

Shuhong Cao
shuhongcao66@gmail.com

Heterosigma akashiwo in the Salish Sea

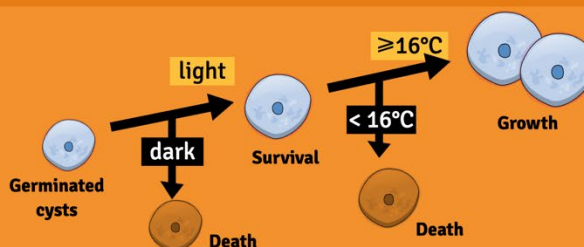
Salish Sea:
spans 18,000 square kilometres
Includes Strait of Georgia, Strait of Juan
de Fuca, Desolation Sound, Puget Sound
and all rivers that flow into the ocean



Temperature, Salinity & Light

Temperatures above 16°C and
sufficient light are the
prerequisites for the survival of
germinated *H. akashiwo* cysts

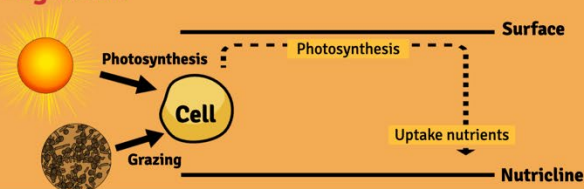
It's growth prefers low salinity



Mixotrophy & Diurnal Vertical Migration

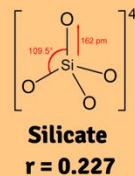
H. akashiwo can perform photosynthesis and
ingest other species

H. akashiwo keeps its cells afloat in the surface
during the day to take advantage of light, and
dive to the bottom at dusk to ingest higher
concentrations of nutrients in that layer



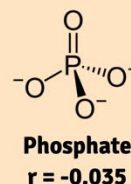
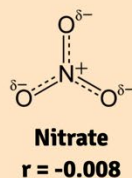
Factors and Nutrients

In the Salish Sea, *H. akashiwo*
concentrations in summer have
a strong positive correlation
with stratification and silicate
concentration



Factors and Nutrients

Although nitrate and phosphate are
essential nutrients for the growth of
H. akashiwo, in summer, they are
negatively correlated with *H.*
akashiwo concentrations in the
Salish Sea



Fraser River

The freshwater of the Fraser River
provides a low-salt environment, strong
stratification, and brings a large amount
of dissolved silicon to support the
growth of *H. akashiwo*



LWS 548 943 2022S1-2 Major Project

Shuhong Cao

Shuhongcao66@gmail.com

Effects of *Heterosigma akashiwo* blooms by Eutrophication in the Salish Sea

Contents

Abstract.....	4
Introduction	5
Characteristics of <i>Heterosigma akashiwo</i>	9
Cells.....	9
Name	10
Growing Environment	10
Mixotrophy	12
Diurnal Vertical Migration	13
Allelopathy.....	13
Distribution of <i>Heterosigma akashiwo</i>	14
Mechanism of fish kill by <i>Heterosigma akashiwo</i>	16
Effects of water environmental factors on the growth of <i>Heterosigma akashiwo</i>	17
Temperature, Salinity and Light.....	17
Nutrients.....	18
Results	21
<i>H. akashiwo</i> in the Salish Sea.....	21
Discussion	26
Conclusions	29
References	29

Abstract

Although algal blooms are one of the most common manifestations of marine pollution, little is known about the species that cause some algal blooms and their impact on ecological health. Broader exposure to harmful algae has emerged in coastal environments and food chains around the world, with increasing occurrences and resulting in the death of large numbers of marine life. This exposure is bound to continue as human-induced emissions of coastal pollutants increase. The impact of algal blooms on marine ecology during its occurrence has sounded an alarm to the world: algal blooms threaten aquatic ecological health on a global scale. The world's growing trends in marine pollutant production, emissions, and disposal must shrink and even stop for dealing with these threats.

