Redfield 1958), that N is in excess of P. The carcass amendment NDS inconsistently increased algal biomass, but we observed a strong response when carcasses were added to the entire site. Differences may be attributed to the scale of the experiment and the available nutrients in amendments. We were limited to adding 200 g ground Lake Trout carcass to the agar because the NDS would not hold together when more was added. The carcass amendment contained fewer nutrients compared with the N and P amendment, which likely attributed to differences between them.

The higher algal biomass of all NDS amendments at the carcass site was likely because of the high number of decomposing carcasses surrounding the experiments and mineralizing nutrients. Some whole carcasses in the littoral zone appeared to decompose slowly, as evidenced by occasional carcasses we observed at our site 1 y after adding them. Therefore, carcasses likely fertilize sites for long periods, although N (primarily in muscle) may be mineralized more quickly than P (primarily in bone; Nobre et al. 2019). Depositing carcasses at spawning sites may increase algal biomass and nutrient cycling while carcasses are present, but the magnitude of the effect likely diminishes as carcasses decompose. Adding carcasses appeared to stimulate primary producers, as we observed the spring following the carcass treatment (Lujan 2020). In addition, periphyton biomass did not differ pre- vs post-treatment at our control site ~12.5 km across the lake, indicating that the effects of carcass treatment are unknown and, we suspect, localized to the benthic area surrounding the carcasses. Additionally, adding carcasses increased mortality in some benthic invertebrates when they were within the treated spawning site (Briggs et al. 2021).

Analog pellet additions

Algal biomass was lowest in the analog pellet amendment at all sites during the pre-treatment period and all amendments at the analog pellet site post-treatment, which is likely due to the pellet ingredients. Adding analog pellets, which are made from ingredients common in commercial fish food (Koel et al. 2020c), to an entire spawning site reduced algal biomass to concentrations below the controls, negating the effects of individual NDS amendments. The effects of analog pellets on aquatic ecosystems varies depending on the type of pellets, amount of pellets added, and the ecosystem to which they are added. For example, periphyton was N limited after the addition of pasteurized salmon analog pellets to streams in the upper Salmon River, Idaho, USA, and pellets did not alleviate nutrient limitation (Ebel et al. 2014), the opposite of what we observed in Yellowstone Lake. Similarly, Marcarelli et al. (2014) added pasteurized fish meal, along with carcasses from a local hatchery, to a stream, and they recorded N limitation in the control and analog pellet sites after treatments. Not only can analog pellets increase nutrient concentrations, they can also increase the length and biomass of some invertebrate taxa (e.g., chironomids), as discovered using flow-through stream channels in Cedar River, Washington, USA (Kiffney et al. 2014). Using analog pellets to treat aquatic ecosystems may alter nutrient availability, leading to bottom-up effects. We assume that adding more pellets is equivalent to adding more nutrients, leading to larger observed effects; however, how pellets alter ecosystems likely depends on many characteristics such as pellet ingredients, ecosystem type, water volume, and trophic state, among others.

Previous studies where analog pellets were used to supplement streams reported higher biomass of periphyton, in contrast with our results, and this difference may be because of the variety of ingredients used to make pellets, the amount applied, or both (Kohler et al. 2012, Ebel et al. 2014, Marcarelli et al. 2014). In our study, the analog pellets used in whole-site treatments and the NDS amendments in 2019 were composed of 6 ingredients, including soybean oil, vitamin E, and estrogen, which we found to suppress algal biomass and which may have individual or combined negative effects on algal growth. Soybean oil contains vitamin E, which is a natural antioxidant (Niki and Traber 2012), and antioxidants appear to depress primary producers. This reduction in growth is similar to the effect of vitamin E in food to reduce microbial activity and extend shelf life (Niki and Traber 2012). Another compound in soybeans that we did not test was phytic acid. Phytic acid is a well-known antioxidant (Graf and Eaton 1990) that binds to P, making the element unavailable to primary producers. We expect that phytic acid would reduce algal biomass; however, we are not aware of any studies that have examined this compound. Additionally, we found that estrogen NDS suppressed algal growth; however, we used ethinyl estradiol, which is 1000× more potent than the phytoestrogens in the analog pellets from soybeans (Kuiper et al. 1998). The extent to which phytoestrogens reduce algae may be less than ethinyl estradiol.

