**Enduring regime shifts in zooplankton functional traits to restoration efforts in alpine lakes stocked with non-native sportfish**

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**Abstract**

Human manipulations of non-native sportfish in sensitive alpine lakes can result in catastrophic, long-term changes to ecosystem regime and function. Addition of novel sportfish predators in alpine lakes impoverished biodiversity and functional traits (i.e., grazing pressure) of natural zooplankton communities through size-selective predation. Recovery actions such as stocking termination, gill-netting, and extirpated species reintroductions have had short term successes in returning taxonomic diversity. However, studies of such recovery efforts focus mainly on immediate trends of taxonomic diversity. As such, there exists limited knowledge of how shifts in community regime and ecosystem function (via species functional traits) develop and recover over long-time periods, after sportfish introductions and restorative efforts. We explore this knowledge gap by comparing 50 years of zooplankton abundances, traits, and environmental data of ~~one natural fish-present, and~~ three uniquely stocked and recovered alpine lakes. By linking trait-based and taxonomic community trajectories with alternative state theory, we confirm the identities of distinct fishless and fish-present community regimes in alpine lakes. However, novel fish predators have long-term, destabilizing effects on community state and function compared to natural fish-present communities. Disruption of community trait structure can impair recovery actions by increasing resilience to recolonizations by extirpated species. Regardless of the technique employed, community regime and functional recoveries of sensitive alpine zooplankton are possible given decadal time to stabilize.

**Keywords:** *functional traits; RLQ analysis; ecosystem function; community trajectories; non-native sportfish; alpine lakes*

**Introduction**

Catastrophic shifts in ecosystems often result from changes in dominant life forms (Scheffer et al. 2001). the introduction of non-native sportfish in fishless mountain lakes biologically impoverished these ecosystems through size-selective predation that extirpated top resident predators, namely large invertebrates (McNaught et al. 1999; Knapp et al. 2001; Parker et al. 2001). Subsequently, various restoration strategies were attempted to return these lakes to their natural fishless states. These actions include termination of stocking support for non-reproductive exotic fish populations (Knapp et al. 2001), active removal of introduced fish (Parker et al. 2001), and reintroduction of extirpated keystone species (McNaught et al. 1999). However, current restoration efforts around the globe focus mainly on immediate re-establishment of taxonomic diversity and provide limited evidence of specific functional changes like grazing pressure and secondary production (Parker and Schindler 2006; Williams et al. 2016; Tiberti et al. 2018). Moreover, knowledge gaps remain how these shifts in community function develop and recover over long-time periods, after sportfish introductions and the potency of restoration efforts to recover these fragile systems.

To date, most studies of the ecological impacts of fish stocking in mountain lakes have focused primarily on directional taxonomic trends over short periods, rather than long-term community shifts and trait structure of their prey communities (Parker et al. 2001; Knapp et al. 2001; Mouillot et al. 2013; Tiberti et al. 2018). While integral to defining taxonomic responsiveness to recovery, these investigations lack the scale or ecological realism to consider potential shifts between alternate stable states, and whether these shifts take place along the same or different pathways (i.e., hysteresis; Scheffer et al. 2001; Lamothe et al. 2019). Also, many studies lack direct comparison of species traits to characterize differences in stability, resistance, and resilience of sensitive alpine lakes (Standish et al. 2014; Lamothe et al. 2019). Species traits determine biological performance, responses, and interactions of individuals to shifting ecosystem conditions (Hébert et al. 2017).

In response, community ecologists have been increasingly focused on developing trait-based approaches to understanding how changes in species and their traits (as *functional* traits) affect community and ecosystem level functionals, including implications across aquatic-terrestrial food webs (Hébert et al. 2017; Koel et al. 2019). However, directly linking zooplankton traits to the ecosystem processes of sensitive alpine lakes has been problematic. This difficulty is due to the island-like distribution of alpine lakes permitting significant levels of local genetic adaptation and low functional redundancy (Hébert et al. 2017; Loewen et al. 2018; Tiberti et al. 2018). Also, the expense of sampling alpine lakes due to their isolation often prevent adequate sampling to make solid conclusions (Pacas and Taylor 2015). Preserving cryptic taxa and their unique traits expressions that shape community resistance and resilience to anthropogenic stress and climatic change in these alpine systems are vital to preserving biodiversity.