In terms of the impact of algal blooms on coastal ecological health, most of the research so far has been limited to some specific stages of the red tide phenomena. It is often limited to a single species and its reproduction, growth and exposure path. This practice obscures the fact that at each stage of the occurrence of algal blooms, there are distinct and complex interactions that affect marine ecological health: the activities and health of marine life, the functioning of the food chain, the quality of water bodies, and even air quality and terrestrial life in the region.

This study details the effects of the reproductive and life cycle of a specific species responsible for harmful algal blooms in the waters of the Salish Sea, and on the levels of various substances in the water. It reveals numerous exposure pathways that affect the health of marine life in the region. This study briefly describes the effects of ingestion, inhalation, and exposure to aquatic organisms, as well as toxic chemicals associated with these algae, such as reactive oxygen species (ROS), which can cause respiratory failure. This study further shows that human activities and pollutant discharges may exacerbate the exposure and risk to these waters by the harmful algal bloom. Despite some gaps in the data, the evidence gathered in this study is sufficient to conclude that measures are urgently needed to reduce human exposure to the waters and make them ecologically free from the red tide pollution.

Introduction

Algae, as the primary producers in the marine ecosystem, as they provide food for other aquatic organisms at the bottom of the food chain and maintain the material cycle of the water ecosystem. By absorbing carbon dioxide in the atmosphere through photosynthesis, that is carbon sequestration, algae also play an important role in the global carbon cycle. However, under certain conditions, certain species can proliferate and aggregate to form algal blooms. Algal bloom describes the ecological abnormal phenomenon that microalgae over-proliferate or accumulate in the water body, causing the water body to change color. The color of an algal bloom varies depending on the main plankton species that blooms. *Noctiluca scintillans*, for example, turn water into a pink or dark red color when they bloom. While a green tide could be formed when the genus *Ulva* species dominates in a bloom, and a golden tide could be formed with the bloom of the genus *Sargassum* species (Smetacek & Zingone, 2013). Among many species of plankton that can cause algal blooms, flagellates and diatoms are considered to be the dominant species. When specific microalgae reach high levels of enrichment in an outbreak, this bloom can take on a detrimental profile by producing harmful or toxic substances that cause a variety of adverse effects, including disruption of the food chain, death of marine life, and severe human health hazards. These types of algal blooms are known as harmful algal blooms (HABs).

Harmful algal blooms are ecological anomalies with complex causes. The primary condition for the occurrence of harmful algal blooms is biological proliferation must reach a certain density under optimal growth conditions for specific microalgae and reduced pressure from predation. Once a HAB forms, it will cause serious harm to other organisms living in the marine environment and even the residents living along the coast of the region. The high density of algal bloom organisms can block the respiratory organs of fish and shellfish. Some secrete toxins, which causes the death of a large number of fish and shellfish as a result of the bloom. The oxidized and decomposed remains of algal bloom species in seawater will consume a large amount of dissolved oxygen in the water and cause hypoxia. Humans also experience health problems from consuming contaminated food or at least in some cases with direct contact with water or aerosols containing contaminants (Caron et al. 2010).

Heterosigma akashiwo is a harmful algal bloom species widely distributed in the world's coastal waters, and it is one of the most important fish-killing algae species in British Columbia (Jack et al., 2010), of which the Salish Sea has historically had the strongest and longest-lasting *H. akashiwo* bloom. The Salish Sea spans 18,000 square kilometres of ocean which contains the waters around Strait of Georgia, Strait of Juan de Fuca, Desolation Sound, Puget Sound and all rivers that flow into the ocean, as shown in Figure 1 (Map of the Salish Sea & Surrounding Basin. Maps by Stefan Freelan, n.d.). The land and cities along the coast are home to more than 7 million people. In recent years, under the background of population expansion, industrial and agricultural development and human activities have brought great pressure on the aquatic ecosystem, including the impact of organic pollutants to water bodies caused by production and urban runoff pollution that feed the algae and has caused the rising scale and frequency of harmful algal blooms in this region.

