Abstract: Didymosphenia geminata diatoms, or Didymo, was first found to be an invasive species that could have negative impacts on the environment due to the aggressive growth of its polysaccharide-based stalks. The stalks' adhesive properties have prompted park officials to alert the general public to limit further spread and contamination of this algae to other bodies of water. Although the negative effects of Didymo have been studied in the past, recent studies have demonstrated a potential positive side to this alga. One of the potential benefits includes the structural component of the polysaccharide stalks. The origin of the polysaccharides within stalks remains unknown; however, they can be useful in a waste management and agricultural setting. The primary purpose of this study was to describe both the harmful and beneficial nature of Didymo. Important outcomes include findings related to its application in various fields such as medicine and technology. These polysaccharides can be isolated and studied closely to produce efficient solar power cells and batteries. Though they may be harmful while uncontained in nature, they appear to be very useful in the technological and medical advancement of our society.

Keywords: polysaccharides; biomineralization; chitin; algae; diatom; didymo; biosilica; biopolymers; environment

1. Introduction

Didymosphenia geminata, commonly known as Didymo, is a relatively new alga that has been found to affect stream systems throughout the world. When Didymo grows into stalks, it can produce negative environmental and ecological impacts. In the International Workshop on D. geminata in 2008, it was stated that Didymo stalks are composed of proteins, sulfated polysaccharides, and some uronic acid. Figure 1 provides a closer look into the stalks’ ability to grow to a length of 500 µm or more [1]. The structure of biomineral components of this species is different from other freshwater [2] and marine algae [3]. For example, polysaccharide-based stalks of Didymo are reinforced with unique calcitic nanofibers [1]; however, the cell is still made of biosilica. These algae tend to form a dense mass through cell division that can be seen as clumps in rivers (as seen in Figure 2). These diatoms can cover up to 100% of a substrate and form a thick layer of more than 20 cm, which can cause physical and biological problems in streams. Moreover, Didymo is able to cover areas over 20 km and can be present in streams for many months. This causes disturbance in the benthic regions and does not allow for the growth of other algae. These stalks also interfere with the fish communities underwater and have been a nuisance to humans due to their ability to clog water filters and hydro plants. Even though Didymo has caused disruptions regarding different underwater communities and human activity, there may now be a promising use of these stalks and their ability to act as reservoirs of mineral ions due to their microcapillary action.
Their structure is considered to be helpful when it comes to metal removal applications due to the large amounts of complex extracellular biopolymers. Due to this characteristic, they are being considered as a fibrous absorbent of lead (II), nickel (II), and cadmium (II) in wastewater systems [5]. As more research was done on this species of algae, it was found that Didymo would be transferred to different parts of the river by sticking to the bottom of fishermen’s boots. As the fishing industry grew in Vancouver Island, so did the amount of Didymo sightings. It was during this time that a connection was made between fishermen’s boots contaminating more of an area than normal [6]. It was further discovered that Didymo was not only transferred through fishermen’s boots, but also through people coming to the rivers for recreational activities. Their swimwear and fishing equipment would be used as a vessel for Didymo transference to other parts of the river, as people coming to the rivers for recreational activities. Their swimwear and fishing equipment would be used as a vessel for Didymo transference to other parts of the river, as people themselves explored the area. To limit the spread of Didymo, proper signage was made for public awareness. As Figure 3 shows, certain steps needed to be taken by the visitors [7]. The proper management of sensitive areas can help avoid problems in the future and can help protect the environment.

This paper focuses on the harmful and beneficial nature of Didymo and its stalks. By analyzing both sides of its nature, future measures can be taken according to the overall consensus of its status. For example, if the negative impacts of Didymo exceed its positive impacts, communities with an infestation would need to produce and utilize a permanent solution for its removal. However, if the benefits of Didymo appear to outweigh its disadvantages, more research should be devoted to how to utilize these polysaccharide stalks in industries outside of agriculture.
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Figure 3. Didymo information signs posted at the Battenkill River and other streams in New York recommend drying fishing gear or cleaning with household cleaner/disinfectant. Photo credit: Samantha Root [7].