Relative effects of site conditions and treatments

Algal biomass appeared to vary more because of whole site treatments and NDS amendments than differences in site conditions. Higher nutrient concentrations, light intensity,

and temperatures are known to increase algal biomass (Singh and Singh 2015, Hao et al. 2020), and variation in these factors among sites provided opportunities to explore their potential effects as compared with treatment and amendment effects. For example, NH_4^+ concentrations increased post-treatment at all sites. However, despite higher N concentrations, algal biomass did not differ at the control site between pre- and post-treatment; therefore, we attribute differences in algal biomass to whole-site treatments. Analog pellets likely reduced light reaching NDS because they dissolved quickly in water and were suspended in the water column, which may have been at least partially responsible for lower algal biomass at that site. However, our prediction that NDS placed at the deepest site would have the lowest biomass because less light is available at deeper depths (Vadeboncoeur et al. 2014) was not supported. Site depth varied between 3 and 7 m, and the control site was the deepest of the 3 sites but had intermediate algal biomass, suggesting that a difference of 4 m did not have an appreciable effect.

Higher water temperatures have also been shown to increase growth and biomass of algae (Bouterfas et al. 2002), and water temperature at the analog pellet site was highest pre-treatment, which corresponded with higher algal biomass. This site cooled by 4.6°C between the pre- and post-treatment periods, which may have reduced algal growth, but we attribute suppressed algal growth to the pellets because algal biomass was lower in the analog pellet amendments compared with the control amendments at all sites regardless of temperature increases or decreases pre- to post-treatment. Additionally, algal biomass was lower when analog pellets were added to the entire site compared with algal biomass at the control site. Post-treatment water temperatures were higher than pre-treatment temperatures at the control and carcass sites, but patterns in biomass growth differed between these sites. Algal biomass increased post-treatment at the carcass treatment site, but despite the control site warming by 1.7°C post-treatment, algal biomass did not differ between periods. Because of this difference, and because adding carcasses to the entire site increased algal biomass relative to the control site and pre-treatment estimates site, we attribute the overall higher algal biomass at the carcass site to the whole-site carcass treatment. The conditions among sites appeared to have had minor effects on algal biomass compared with amendments and treatments.

Broader implications

Using carcasses or analog pellets to supplement or treat aquatic ecosystems can present challenges, such as availability and transportation, and unforeseen consequences, such as disease transfer. Carcasses are usually obtained from sources within the watershed, as was done in our study, therefore reducing the potential for introducing pathogens. Analog pellets have the advantage of being composed of pathogen-free fish meal, fish made into cakes (Wipfli et al. 2004, Kohler et al. 2008, Marcarelli et al. 2014), or only plant-based (soy and wheat gluten) materials (Koel et al. 2020c). For suppression treatments in the study lake, Yellowstone National Park manufactured plant-based analog pellets to avoid pathogen risks and because the supply of Lake Trout carcasses is limited in autumn; however, the effects of individual ingredients on primary producers should be further examined.

Suppression actions at spawning sites alleviated nutrient limitation in periphyton; however, algal biomass responded differently to the addition of carcasses compared with analog pellets. We observed an increase in nutrient concentrations and periphyton biomass at the carcass site, suggesting the low concentrations of oxygen did not affect the growth of periphyton. Nutrients provided by carcasses may cause bottom-up effects, leading to invertebrates at lower trophic levels increasing in growth and biomass (Kaylor et al. 2020). In contrast, the analog pellets suppressed algal biomass below concentrations measured in the controls. The analog pellets appeared to break down quickly and become suspended in the water column, which may also have suppressed phytoplankton biomass. Although our results indicated that analog pellets may not cause algal blooms, the analog pellets appeared to suppress periphyton below natural levels. Periphyton and phytoplankton are vital food sources for most aquatic invertebrates, which in turn feed both fish in the lake and terrestrial animals around the lake margin (e.g., Epanchin et al. 2010). Estimating how these suppression techniques may alter the foodweb base is vital to designing the best protocols to removing invasive species. The possibility for unintentional, lake-wide effects is limited because carcass and pellet analog treatments are confined to the small fraction of the lake (<0.03%) where Lake Trout spawn. Additionally, primary production in lakes tends to be dominated by either benthic or pelagic primary producers (e.g., Vadeboncoeur et al. 2001). Phytoplankton in the pelagic zone dominate Yellowstone Lake as indicated by higher NH_4^+ uptake by phytoplankton compared with periphyton (Lujan 2020). We conclude that the combination of the small spawning areas and low periphyton production will result in treatments having a minor effect on Yellowstone Lake as a whole. Estimating how suppression techniques may alter other trophic levels is critical to avoid unintentional consequences of management actions.

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project and edited the manuscript. Additionally, TMK facilitated transfer of funding and arranged logistics for field work.

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