During the late 1980s and 1990s salmon farms in British Columbia and Washington State lost more than \$35 million to harmful algal blooms dominated by *H. akashiwo*. (Horner et al., 1997). Two massive *H. akashiwo* blooms in 2014 and 2018 killed a total of more than 500,000 local fish (Huffman & Jason, 2018). The recurring HABs led by *H. akashiwo* have caused a large economic and operational impact on the commercial fish aquaculture industry along the Salish Sea coast. With the rising demand and continued growth of local fish aquaculture, a better understanding of harmful species is therefore required to alleviate the economic hardships they cause.



Figure 1. Map of the Salish Sea & Surrounding Basin. Maps by Stefan Freelan. (n.d.)

Carrying out research on the occurrence mechanism of *H. akashiwo* bloom in the Salish sea is particularly important in view of the harmfulness of the occurrence trend of *H. akashiwo* blooms.

In the past few decades, research and predictions on the blooms produced by *H. akashiwo* in the Salish Sea have been carried out from different aspects. A sixteen-year monitoring study carried out by Brown et al. (2018) in Departure Bay demonstrates the effects of Fraser River flow, salinity, and temperature on *H. akashiwo* blooms (Interannual variation of the toxic raphidophyte *Heterosigma Akashiwo* in ... n.d.). Cochlan et al. (2013) demonstrated that differences in growth stage or nutrient availability of *H. akashiwo* affected the toxicity of its blooms. Strom et al. (2013) showed that the adaptation of *H. akashiwo* to low salinity and the reduced predation pressure allow blooms to start more at low salinity. The study of *H. akashiwo* DNA sequence by Blanco et al. (2013) showed that its strong adaptability to changing environmental conditions contributes to the dominance of *H. akashiwo* over other phytoplankton species in the presence of harsh environmental conditions. Leftley et al. (1993) suggests that physical factors such as water temperature and vertical stratification of the water column are the most important factors in promoting *H. akashiwo* blooms in most BC waters of the Salish Sea. Nevertheless, research on the nutritional dynamics of *H. akashiwo* and its potential link to eutrophication in the Salish Sea culture has not discovered much.

This study summarizes the research progress of *H. akashiwo* focusing on its species identification, basic biological characteristics and ecological behavior, focusing in order to provide reference for the research on the mechanism of algal blooms of this species and the prevention and control of blooms. In addition, by determining the changes in land use types and pollutant sources in Cities along the coast of the Salish Sea in recent years, combined with the accumulated effects of various water environmental factors on the growth of *H. akashiwo*, this study provides a reference for determining whether human activities recently have a great role in promoting *H. akashiwo* algal bloom along this coastal region. Recommendations on management practice of nutrient emissions from human activities along the coast of the Salish Sea are also suggested based on the results.

Characteristics of *Heterosigma akashiwo*

Cells

The unicellular alga *Heterosigma akashiwo* belongs to the *Raphidophyceae*. Its shape is often oval with varying degrees of flattening, with individual diameters ranging from 8 μm to 25 μm . *H. akashiwo* has no cell wall, instead it is coated with a periplasmic membrane. Each cell has two flagella of unequal length, which often bend or extend perpendicular to the long axis of the cell by turns when the algae move, as shown in Figure 2. Each cell possesses a nucleus and 10-25 chloroplasts (Engesmo et al., 2016) *H. akashiwo* appears golden-brown under light microscopy due to the presence of its intracellular fucoxanthin and violaxanthin (Martinez et al., 2010).