2. Current Status of Didymo in Canada

Although the Vancouver Islands have seen Didymo in their rivers for the last few decades (since the 1990s) [6], on the other side of Canada, in Montreal, they were discovered much later. In 2006, Didymo was first found in the Matapedia River in Montreal [8]. Another river in Quebec, the Causapscal River, remained Didymo-free and was used as a control site to observe the difference in aquatic food webs. Since the Matapedia River is a source of aquatic salmon in the region, there was fear of a lack of availability of salmon which is an important source of food in the area, and therefore studies were done between the different rivers. Comparisons between the different environments have shown that the macroinvertebrate densities have increased in rivers where Didymo was present compared to the control site where Didymo was not found. The structure of the community in the region did change when certain flies (mayflies, stoneflies, and caddisflies) were seen to decline by about 30–50% where Didymo was present, therefore showing an inverse relationship between this particular species of flies and Didymo [8].

As seen in Figure 4, other areas where Didymo was found and surveyed in Canada were the Eastern Rocky Mountains, specifically, the Athabasca River (surveyed 2003–2007), Bow River (surveyed 2002–2007), and Red Deer River (surveyed 2004–2006) [9].

The commonality between these three rivers was that they were in the middle of forests and running downstream. In 18 rivers from the South Saskatchewan River Basin, 80% of 50 sites showed Didymo growth. Dense growth has also been recorded in almost half of the sites in Alberta. In Alberta’s Bow River and Red Deer River, a negative relationship was seen between Didymo biomass and mean discharge. This negative relationship could be due to the changes in temperature due to changes in flow at a dam outflow. These changes can allow for various types of nutrient growth and can change the chemistry of the water [9]. More research needs to be done in other provinces within Canada, especially Ontario, where a large amount of the population live around water bodies and could potentially contaminate these areas fairly quickly. As can be seen in Figure 5, there has not been much research in the northern parts of Canada and a future research project is needed in order to properly portray the appearance of Didymo. With the help of GIS techniques and remote sensing, a closer look can be taken at Didymo in certain areas, and a record of past observances can allow us to better monitor potential areas of rapid growth.
Figure 4. Didymo presence in British Columbia and Alberta [10].

Figure 5. Worldwide distribution of records for *D. geminata* [7].
In Figure 4, we can also notice that some areas (blue) are showing new growth of Didymo and it is essential to make note of these in further studies to see how fast this spreads. There may even be a chance to observe some blooming Didymo that are not currently present, which may lead to further study of the area to learn about what may have stopped the growth. These data can be beneficial to data analysts for modeling future increases or decreases in Didymo within the area. An issue with collecting data within Canada comes when satellites are not able to detect the growth on land due to snow cover. This may be the reason why, in Figure 5, only the southern parts of the provinces are shown. Therefore, it is essential to keep in mind that a project done on Didymo in the northern areas of Canada has to be done during the summer months. This will allow us to properly observe growth on land without natural interruptions.

3. How Harmful Is This Species to the Canadian Environment and Public Health?

In terms of the negative effects that Didymo can have on the environment, several studies have focused on the impact that *D. geminata* has on native species and biodiversity in different regions. Within the last decade, *D. geminata* has spread outside of its native regions to the rivers and lakes of several countries including New Zealand, Argentina, and southern Chile. In the benthic diatom communities in the Chilean rivers, it was found that community heterogeneity decreased in the presence of *D. geminata*, making the site more homogenized. This result was observed through the increase in small stalked diatom density and the decrease in species turnover in the invaded rivers. The authors did not observe species exclusion; however, it was observed that *D. geminata* is inclined towards a particular group of diatom species. This inclination can displace other diatom species and, therefore, cause different types of impacts, such as alterations in fluvial trophic webs [11].

There are several studies that have commented on the possible effects that *D. geminata* could have on fluvial tropic webs. For example, in British Columbia, it was observed that a salmon channel covered with *D. geminata* showed reduced zoobenthos heterogeneity in comparison to the mainstream. Due to the presence of *D. geminata*, the distribution of salmonid food organisms increased in the channel from 57% to 93% Chironomidae [9]. This was a similar observation found in the Matapedia River Valley (located in Quebec). Even though there is currently no scientific evidence that suggests this alga has significant effects on the Atlantic salmon population in Eastern Canada, Gillis and Chalifour [8] reported that the presence of *D. geminata* could act as an additional stressor to this population. They discovered that benthic macroinvertebrates, such as mayflies, stoneflies, and caddisflies, diminished due to the presence of *D. geminata*, while chironomid proportions had increased and become the dominant taxa. This finding confirms that this community has a less homogenous macroinvertebrate distribution due to the presence of this alga [8]. More research would have to be done to determine the long-term impacts that *D. geminata* has on salmonid populations, but there is clear evidence that the presence of *D. geminata* does change the distribution of salmonid food organisms, which, in turn, could have impacts on the diet and physical condition of the salmonid population.