Recently, a more functional approach to community ecology in the context of extreme environmental changes has taken place based on specific multivariate analyses (Litchman et al. 2013; Mouillot et al. 2013; Lamothe et al. 2019). For example, RLQ analysis enables simultaneous ordination of three data matrices (R = environmental variables; L = species abundances; Q = species traits) to identify explanatory variables of functional shifts across communities (Mouillot et al. 2013). This method has been used recently in both controlled experiments and surveys to assess the trait-based consequences to ecological function and community resilience against known environmental changes (Mouillot et al. 2013; Loewen and Vinebrooke 2016; Redmond et al. 2018). However, the temporal scales of most previous RLQ studies are limited to only a few months (MacLennan and Vinebrooke 2016; Loewen and Vinebrooke 2016). Ordination methods such as non-metric multidimensional scaling (NMDS) have been employed to illustrate how community compositions change over time via temporal trajectories (Matthews et al. 2013; Litchman et al. 2013; Matthews and Marsh-Matthews 2016). These trajectories have recently been further adapted to characterize these taxonomic responses to ecosystem disturbances and their potential recoveries (Matthews et al. 2013; Lamothe et al. 2019). Despite the reliance of judging community recoveries to a previous regime state, the direct link between ordination trajectories and the alternate stable state theory has only been freshly argued (Lamothe et al. 2019).

By combing NMDS community trajectories, alternative stable state theory, and trait-based RLQ, our study aims to characterize the: (1) multi-decadal taxonomic and functional trajectories of alpine zooplankton communities in the presence of either non-native sportfish following their introduction and subsequent elimination (2) relative influences of natural fish population decline, gill-net removal of introduced fish, and reintroduction of extirpated keystone species as management actions on these trajectories, and (3) environmental factors that best explained shifts in the functional structure of the communities based on their species traits. We predicted that the introduction of non-native sportfish into a fishless alpine lake would displace and destabilize zooplankton communities relative to those in the presence of a native fish population, or undisturbed by introductions. We rationalize this as highly localized adaptation common in alpine lakes, so communities which evolved in the absence of visually feeding fish will have low resistance to and poor resilience to the novel predators. We also expected that the taxonomic trajectories after fish-stocking and subsequent action would not match (i.e., hysteresis; Lamothe et al. 2019) because of differences in underlying ecological drivers and alpha diversity. Active removal of introduced fish was not expected to accelerate taxonomic recovery by zooplankton communities owing to mate limitation (i.e., Allee effect; Kramer et al. 2008) constraining re-establishment of the keystone alpine zooplankton species *Hesperodiaptomus arcticus*. Our final prediction was that the active reintroduction of an extirpated species accelerates, but is not necessary for, the recovery of a previously stocked lake’s function or community state. The documented cascading trophic effects that *H. arcticus* can trigger in aquatic mountain lakes, restructuring community compositions to lower resistance to a regime shift that favors further recolonization and dominance of previous keystone zooplankton species.

**Methods**

*Study Area*

Based on several published reports (Anderson and Donald 1978; Parker et al. 1996, 2001; McNaught et al. 1999; Wilhelm et al. 1999; Parker and Schindler 2006) and more recently collected data (2013-2022), we compiled chronological datasets of zooplankton species abundances and environmental metrics for four alpine lakes located in the Cascade Valley of the Eastern Front range of the Rocky Mountains in Banff National Park, Alberta, Canada. The four lakes are each located at a similar elevation (2226 - 2353 m asl) in hydrologically independent cirque valleys, yet share several key environmental characteristics, including low nutrient levels (4 - 6 µg/L total phosphorus) and similar surface water temperatures following ice-out (9.7 ºC - 12.2 ºC).

Pipit (51°37'N 115°51'W), Bighorn (51°28'N 115°45'W), and Snowflake (51°35'N 115°49'W) lakes are naturally fishless but were uniquely stocked with a variety of non-native sportfish and has a unique history of resource management practices (Table 1). In contrast, Harrison Lake (51°33'N 115°48'W) contains a native Bull Trout (*Salvelinus confluentus,* Suckley 1859) population, providing a case study of a relatively unperturbed alpine lake with a natural fish community. These attempts at restoration to a naturally fishless state includes active removal of introduced fish via gill-netting (Bighorn Lake), passive extirpation of introduced fish (Pipit Lake) and deliberate reintroduction of an extirpated keystone zooplankton species (Snowflake Lake).