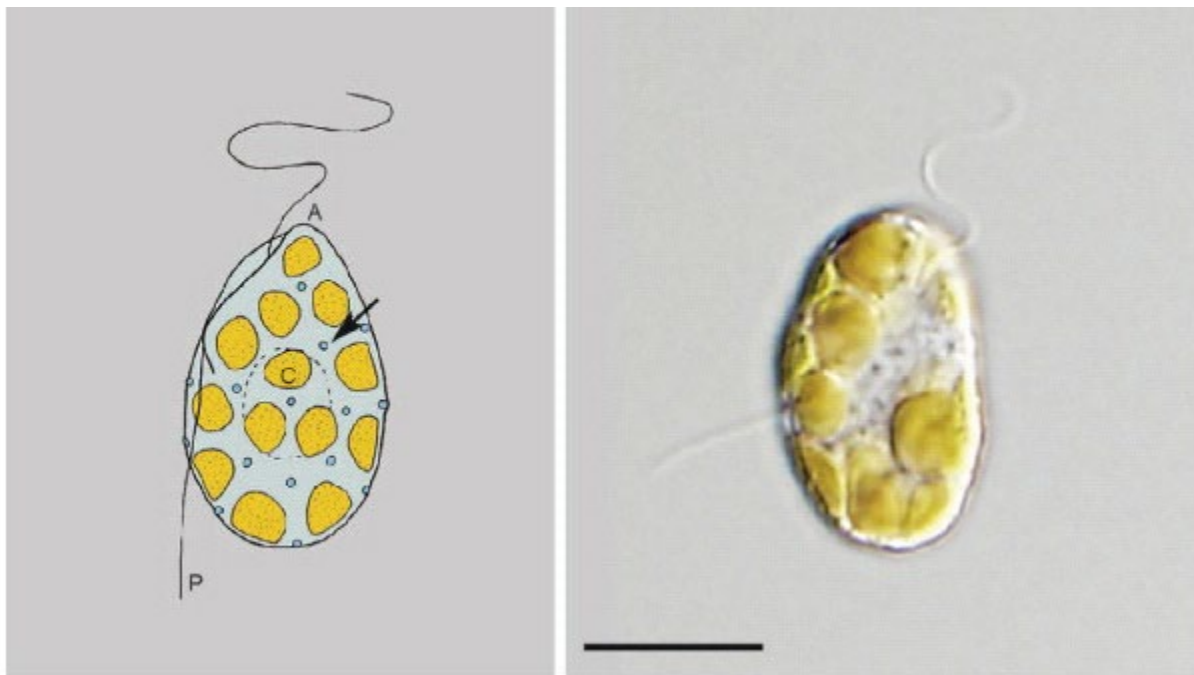


Figure 2. (Left) The morphological features of *H. akashiwo* under light micrographs (Line drawing). Mucocysts are pointed out by the arrow, the central nucleus is cycled by the dashed line. (Right) *H. akashiwo* under light micrographs. Peripheral chloroplasts are shown as yellow blocks, mucocysts are shown as dots, and the central nucleus is visible as a grey area (Engesmo et al, 2016).

Name

The naming and classification of *Heterosigma akashiwo* has generated controversy in the academic community over the past few decades. According to records, *Heterosigma akashiwo* was first discovered by Hulburt in 1965 in Massachusetts, USA. Being mistakenly recognized as an *Olishodicus luteus*, Hulburt didn't realize it was an entirely new genus and named his discovery *Olishodicus carterae*. Two years later, in 1967, the protozoologist, Hada discovered a similar species in Japan and named it *Entomosigma akashiwo* Hada, and then in 1968 first proposed the name of the genus as *Heterosigma* and changed it to *Heterosigma inlandica* (Hara & Chihara, 1987). However, according to the botanical codes of nomenclature, the above names are invalid because they have not been assigned the holotype of the species, which is mandatory. It wasn't until 1987 that Hara and Chihara validly named the species *Heterosigma akashiwo* (Hara & Chihara, 1987). In addition, Engesmo et al. (2016) discovered a new species in 2016 through the identification of developmental phylogeny and morphology of 24 *Heterosigma* from four regions of the world and named it *Heterosigma minor*. Compared to *Heterosigma akashiwo*, *H. minor* possesses a more uniform cell volume and shape. Its cells are also smaller and rounder than *H. akashiwo*. This resulted in individual cells of *H. minor* carrying fewer chloroplasts, and fewer and sparser mucous cysts covering the surface compared to *H. akashiwo* cells.

