**Cleaning Up the Green: A Review of Current Biological Manipulation Methods to Restore Water Quality in Temperate Eutrophic Waters**

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 Arguably the most pressing threat to freshwater ecosystems worldwide is eutrophication: the overproduction of phytoplankton and algae in a body of water brought about by excessive nutrient input. The harmful consequences of eutrophication are well documented and publicized, specifically the anoxic “dead zones” that are created when the algae eventually die and decompose (Dodds, 2006; Sinha et al., 2017). This issue is pervasive across the world but is worst in areas where extensive agricultural runoff containing nutrients such as nitrogen and phosphorus pollutes watersheds (Dodds, 2006; Paerl et al., 2006). In the coming decades, land development along with increased precipitation due to climate change will only exacerbate nutrient loading leading to eutrophic conditions (Sinha et al., 2017).

Currently, most treatments of these conditions concentrate on managing the nutrient input through either physical or chemical means (Paerl et al., 2011; Sinha et al., 2017; Walker et al., 1989). In contrast to these expensive and often destructive interventions, manipulating the biological communities within the aquatic ecosystem has been shown to be an effective, relatively natural means of restoring water quality in eutrophic waters. This type of intervention only requires manpower and often works in tandem with fishery maintenance (Burns et al., 2014, Lazzaro, 2007). Additionally, its effects have been shown to last longer than chemical interventions, although repeated interventions are always necessary in eutrophic water management (Søndergaard et al., 2008; Walker et al., 1989). The extent to which water quality has been improved is often measured by water sample measurements for total nitrogen, total phosphorus, and chlorophyll a content as well as Secchi depth measurements to estimate primary production and turbidity (Carpenter et al., 1985; Walker et al., 1989).

 Biological manipulation – often referred to as biomanipulation – can take on many different forms depending upon the biotic and abiotic factors of the target body of water. It is widely understood that the structure of a food web plays a significant role in the production of phytoplankton and algae at a given nutrient loading rate (Carpenter et al., 1985). The goal of biomanipulation is to use a base knowledge of trophic interactions to limit the rate of primary production. The methods of biomanipulation, consequently, vary with geographic location and ecosystem compositions (Burns et al., 2014; Lazarro, 1997; Walker et al., 1989). Due to the relative complexity of fish communities and food webs in tropical and subtropical climates (Lazzaro, 1997), the majority of biomanipulation studies have taken place in temperate and boreal climates in the Northern hemisphere where food webs are well understood (Burns et al., 2014; Sierp et al., 2009). Differing opinions on the strength of food-web interactions on eutrophication has in turn led to a wide array of biomanipulation interventions that address various trophic levels. Three techniques, however, have emerged as the most common: fish manipulation, filter-feeding organism manipulation, and submerged macrophyte manipulation.

*Fish Manipulation*

 Fish populations can provide a wide variety of ecological impacts. There are, therefore, several different ways in which fish are manipulated to mitigate the impact of eutrophication. One of the most common forms of fish manipulation involves the addition of predatory fish that feed on smaller fish which support primary productivity through services such as zooplankton suppression and nutrient cycling (Lazzaro, 1997; Søndergaard et al., 2008). This form of manipulation relies on the assumption that the food web structure is cyclical; smaller fish and zooplankton act as important nutrient sources for phytoplankton (Vanni & Findlay, 1990). Findlay and others (2005) demonstrated in a whole-lake experiment that the addition of Northern pike (*Esox lucius*) could effectively control yellow perch (*Perca flavescens*) populations and thus phytoplankton production through top-down trophic interactions in boreal lakes in Ontario. This supported past experiments conducted in enclosures (Vanni & Findlay, 1990), thus strengthening the assumption that top-down predator manipulation creates strong water quality improvement effects. These conclusions, however, are potentially overreaching yet narrow in scope. Food-web interactions are innately complex, and it is likely that there are multiple confounding variables that lead to the observed results beyond the theoretical interactions proposed (DeMelo et al., 1992).

 Indeed, there is evidence that rather than a predicted top-down trophic cascade suggested by numerous studies (Carpenter et al., 1985; Findlay et al., 2005), there can be much more complex ecological actions leading to the expected water quality restoration. In a biomanipulation study in a eutrophic lake in China, researchers observed that the removal of benthivorous fish – fish that feed along the lake floor and thus stir up sediment and nutrients – were likely the mechanism behind restoration of water quality (Liu et al., 2018). Although piscivorous fish were also added to induce the top-down trophic interactions on planktivorous fish described by Findlay and others (2005), the researchers observed that zooplankton density was relatively low and that their grazing on phytoplankton was minimal. In fact, the removal of benthic feeding fish likely created suitable environment for submerged macrophytes to outcompete the phytoplankton. Other studies have confirmed that fish biomanipulation does not impact zooplankton biomass through cascading trophic interactions as other studies assume (Settubal and Riccardi, 2020).