To represent the naturally fishless alpine lake condition, we further collected chronologic zooplankton and environmental data (1975/200X to 2017 or 2018) for never stocked, but human visited, alpine lakes at similar relative latitude with similar environmental conditions and elevations in the Canadian Rockies. These lakes are Sentinel (51°20'04.7"N 116°13'14.9"W; aka Minnestimma Lake) and Eiffel Lake (51°19'16.9"N 116°14'37.9"W) of Banff National Park. And Oesa, Opabin, and Hungabee lakes (respectively: 51°21'14.5"N 116°18'08.0"W; 51°20'27.4"N 116°18'43.2"W; 51°20'37.0"N 116°19'07.0"W) of Yoho National Park.

*Limnological Sampling*

Over the past 50 years, each lake was sampled a minimum of 16 times consistently using consistent methods during the ice-free season (i.e., late June to early September). Zooplankton were collected by performing vertical hauls of the entire water column using a 63µm conical net at the deepest locations in each lake. Zooplankton samples were taxonomically enumerated (individuals/L) to the finest possible taxonomic level using the reference key by Edmondson (1959). Surface water samples for chemical analyses were collected using acid-washed 1 L capacity Nalgene containers.

Now, zooplankton taxa found were each assigned a set of six characteristic traits (Table 2) that influence their functional roles within lake ecosystems (i.e., functional traits; Barnett et al. 2007; Hébert et al. 2016, 2017; Redmond et al. 2018). The trophic group that a species belongs to determines the composition of its prey community and the influence it has on the stability of a trophic food web (Shanafelt and Loreau 2018). Similarly, feeding method determines a species prey preference (e.g., edibility of diatoms; Tonno et al. 2016) and feeding rates (e.g., active vs passive foraging; Colina et al. 2016), influencing the available biomass of multiple trophic levels (Vanni 2002). The reproductive strategy employed by a species influences the phenology and biomass availability of subsequent generations impacting their top-down and bottom-up trophic impacts (Winder and Schindler 2004; Kramer et al. 2008; Dupuis and Hann 2009). Pigmentation and motility are best associated with how effective a species is at predator avoidance, influencing their availability as a food source for predators (Litchman et al. 2013). Zooplankton body size has been suggested to mediate these functional trait interactions, as the strength of each interaction scales with increasing body size (Hébert et al. 2017). *Statistical Analysis*

Mean zooplankton community biomasses in each lake over the study period were examined to both assess and exemplify how an aggregate ecosystem function may be affected by the functional composition of the community. Using species abundances (individuals/L) and average length measurements acquired during our literature review for traits, we obtained average community biomass (µg/L) estimates of each sampling year using standard length-weight regressions (Watkins et al. 2011). Replicate samples per year were averaged and treated as annual biomass potential for each lake and plotted over time.

We created separate chronological trajectories of the functional and taxonomic identities of each lake in comprehensive NMDS ordinations using aggregated yearly average taxa abundance of both study and reference lakes. While following traditional methods for taxonomic ordination, the NMDS of community traits required the creation of a community weighted annual mean (CWM) matrix (Zelený 2018) of each lakes sampled year and representation of the traits described above. Both modes of abundance matrices were untransformed entering NMDS analysis. We chose NMDS as it suffers less from the spatial distortion of other gradient analyses and allows the assessment of similar and dissimilar community compositions while preserving statistical integrity (i.e., NMDS stress value; Clarke and Warwick 2001). These ordinations were built upon Bray-Curtis distances as it ignores issues of double zeros when measuring differences between community compositions, reducing overweighting of rarer taxa (Legendre and Legendre 1998; Clarke and Warwick 2001). In this way, truer ecological distances can be preserved in the unconstrained two-dimensional NMDS space, while allowing for more accurate comparisons of community compositions at specific time points within and between lake histories and natural fishless conditions (Legendre and Legendre 1998; Clarke and Warwick 2001; Mathews et al. 2013).

The natural fishless condition of alpine lakes is represented in both taxonomic and trait ordination space by constructing statistical ellipses (95% CI) encircling our unmanipulated reference lakes. These ellipses allow the visual deduction of representative taxa and community compositions of alpine lakes. We then isolate and visualize the Bray-Curtis distances between the fishless reference condition centroid and each lakes aggregated sample point. This allows us to better compare and quantify the ecological impact, recovery rate, and natural stochasticity of the taxonomic and trait states of our alpine lakes.