Growing Environment

H. akashiwo can survive and reproduce well in a wide range of salinity and temperature. According to Martinez et al (2010), *H. akashiwo* grows best in the salinity range of 25 ppt-30 ppt. However, multiple studies have shown that it can survive at reduced growth rates in salinities from 10 ppt to 40 ppt, with minimum growth values as low as 5 ppt or above 40 ppt (Honjo et al., 1993; Haque & Onoue, 2002; Tomas, 1978). Since the average salinity of seawater is 35 ppt, *H. akashiwo* grows in the highest rate at salinity below the average. Changes in salinity in offshore surface waters are generally affected by factors such as seawater evaporation, rainfall, and stratified mixing. Therefore, the environmental changes of regional seawater salinity reduction caused by different factors should also be considered when discussing *H. akashiwo* blooms.

H. akashiwo generally has the highest growth rate in summer and autumn in temperate coastal waters and is dormant on the seafloor in winter. The main factor limiting its recovery and bloom is considered to be temperature. *H. akashiwo* proliferates in the temperate zone near the seabed in summer when the water temperature is at 15 to 25°C, where 23°C is the optimum temperature for *H. akashiwo*. The growth and viability of *H. akashiwo* decreased significantly in the environment below 14°C and above 30°C (Martinez et al., 2010). According to the data records of previous years (Table 1), the average water temperature of the Salish Sea, from June to August, in summer is 15 to 18 degrees, where August is the month with the highest water temperature at 18.1°C. The meeting of the temperature requirements provides one of the most optimum conditions for the occurrence of *H. akashiwo* bloom in the summer.

	Max. Water Temperature (°C)	̅. Water Temperature (°C)	Min. Water Temperature (°C)
January	6.6	6.5	6.4
February	6.7	6.6	6.4
March	8	7.2	6.7
April	10.4	9.1	8.1
May	13.9	12.4	10.5
June	16.3	15.1	14
July	17.8	17.1	16.3
August	18.1	17.4	16.2
September	16.3	14.8	12.9
October	12.9	11.3	9.9
November	9.9	8.8	7.7
December	7.7	7.2	6.7

Table 1. Salish Sea Water Temperature by Month

Admin. (2020, November 23). *Nanaimo water temperature*. WATERTEMPERATURE.NET. Retrieved June 28, 2022, from <https://www.watertemperature.net/canada/nanaimo.html#waterC>

Mixotrophy

In terms of nutrient acquisition, *H. akashiwo* has an mixotrophic feeding ability. The mixotrophy of a plankton means that such species can ingest energy and carbon from the outside world through means other than only performing photosynthesis, shown as GM3 in Figure 3.

According to Nygaard et al. (1993), *H. akashiwo* can obtain required phosphorus by ingesting heterotrophic bacteria and cyanobacteria under phosphorus-limiting conditions. Subsequent studies further confirmed the conclusion that *H. akashiwo* is a mixotrophic species and showed that the predation rate of this species is proportional to the concentration of prey in the system (Jeong et al., 2010). Thus, the mixotrophy of *H. akashiwo* expands its range of nutrient sources relative to autotrophic (only obtains energy and accumulates carbon through photosynthesis) phytoplankton and favors this species to increase in value more rapidly than other species under nutrient-limiting conditions, making it easier for *H. akashiwo* to take advantage of interspecies competition and generate blooms under such conditions.