While it has been generally accepted that *D. geminata* has the potential to alter ecosystems, it is not considered to be directly harmful to human health. However, while there is no direct impact of *D. geminata* on human health, there is a growing literature that demonstrates that the changing of ecosystem structures can have a negative impact on public health [12]. This is an area of research that is at the intersection of health and ecology. While the presence of *D. geminata* in a community would not necessarily lead to a risk in exposure to infectious diseases, a decrease in biodiversity or the abundance of a species could have other significant human health impacts. A lack of biodiversity could contribute the diminishing of marine wildlife and cause a nutritional crisis in certain communities [12]. More research would have to be done to determine whether the change in the ecosystem structure due to *D. geminata* has negative impacts on public health.

As we now aware, *D. geminata*, forms brownish-whites mucilaginous stalks that have been reported to be as thick as 20 cm [13]. As Didymo spans many kilometers, it
can monumentally degrade aquatic ecosystems and cause a drastic change in benthic communities [13], such as replacing invertebrate species like mayflies and stoneflies with much smaller midges [13]. Furthermore, shallow cold-water streams, rivers and lake margins with rocky substrates that have constant flows and low nutrients allow Didymo to grow favorably [13]. Didymo has been known to be a significant nuisance for local trout and salmon populations, being the ideal habitat for those species of fish [13]. Previous studies have found that Didymo has an effect on the microenvironment by reducing fish populations [14]. Due to the nature of fish spermatozoa, within the ejaculate, they are immobile, and only after osmotic shock in water can they begin to move or swim [14].

Overall, while there are distinct patterns in how the presence of *D. geminata* affects the biodiversity of certain communities both internationally and locally, there has not been enough research done to demonstrate the longitudinal effects that this species has on trophic food webs and public health. This area of research would have to be explored further.

4. Benefits of Didymo

While most of the research surrounding *D. geminata* has focused on its structure, management, and possible negative impacts, there has been recent research published that demonstrates the possible positive effects that Didymo can have on the environment. In a paper written by Wysokowski et al. [5], the authors state that the polysaccharide stalks of *D. geminata* have the ability to absorb harmful metal ions, such as Pb (II), Ni (II), and Cd (II). These stalks contain large amounts of complex extracellular biopolymers, which allows them to remove metals comparable to other polysaccharides. The authors used a pseudo-second-order kinetic model to describe the absorption kinetics of the stalks and found that *D. geminata* demonstrated an extraordinary sorption capacity for Pb (II) ions of 175.48 mg g$^{-1}$ and a high sorption capacity for Cd (II) ions of 145.86 mg g$^{-1}$. They also observed a sorption capacity of Ni (II) ions of 130.27 mg g$^{-1}$. In terms of applicable use, after performing tests on industrial wastewater, the authors suggest that this alga can be used for the purification of industrial wastewater, especially water that has a high content of Pb (II) ions. Due to *D. geminata’s* effectiveness of absorption, this biological material can be competitive with manufactured and low-cost adsorbents [1].