In order to combine the trait and taxonomic approaches, we employ Procrustes analysis to determine the congruence between each lakes yearly sample point positions in both ordination types through fish presence, decline, and absence. Here we highlight years of high variance in both zooplankton diversities, explore redundancy, and consider the relative importance of stochastic vs deterministic processes of community assembly within each lake’s history. To describe major trait-environment relationships in functional space, we used RLQ analysis. Three-table RLQ combines data matrices of mixed factor environmental variables (R), species abundances (L), and categorical species traits (Q) (Mouillot et al. 2013). By using RLQ, we could visualize and evaluate the change in functional turnover patterns of specific community groups in response to environmental variables in ordination space (Mouillot et al. 2013; Dray et al. 2014). To accurately quantify relationships in functional space, we selected a subset of our data (1991 - 2002) that had relatively consistent and replicated environmental measures for RLQ analysis. As such, we only selected variables that were available for every sample such as fish presence-absence, water column temperature profiles (TEMP), total dissolved phosphorus (TDP) and nitrogen (TDN) and dissolved organic carbon (DOC).

We then performed the RLQ analysis following protocols outlined by Dray et al. (2014) and Thioulouse et al. (2018) Species abundances (L) were untransformed annual mean taxa abundances, to preserve variation within the relatively small subset of years.Our environmental matrix (R) remained as a qualitative factor (i.e., fish presence or absence) with quantitative abiotic gradients, analyzed using the Hill-Smith method to handle the mixed nature of the data. Zooplankton body sizes were restructured to size classes (Small: <1 mm; Medium: 1 – 2 mm; Large: >2 mm) to unify all traits (Q) as qualitative data, reducing the potential for artificial inflation or overlap of variation during multiple correspondence analysis.

All statistical analyses were performed within R v4.2.2 (R Core Team 2022) and R studio v2022.12.0.353 (Posit team 2022). All distance measures, NMDS ordinations, and Procrustes analyses were performed using functions of the R package vegan v2.6-4 (Oksanen and others 2022), while RLQ analysis was created using functions found in the ade4 package v1.7-13 (Dray and Dufour 2007).All results are visualized using the ggplot2 R package v3.4.0 (Wickham and others 2022) with minor modifications made in Adobe Illustrator CC (Adobe Systems Incorporated 2015).

**Results**

*Community Trajectories*

NMDS revealed distinct trajectories by the zooplankton communities in each of the four study lakes, with routes centered around two relatively stable states that contrast along the x-axis (Fig. 1). In general, the presence of an established fish population was indicated by communities positioned to the right of the origin along the x-axis (e.g., Harrison Lake, Fig. 1a). In contrast, communities that were indicative of a naturally fishless state appeared to the left of the origin. The NMDS was generated with a stress value of 0.18 at 2 dimensions, falling below the accepted standard of 0.2 that indicates an acceptable NMDS fit (Legendre and Legendre 1998; Clarke and Warwick 2001). Below, the trajectory of each of the four lakes was plotted separately out of the NMDS to better illustrate and explain community changes through time (Fig. 1).

Harrison Lake travelled the least total distance in taxonomic ordination space of any of the four lakes, indicating a relatively stable zooplankton community consisting mainly of small-bodied cyclopoids and cladocerans in the presence of a natural Bull Trout population (Fig. 1a). Nevertheless, a marked taxonomic shift can be seen to have occurred between 1966 and the mid-1970s, which was marked by a decline in the *Diacyclops thomasi* population and increase in the abundance of other cyclopoid copepods (generalized group CYCLO; Table 2). Thereafter, the lake moved relatively little in ordination space as the community remained taxonomically stable until the early 2000s when *Eucyclops* began to steadily increase in abundance. As a result, Harrison Lake displays an overall gradual directional trajectory (Matthews et al. 2013; Lamothe et al. 2019) in ordination space between 1966 and 2015.

Pipit Lake showed a more dynamic trajectory, consisting of two distinct regime shifts (Fig. 1b). Initially, the 1966 sample characterized dwindling populations of *D. middendorffiana*, *H. arcticus*, and other calanoids, the remnants of a previous fishless state of the lake before 1964 and the following 2-year stocking regime (Fig. 1b). Thereafter, the lake community shifted quickly in ordination space within two years towards another state characterized by the smaller cyclopoid *Acanthocyclops vernalis*. Afterwards, the community remained relatively stable until ~1977, which coincides with the last anecdotal report of fish presence in Pipit Lake (Parker and Schindler 2006). Once again, there occurred a substantive shift in the lake’s trajectory between 1977 and 1991 mainly as a result of the natural recovery by the *H. arcticus* population. Thereafter, the lake community remained relatively stable throughout 1990 - 2013, where it closely resembles the composition of 1966. Thus, Pipit Lake showed an overall trajectory in ordination space that can be defined as being saltatory directional with recovery (Matthews et al. 2013; Lamothe et al. 2019).