 Many biomanipulation interventions instead remove planktivorous fish directly rather than introducing their predators. Søndergaard and others (2008) conducted a massive removal of different species of fish that both cycle nutrients from the lake floor and feed upon zooplankton, then observed the effects over a long period (10-15 years) in over 36 Danish lakes. They concluded that repeated interventions were necessary, as the reduced nutrient loading effects of fish removal lasted only 6-10 years. The scale of this study is uncommon in the field, as most studies are limited to short term, single lake experiments (Chen et al., 2020; Findlay et al., 2005; Setubal & Riccardi, 2020). While this study provides a compelling example of the efficacy of fish removal, there is potential bias in the sample selection as the authors selected lakes based upon the existing level of eutrophication and proximity to developed land where nutrient loading is high. This limits the external validity of the findings beyond those conditions. Nevertheless, fish manipulation has become a popular method of biomanipulation treatment in Northern European freshwater lakes as well as temperate Chinese lakes (Burns et al., 2014; Liu et al., 2018; Sierp et al., 2009).

*Filter-Feeding Organism Manipulation*

 A more direct treatment of eutrophication involves the addition of organisms that filter the water and feed upon phytoplankton and algae. Filter-feeding mollusks such as mussels (*Hyriidae*) can effect significant decreases in nitrogen, phosphorus, and chloryphyll a content in eutrophic water, thus leading to lower turbidity (Gao et al., 2017; Soto & Mena, 1999; Waajen et al., 2015). Unlike fish manipulation experiments, whole-lake experiments involving the addition of mussels are scarce. Rather, researchers have often utilized in situ enclosure experiments (Waajen et al., 2015) or ex situ tank experiments (Soto & Mena, 1999) to effectively observe their isolated filtration rates within eutrophic water. An advantage of this method of biomanipulation is that is does not rely upon complex food web interactions to improve water quality. Because of this, the profound impacts of mussel addition are easy to observe (Waajen et al, 2015). Additionally, mussels are generally hearty and highly adaptable, allowing them to be used in biomanipulations in a variety of climates (Gao et al., 2015). A drawback, however, is that little is understood about the long-term effectiveness of these manipulations (Gao et al., 2017; Soto & Mena, 1999; Waajen et al., 2015). Scaling up toward whole-lake manipulations and long term (5-10 years post-treatment) observation is a logical next step in mussel manipulation research.

 While mussels’ ecological impacts are mostly limited to grazing upon plankton and detritus, filter-feeding fishes can effect a far greater influence on the biotic and abiotic components of a lake. Silver carp (*Hypophthalmichthys molitrix*) addition has been commonly used to produce similar phytoplankton suppression effects as mussel addition through filter feeding (Hu et al., 2017; Gao et al., 2017). However, carp increase nutrient release from sediment via foraging activity, thus increasing phytoplankton production, and negating positive effects from their filtration (Gao et al., 2017). Researchers have therefore concluded that filter feeding fish manipulations alone are insufficient in treating eutrophic bodies of water and should be used in tandem with other manipulations, if at all (Hu et al., 2017; Gao et al., 2017).

*Submerged Macrophyte Manipulation*

 Biomanipulations can be effective when manipulating organisms that occupy the same trophic level as phytoplankton and algae, namely large submerged marcophytes along the lake floor. By competing with phytoplankton for available nutrients, macrophytes effectively suppress eutrophic conditions in temperate, shallow bodies of water (Chen et al., 2020; Sayer et al., 2010, Søndergaard & Moss, 1998). In contrast to the easily isolated effects of filter feeding mollusks, most research on macrophyte manipulation is done via whole-lake experiments in combination with other biomanipulations (Chen et al., 2020; Settubal & Riccardi, 2020), which limits the possibilities to compare methodologies between different types of manipulations.

The effects of macrophyte manipulation could also extend beyond trophic competition with other primary producers. Settubal and Riccardi (2020) conducted a long term (24 years monitoring) biomanipulation study in Italy which speculated that the change in habitat brought about by macrophyte management led to significant changes in zooplankton communities that in turn improved water quality. It should be noted that this biomanipulation included fish addition that could have confounded the results. In addition, the study had no control lake for comparison.

 Chen and others (2020) conducted a combined biomanipulation project in a shallow, eutrophic lake in subtropical China that involved the addition of submerged macrophytes, filter-feeding fish (*Hypophthalmichthys molitrix*), filter-feeding mollusks (*Hyriidae*), and zooplankton. The researchers observed substantial and quick nutrient reduction and water quality improvements. They attributed the improvements to positive feedback effects within the constructed community, namely that the presence of the filter-feeding organisms created more suitable habitat for submerged macrophytes to flourish, a finding supported by past research (Gao et al., 2017, Sayer et al., 2010). Both the short-term observation and the complex interactions in this study make continued observation and replication critical in confirming the validity of this combined manipulation.

*Future Directions*

 There is substantial research on the various types of biomanipulation treatments, with most studies presenting results of the application of either one technique or a combination two or more (Dodds, 2006; Sierp et al., 2009). Few studies provide a direct comparison of the success rate of the different manipulation methods discussed in this review. Geographic and climatic differences among study sites make effective comparison among these methods difficult. As mentioned earlier, food webs can show dramatic diversity by location, so the chosen methods of biomanipulation tend to be tailored to the region and climate (Burns et al., 2014; Lazarro, 1997; Walker et al., 1989). Nonetheless, there is little consensus on which methods or combinations of methods of manipulation are the most effective at alleviating eutrophic conditions. The purpose of this study was to compare and contrast the success rate of the three most common forms of biomanipulation – fish, filter-feeding mollusks, and macrophytes – in three separate temperate, eutrophic lakes within the same geographic region.

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