The discovery of *D.geminata’s* biological absorption ability can have profound effects in the treatment of industrial wastewater; however, its methods for commercial use will have to be further investigated. Industrial wastewater is typically treated through wastewater plants. Due to the sheer volume of wastewater, *D.geminata* could be used within this process, so long as it is contained within a closed system. Another positive effect surrounding this freshwater diatom is its role in nitrogen fixation. Even though little is known about the different microbial species associated with *D. geminata*, it is assumed that many different microbial communities are known to co-exist with the freshwater algae [15]. Found in these communities are nitrogen-fixing bacteria, which are commonly found in habitats that *D. geminata* covers [15]. Furthermore, bottom-feeding cyanobacteria can illustrate the environmental conditions of the freshwater body [15]. Previous studies have stated that *D. geminata* and nitrogen-fixing bacteria have a mutualistic relationship, where nitrogen fixers could provide nitrogen to Didymo in a low-nutrient environment [15]. The study goes on to state that it believes the genus Godleya could be one of the cyanobacteria that favor similar habitats and that fix nitrogen for *D. geminata*. With this in mind, our ability to obtain suitable growth patterns of *D. geminata* can allow for these nitrogen-fixing bacteria to proliferate in areas that are low in nutrients. Studies have concluded that due to 78% of the earth’s atmosphere is made up of nitrogen but exists as dinitrogen [13]. Moreover, to allow living organisms to use nitrogen, it must be transformed into ammonia or nitrate via endosymbiotic bacteria [16]. Ultimately, if *D. geminata* is sustained with less environmental impact it can benefit aquatic plants growing in unfavorable low-nitrogen environments by providing enough nitrogen to grow.

\[
\text{N}_2 + 8e^- + 8H^+ + 16\text{ATP} \rightarrow 2\text{NH}_3 + \text{H}_2 + 16\text{ADP} + 16\text{P}_i
\]
Not only do the polysaccharide stalks of *D. geminata* have incredible absorption abilities, it was also found that the structure of the stalks allows the algae to be both stable and flexible under various flow conditions. Ehrlich et al. [2] discovered that the stalks contained nanostructured calcite-based scaffolds. This structural attribute could further provide opportunities for biometric approaches in developing unique and innovative biomaterials with adhesive properties. While *D. geminata* has been termed as a “nuisance species,” there have been reports that show the possible positive effects on the environment and in the development of biomaterials.

This group has been extensively studied by researchers since the 18th century because of its unique and complicatedly patterned frustules (silica cell walls). The mechanical structure of frustules was well investigated, revealing interesting characterizations and patterns [17–19]. Interestingly, the frustule is mainly composed of chitin [20] in biogenic silica, which is different than other chitin-based algae [1,21] in nature. Because of their unique mechanical properties, they have a large variety of applications, such as in the modeling of mechanical properties [19,22], drug delivery [23,24], electronics [25], the modifier of resin [26], and biomimetics [27].

5. Management of *Didymosphenia geminata*

*D. geminata* has the potential to have catastrophic effects on local freshwater environments, due to the high isolation and endemism found in species living in rivers, streams, lakes and ponds [28]. In recent years, since this discovery, many different research programs have investigated different potential control products to manage the spread of Didymo [28]. A previous study performed rigorous testing to identify the best chemical control compound [14]. Such testing looked at the toxicity of chemicals such as chelated copper compounds known as Germex, EDTA, Hydrothol 191, and Organic Interceptor, otherwise known as pine oil formulation [17]. Numerous factors went into the testing process to determine which of these chemicals had the best effect on Didymo [17]. These factors included effectiveness, non-target species impacts, stalk removal, degradation profile, risks to health and safety, ease of application, neutralization potential, cost, and local regulatory requirements [14]. The study found that both Gemex and Organic Interceptor were the best in terms of biocidal efficacy on Didymo [28]. However, when Organic Interceptor was exposed to species of fish, the researchers noticed a higher mortality rate under laboratory conditions [28]. However, Germex was noted to have negative effects on invertebrates, allowing stakeholders to approve the chemical as the best option to tackle this freshwater diatom, but further testing is required. Other household products have also been discussed for controlling the spread of *D. geminata*. Among several common decontamination treatments that were tested, the study by Root and O’Reilly [7] found that dish liquid detergent was the most effective, followed by bleach, Virkon, and salt. The above decontaminants were highly effective on the *D. geminata* cells still attached to their stalks [7].