Bighorn Lake showed an even more dynamic trajectory consisting of three distinct phases (Fig. 1c). The trajectory began with a dominance of *H. arcticus* in 1966. Initially, the now stocked lake moved substantially in ordination space as *H. arcticus* was quickly superseded by *D. thomasi* between 1966 and 1971 following the introduction of sportfish. Thereafter, *A. vernalis* and other small-bodied cyclopoids became more abundant in the presence of a successfully reproducing fish population. Upon gill-net removal of the fish during 1996 to 2001, the trajectory shifted erratically with increased abundances of chydorids, as well as, larger bodied calanoids and *D. middendorffiana*. Following the complete removal of fish by 2002, the lake moved substantially, but not exactly, towards its position in 1966 as *D. middendorffiana* and *H. arcticus* superseded smaller cyclopoid copepods. Here, Bighorn Lake displayed a net trajectory that can be described as directional hysteresis recovery, displaying less obvious recovery than shown in Pipit or Snowflake Lake (Lamothe et al. 2019).

Snowflake Lake showed a unique trajectory compared to those of the other three study lakes (Fig. 1d). It began with a large distance travelled between 1966 and 1968 as *L. tyrrelli* and other calanoid copepods declined following the introduction of sportfish. Thereafter, the trajectory shifted substantially in the reverse direction with the increased abundance of *D. thomasi* and *D. pulex* during the 1970s. Although stocked fish population had collapsed by 1984 (Parker et al. 1996), the lake trajectory did not shift leftwards to the fishless state because of continued high abundances of *D. pulex*, *D. thomasi,* and other cyclopoids.After the aerial reintroduction of *H. arcticus* in 1992 (McNaught et al 1999), the trajectory began to steadily move leftward until both *H. arcticus* and *D. middendorffiana* became re-established in 1997. After 1998, the trajectory began to move downward towards its initial point as the *L. tyrrelli* population recovered, causing the community trajectory to cycle around a composition of *H. arcticus*, *D. middendorffiana*, and *L. tyrrelli* until the final sample point in 2013. Although the trajectory of Snowflake is unique, it can also be described as saltatory directional with recovery (Matthews et al. 2013; Lamothe et al. 2019).

*RLQ Analysis*

RLQ analysis revealed strong selection by fish presence for zooplankton species of small body size, lack of body pigmentation, and raptorial feeding (Fig. 2). Here, the first and second axes of the RLQ captured 98.6% of the co-inertia of the model with the first axis alone accounting for 96.5%. The first RLQ axis significantly captured the covariance between the environmental and trait matrices (covariance = 0.7798) and maintained a moderate correlation to the unconstrained ordination of site abundances (correlation = 0.5194). RLQ analysis identified a significant link between the environmental (R) and abundance (L) tables (model 2; *P*= 0.00002), and a non-significant link between the abundance (L) and traits (Q) tables (model 4; *P*= 0.18478). As such, species and trait coordinates were normalized to model 2 for visualization and interpretation (Fig. 2).

*Zooplankton Community Biomass*

The four lakes also showed distinct levels of average zooplankton community biomass over the past five decades (Fig. 3). Mean annual zooplankton biomass was the highest in Harrison compared to the other three lakes, but also more variable as it declined between the 1960s to 1997 (Fig. 3a). Zooplankton biomass remained relatively low in the presence of stocked fish in Pipit Lake, but thereafter showed a slight overall increase as the lake returned to its naturally fishless state (Fig. 3b). Similarly, zooplankton biomass in Bighorn Lake remained low in the presence of stocked fish, but thereafter, increased and became more variable during and following the removal of the fish between 1996 to 2001 (Fig. 3c). In Snowflake Lake, zooplankton biomass remained relatively stable except for a brief spike around the time of aerial import of zooplankton from nearby Pipit Lake in 1993 (Fig. 3d).