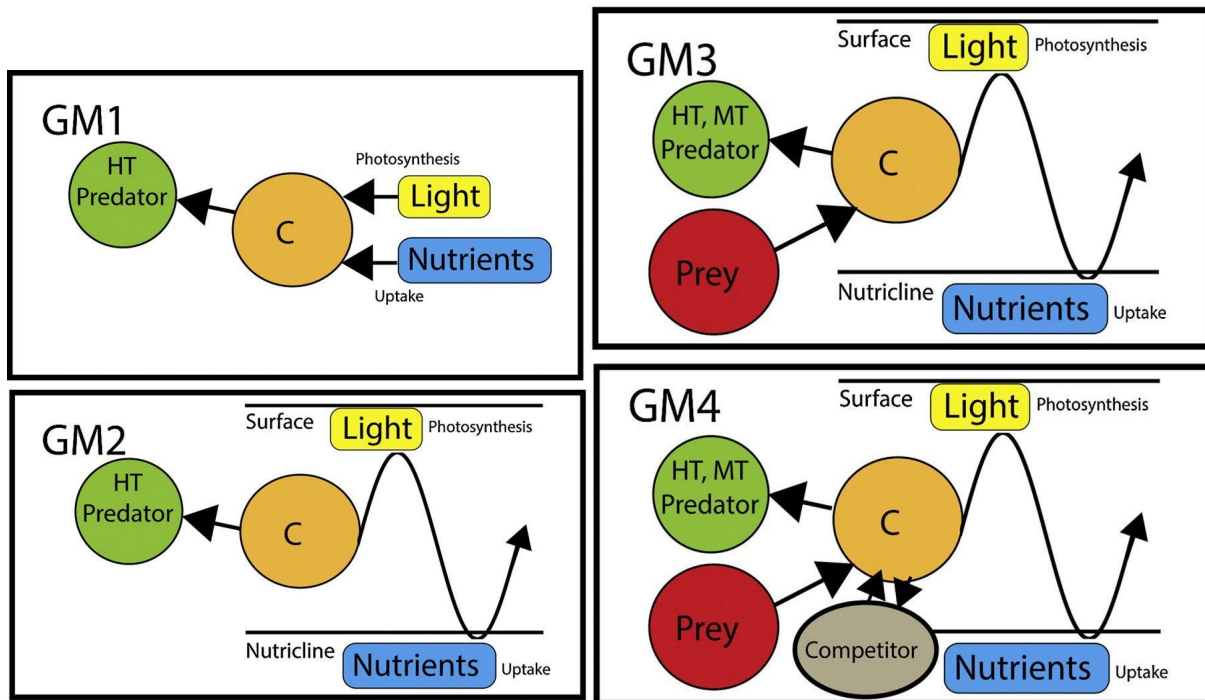


Figure 3. The red-tide formation categorized by nutrition, behaviors, and interactions of red-tide organisms. GM1: Autotrophy and photosynthesis. GM2: With vertical migration. GM3: Mixotrophy. GM4: Inhibition through interspecies competition (Jeong et al., 2015)

Diurnal Vertical Migration

Another characteristic of *H. akashiwo* that facilitates its growth is the diurnal vertical migration behavior that is widely present in phytoplankton. Species with this behavior keep their cells afloat in the ocean's surface water during the day to take advantage of light, and dive to the bottom water at dusk to ingest higher concentrations of nutrients in that layer. By simulating a summer seawater environment with weak water mixing, Watanabe et al., (2008) showed that *H. akashiwo* acquires phosphorus from the bottom water body through vertical diurnal migration and stores it as polyphosphate. It is then utilized through photophosphorylation by cells in surface water during the day. Compared with non-flagellate algae that do not have vertical diurnal migration, such as diatoms, *H. akashiwo* has the advantage of greater interspecies competition in terms of utilizing light and nutrient uptake. In addition, diurnal vertical migration behavior is also thought to be more effective in evading zooplankton predation. Given that this trait plays an important role in the growth of *H. akashiwo*, special attention should be paid to the contribution of vertical diurnal migration when focusing on the formation of its blooms.