However, due to the nature of Germex and other toxins which could have adverse effects on nontarget algae by disrupting the base of the food web, many researchers have looked at novel biological control methods [29]. Studies surrounding the use of Germex have proven that whole-stream chemical poisoning can have major implications for the surrounding environment, causing more ecological costs than benefits. Short-term applications of Germex, repeated weekly, can cause major damage to local trout populations [29]. Currently, most management policies to prevent *D. geminata* from uncontrollable blooms have adapted the “Check, Clean, Dry” marketing campaign used in New Zealand [29]. While many of these decontamination programs are elected to be the first line of defense against unwanted organisms, their efficacy has not been proven to slow down the spread of free-living microorganisms such as *D. geminata*. With this in mind, scientists studying *D. geminata* in New Zealand have noted that diatoms do not develop in streams and rivers with higher than normal concentrations of inorganic phosphorus not exceeding 2 micrograms per liter [18], thus suggesting an alternative control method for *D. geminata* through phosphorus augmentation [29]. Phosphorus augmentation may only provide short-term
control, because it needs continuous application to control the spread of bloom. If that is the case, increasing phosphorus concentrations can be counter-intuitive, as most freshwater bodies would like to keep phosphorus concentrations low in order to prevent cultural eutrophication. Moreover, increasing phosphorus concentrations can be costly to maintain a continuous flow of inorganic phosphorus. Such practices can only be suggested if the stream that D. geminata inhabits is threatening other species living in that freshwater body, as additional phosphorus can only be recommended for a short period [29].

The economic impacts of Didymo are currently unknown. With its ability to be used in wastewater management and agricultural practices, we can see it potentially having great prospects in the coming years. However, it is hard to say if its effects on certain species in river ecosystems will reduce the amount of economic growth that region could have without access to essentials like salmon. Further research on these topics is necessary to make an informed prediction.

Diverse aspects of the structural biology of D. geminata have been recently described [17, 18, 30, 31]. Thus, D. geminata stalks are examples of unique biocomposites, which contain calcite [1], some proteins, and polysaccharides, which are still poorly investigated. According to Bothwell and Spaulding [32], these stalks are “composed primarily of sulfated polysaccharides with significant uronic acid content, and protein. Monosaccharide analysis has revealed predominately galactosyl and xylosyl residues and linkage analysis has shown predominantly 3,4-Gal and 4-Xyl. The polysaccharide portion of the stalk therefore appears to be primarily sulfated xylagalactan, which has been reported for stalks of related diatoms Gomphonema and Cymbella, where it was shown to be intrinsically hydrophilic and linked by ionic cross-bridging”.

Preliminary experiments which have been carried out in our lab for the identification of the nature and origin of the polysaccharides within Didymo stalks showed an absence of chitin. However, possible localization within siliceous cells, similar to the phenomenon reported in T. pseudonana diatoms [10, 33], has yet to be studied. We also suggest that, due to the confirmed presence of xylose within D. geminata stalks [32], corresponding analytical studies should be carried out in the near future with the aim of identifying xylosamine as a structural alternative compound to chitin.

As seen in Figures 6 and 7, the scanning electron microscope (SEM) images show the Didymo stalks in higher resolution, allowing for the intricate details of the structures to be made clear. It is these structures that are important for the future study and management of this species. These images can help us obtain information about the surface topology and inner structural peculiarities.

Figure 6. SEM image of Didymo siliceous cell tightly bound to the biomineralized polysaccharides-based stalk.
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**Figure 6.** SEM image of Didymo siliceous cell tightly bound to the biomineralized polysaccharides-based stalk.

**Figure 7.** SEM image of the Didymo stalk cross-section confirms its multilayered structure with capillary-like morphology.

### 6. Conclusions

The unique nature of Didymo has been proven to cause environmental and ecological damage. Higher mortality rates in fish, affecting water filtration systems and hydro-plants, are major areas of concern for streams affected by Didymo. This is spreading from coast to coast; it not only occurs in Canada, but is a worldwide problem. Very few benefits come to mind when talking about *D. geminata*, like its ability, when controlled, to absorb harmful metals and co-exist with nitrogen-fixing bacteria. However, managing the species can be expensive using synthetic chemical agents and can further damage the environment. Other solutions, like management policies and using inorganic phosphorus augmentation, can control the spread of the algae. We also applied GIS tools to identify the location and current condition of this alga which could help researchers in modeling and management to either control or collect the beneficial applications. With all of the information provided above, it can be confirmed that *D. geminata* can, in fact, be a “nuisance species”, but, due to its structure (e.g., polysaccharide stalks, chitin-based frustule), scientists have the ability to manage the spread, and possibly use this species to our benefit. Therefore, permanent removal of its polysaccharide stalks is not needed, but its growth should be isolated from the environment so that its negative effects in rivers and streams are minimized while it helps maximize our technological growth in all sectors.

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