**Discussion**

Our study compared the signatures of ecological resilience and resistance exhibited by three alpine zooplankton communities when confronted by, and later released from, the influence of stocked sportfish. Despite differences in their ecological histories over the past 50 years, communities in all three restored fishless lakes (Bighorn, Pipit, Snowflake) recovered taxonomically back to near their pre-stocking community states within two decades of fish absence. However, the taxonomic recovery trajectory of Bighorn Lake differed from that of its initial displacement and has yet to fully return to the fishless basin of attraction exhibited by Snowflake and Pipit. This lag in recovery highlights the potential for hysteresis (i.e., unexpected events or their legacy effects) to occur during similar lake restorations. Here, our findings showed that selective processes likely differed between the stocking and recovery periods, namely size-selection for more conspicuous prey by introduced fish was so pronounced, that they trigged regime shifts to dominance by functionally distinct, smaller zooplankton species. This state persists until extirpated taxa were able to recolonize and refresh the food web under renewed fishless conditions via gill-netting or natural decline. Compared to natural fish populations, stocked fish cause significant destabilization of the fish present regime of naïve zooplankton community function long after their introduction. Despite being generally stable, Harrison Lake did show a gradual directional trajectory and declining average zooplankton biomass, possibly reflecting the five-fold increase in Bull Trout abundance that had occurred since implementation of strict catch-release regulations in 1980 (Parker et al. 2007).

*Non-native Sportfish Destabilize Ecological Function*

The most striking contrast of the study lakes involved the differing zooplankton communities of the post-fish trajectory of Pipit Lake and the native fish-present Harrison Lake, which together best explained the primary NMDS axis. The relatively short oscillations that characterized the general trajectory of Harrison Lake in ordination space between 1977 to 2009 defined a stable basin of attraction for a native fish-inhabited state (Lamothe et al. 2019) that centers around the presence of cyclopoid copepods. In contrast, the saltatory directional recovery of Pipit Lake to the opposite extreme of the x-axis after fish decline confirmed the existence of an equally stable fishless state. Here, tight clustering of the sampled years in ordination space centred around *H. arcticus* and *D. middendorffiana*, signifies a basin of attraction for a fishless lake regime (Lamothe et al. 2019). As such, our findings corroborate earlier reports by showing that Pipit Lake has stabilized to become representative of other naturally fishless lakes within the study area (Parker et al. 1996, 2001; McNaught et al. 1999; Parker and Schindler 2006).

The lack of overlap between the fish-present communities between stocked lakes and that of Harrison Lake supports how the impact of a novel predator on a prey community implicitly differs from that of a native predator (Loewen and Vinebrooke 2016). Our results also revealed that the introduction of non-native sportfish into fishless alpine lakes destabilizes prey communities both taxonomically and functionally, relative to those in the presence of a naturally reproductive native fish population. The fish present signatures of our stocked lakes only transiently occupy similar community points to Harrison Lake, denoting the importance of local adaptations by zooplankton to the presence of vertebrate predators and the importance species suppressions have on community stability (Loewen et al. 2018; Shanafelt and Loreau 2018; Lamothe et al. 2019). Zooplankton traits adapt and acclimate in generational exposure to stressors, gradually optimizing local species performance through energetic trade-offs (Hébert et al. 2017).

RLQ analysis revealed fish predation upon gradients of size-selection and visual opacity principally drive the community assemblage and functional originality of alpine lakes, due to associations with other functional traits such as trophic group, feeding method, and motility. Here, body size can be termed as a “master trait” mediating the impacts of other functional traits that have broader functional implications from the community to ecosystem level (Brooks and Dodson 1965; Loewen et al. 2016; Hébert et al. 2017; Redmond et al. 2018). The intense predation pressure exerted by such novel predators on initially abundant prey species can result in erratic shifts towards other functionally distinct species (Hébert et al. 2017). These differences in species trait expression, as well as predator-prey cycles between fish and individual zooplankton species, lead to novel direct and indirect interactions among zooplankton populations that can destabilize communities by lowering their resistance and resilience (Shanafelt and Loreau 2018; Lamothe et al. 2019). By functional trait RLQ, Redmond et al. (2018) also similarly emphasized the lack of functionally redundant species co-occurring in high elevation lakes, signifying strong niche competitions at both regional and local scales (Mouillot et al. 2013; Redmond et al. 2018).

*Recovery after Gill-netting vs Natural Fish Decline*

Gill-netting of introduced sportfish in Bighorn Lake did not accelerate taxonomic recovery of the zooplankton community, having yet to make a complete recovery like those of Pipit Lake or Snowflake Lake. Nevertheless, gill-netting (active management) did enable saltatory directional recovery with the potential to trend towards the basin of attraction that characterizes a fishless alpine state, best characterized as a hysteresis recovery trajectory (Lamothe et al. 2019). Such recoveries travel unique paths to full recovery due to legacy effects of fish presence lowering thresholds to alternative, non-targeted community states (Lamothe et al. 2019). Bighorn Lake stands apart from the other restored lakes, as its stocked fish population reproduced and persisted for longer than Snowflake or Pipit, making it unlikely that any diapausing eggs survived the 35-year occupation of fish, suggesting total extirpation (Parker 2001; Knapp and Sarnelle 2008). Further, natural emergence and immigration events of large, obligately sexual zooplankton in alpine lakes often fail to re-establish populations after release from top-down control by fish (Kramer et al. 2008; Loewen et al. 2018). Such species struggle to find mates under conditions of low abundance, shifting emergence cues, and competitive release of mesopredators impeding functional recovery via the Allee effect (Kramer 2008; Dupuis and Hann 2009; Tiberti 2018). These speciation filters are increasingly tricky for *H. arcticus* who are climatically sensitive obligate sexual reproducers and may have been eradicated immediately upon emergence or before copulation (Parker et al. 2001). These results suggest that while active fish removals may not accelerate recovery in alpine environments, they do erode thresholds to recovery.

*Extirpated Species Reintroductions*

Our results also revealed a small persistent re-establishment of *H. arcticus* in Bighorn Lake since 2003, without the need for deliberate restocking, an event previously thought not possible in either this lake or Snowflake Lake (Parker et al. 2001). As such we are drawn to consider if reintroduction of extirpated species alone is of any benefit to lowering functional recovery resistance. In Snowflake Lake, the stocked fish population had persisted for approximately 25 years, limiting the chance for re-emergence by *H. arcticus* from resting egg structures (McNaught et al. 1999). The few *H. arcticus* eggs that may had remained viable in the sediment were also heavily predated by *Gammarus lacustris,* which had been released from predation following the decline of the stocked fish population (Parker et al. 1996; McNaught 1999). In addition, high abundances of predatory cyclopoids persisted after fish decline, further suppressing re-establishment of *H. arcticus* or *D.* *middendorffiana* from sedimentary resting stages or dispersal from the regional species pool (Parker et al. 1996, McNaught et al. 1999).

The pulse reintroduction of *H. arcticus* competitively excluded *Daphnia pulex,* and predatorily suppressed small cyclopoid populations (McNaught et al. 1999). This suppression of the smaller predatory copepods and elimination of competitive daphnids also enabled quicker re-establishment of *D. middendorffiana* from natural dispersal. Many cladocerans disperse and colonize more efficiently than copepods in the absence of predators, due to clonal reproduction mostly releasing them from the Allee effect (Kramer et al. 2008; Loewen and Vinebrooke 2016). The inoculation of *H. arcticus* triggered accelerated, directional recovery in Snowflake Lake. Also, the consistent movement of the trajectory suggests the biological filters to *H arcticus*, and *D.* *middendorffiana* establishment was a threshold to community recovery, eroded by the artificial influx of calanoid omnivores (Parker and Schindler 2006; Lamothe et al. 2019). We conclude that reintroduction of *H. arcticus* to Snowflake Lake was necessary to return to a fishless community state. Moreover, *H. arcticus* reintroduction accelerated recovery and promoted stability by opening niche space for the complimenting large-bodied zooplankton species, allowing recovery to the pre-fish state exemplified by Pipit Lake.

*Community Biomass*

A surprising result of our investigation of aggregate functional biomass is the loss experienced in Harrison Lake since the 1960s. This decline has likely been the result of community restructuring to smaller bodied, transparent cyclopoid species suffering from the Allee effect (Kramer et al. 2008). This restructuring and increased reproduction pressure are likely the result of a five-fold increase in the native Bull Trout population reported by Parker et al. (2007), after closure of the nearest access road effectively ceased fishing activities in 1988. Wilhelm et al. (1999) suggest that Harrison Lake’s Bull Trout experience high levels of intraspecific competition for food and unusually low levels of cannibalism. The combination of reduced prey abundance, size, and unchecked population growth caused a size shift to smaller Bull Trout as well, who predominantly are zoo-planktivorous (Wilhelm et al. 1999; Parker et al. 2007). This increased intraspecific competition for zooplankton prey gradually restructured the zooplankton community function towards amplified omnivory and reduced grazing, which lowered overall water clarity (Wilhelm et al. 1999).

The mean annual zooplankton biomasses of our naturally fishless lakes did not significantly change after the non-native fish extirpation. This indifference can be attributed to functional compensation by small-bodied zooplankton after the introduction of non-native predators. These smaller species were freed from competitive exclusion and predation by larger zooplankton, driving aggregate biomass to environmentally dictated capacity (Parker and Schindler 2006). Further evidence for carrying capacities of zooplankton in our lakes stem from the lack of persistent, increased biomass levels once large-bodied zooplankton re-establish after fish removals, even after *H. arcticus* reintroduction event in Snowflake Lake. However, once large species re-establish in fishless lakes, they comprise approximately 90% of the available biomass (Parker and Schindler 2006).

*Ecological function to service*

The removal of non-native fish aids in the preservation of alpine lakes as healthy refuges for large plankters such as *H. arcticus* and *D. middendorffiana* that both directly or indirectly provide energy up aquatic-terrestrial food webs to macroinvertebrates, amphibians, and birds (Loewen et al. 2018; Tiberti et al. 2018; Koel et al. 2019). In mountainous lakes of the northwest United States, limnologists have noted increased nitrogen depositions in alpine lakes by air pollutants stimulating diatom growth and increased trophic states resulting in reduced water quality (Williams et al. 2016; Rhodes et al. 2017). The preservation of large-bodied grazers, such as *D. middendorffiana* which effectively graze diatoms, may increase community resilience to loss of water quality and clarity. This buffering to increasing trophic state is especially crucial in alpine lakes for clean freshwater supply (Parker and Schindler 2006; Rhodes et al. 2017). However, the management practices involved are nuanced by socioeconomic factors (Chiapella et al. 2018), and resistance thresholds to protect biodiversity are hard to predict (Standish et al. 2014; Rhodes et al. 2017).

*Conclusions*

Our discovery of two distinct zooplankton community states in naturally occurring fish present and previously stocked alpine lakes provide a robust framework to judge the taxonomic and functional recovery potential of three distinct scenarios of fish stock removal. This investigation revealed similar levels of resistance and resilience to these management actions, providing valuable insights into the restoration of stocked mountain lakes back to their naturally fishless state. Firstly, natural resource managers should be encouraged by our results, which show that full recovery by zooplankton communities can likely be achieved within 10 – 20 years regardless of fish eradication method from the lakes. The demonstrated decadal timeframe for recovery in these lakes corroborates earlier evidence for other lakes in the Canadian Rockies (Donald et al. 2001) and the Californian Sierra Nevada (Knapp et al. 2001). Secondly, resource managers should also anticipate some ecological surprises during recovery. As our results showed, recovery trajectories may stray from the path travelled to a fish-present state, due to legacy effects of fish (i.e., hysteresis). Thirdly, we demonstrated that introduced fish destabilized zooplankton communities, relative to native fish. This disruption of function lends support to efforts targeting the removal of non-native fish because of their invasive impacts on naturally fishless mountain lakes.

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**List of Tables**

Table 1 Stocking histories of Harrison, Pipit, Bighorn, and Snowflake Lake (Anderson and Donald 1978; Parker et al. 1996, 2001; McNaught et al. 1999; Wilhelm et al. 1999).

Table 2 Matrix of zooplankton taxon, assigned codes, and functional traits (Barnett et al. 2007; Hébert et al. 2016, 2017; Redmond et al. 2018).

**Figure Captions**

**Fig 1** Single NMDS ordination in 2 dimensions, parsed apart to compare temporal community trajectories of four alpine lakes varying in stocking history and recovery action between 1966 to 2015 for **a** Harrison Lake (naturally fish-present), and to 2013 for **b** Pipit Lake (stocking termination), **c** Bighorn Lake (gill-net fish removal), and **d** Snowflake Lake (stocking termination with species reintroduction). Zooplankton taxa are also visualized to provide insight of actual community assemblage at each timepoint. This ordination space was constructed using Bray-Curtis distances between annual average zooplankton abundances of all four lakes. Trajectory segment color represents states of fish status between recorded timepoints for each lake.

**Fig 2** RLQ analysis ordination space of zooplankton abundances (species codes) and functional traits (colored squares), based upon their relationship to measured environmental variables and fish presence (inset vector plot) of four alpine lakes (Harrison, Pipit, Bighorn, and Snowflake Lake) between the years 1991 to 2002. Functional trait conditions are grouped by color.

**Fig 3** Mean aggregate zooplankton biomasses (µg/L; points) of four alpine lakes varying in stocking history and recovery action between 1966 to 2015 for **a** Harrison Lake (naturally fish-present), and to 2013 for **b** Pipit Lake (stocking termination), **c** Bighorn Lake (gill-net fish removal), and **d** Snowflake Lake (stocking termination with species reintroduction). Line color changes, and vertical dashed lines, represent changes in fish status between recorded timepoints for each lake. Star symbol represents year of *Hesperodiaptomus arcticus* reintroduction in Snowflake Lake.