Allelopathy

In addition to mixotrophy and vertical diurnal migration, *H. akashiwo* also performs allelopathy to affect the growth and development of other organisms through the production of secondary metabolites, also known as allelochemicals, enabling them an advantage in competition with other species. The allelopathy of *H. akashiwo* was first observed and proposed by Pratt in 1966. The average cell growth rate of *Skeletonema costatum*, which competes with it in the system, is more dominant than that of *H. akashiwo*. However, the authors observed that its cellular abundance was significantly lower than normal when a *H. akashiwo* bloom occurred, and gradually began to increase until the end of the *H. akashiwo* bloom. Up until 2009, Yamasaki et al. demonstrated that the allelopathic effects of *H. akashiwo* on primary producers such as *S. costatum* and *T. rotula* were due to their secreted allelopathic polysaccharide–protein complexes (APPCs). Although the authors proposed several possible mechanisms by which APPCs inhibit the growth of competitors, there is currently insufficient evidence to prove their authenticity. So far as it has shown through studies, *H. akashiwo* can inhibit the growth of other organisms in the system through allelopathy with APPCs, which mechanism is complex. As the frequency and

hazard of *H. akashiwo* algal blooms increase, its allelopathic effects will increasingly affect the structure of plankton ecosystems in affected regions.

Distribution of *Heterosigma akashiwo*

Heterosigma akashiwo is widely distributed in various shallow regions of the world's oceans and has been recorded worldwide except for the Antarctic, including the coastal areas of the United States, Japan, New Zealand, Australia, China and other countries. Most *H. akashiwo* and the high-risk area of its blooms are distributed in temperate seas due to the limitation of its growth with water temperature. The detailed global distribution map of *H. akashiwo* has been updated by Kok et al. in 2015, as shown in Figure 4. Nevertheless, the existing records show its coverage as far north as the Barents Sea and the White Sea in the Arctic and as far south as the South Pacific near Chile, as well as Australia and New Zealand. Similar to the boreal zone, a small number of records showed that *H. akashiwo* has also been found in the tropics, as in Singapore, Thailand, Vietnam, and Brazil (Tang et al., 2004; Branco et al., 2014; Engesmo et al., 2016). Since overwintering is considered to be an important phase driving *H. akashiwo* bloom in temperate waters (Smayda, 1998), there may be unrecorded differences in *H. akashiwo* in tropical or frigid regions where annual mean temperature changes are small. Although the study by Kok et al (2015) has attempted to investigate the effects of nitrogen loading and light availability on the growth of *H. akashiwo* in the tropics (Singapore), it does not provide enough evidence to show that it is physiologically different from the general temperate species.



Figure 4. Detailed global distribution map of *H. akashiwo*. Grey circles represent recorded fish-kill events, larger circles show records of recurrent blooms, grey triangles represent recorded sightings of the species. The numbers correspond to the List of records from culture records and published literature in the original text (Kok et al., 2015).

As shown in Figure 4 and Table 2, No.13 to No.19 recorded a total of seven fish kills due to *H. akashiwo* blooms in the Salish waters as of 2015. It's also shown by the size of grey circles that the Salish Sea and its surrounding waters were the most important areas of recurrent *H. akashiwo* blooms outside the coast of Japan. The first recorded fish kill due to the *H. akashiwo* bloom in the Salish Sea region occurred in Nanos Bay in 1986, followed by less frequent and short-duration blooms in Puget Sound in 1989. Subsequently, several *H. akashiwo* blooms appeared in Departure Bay and George Strait in the 1990s, with larger areas and a significant increase in duration compared to the previous (Jack et al., 2010).

No.	Locality	Source Record type
13	Barkley Sound, Vancouver	Black et al. (1991)
14	English Bay, Canada	Strain from NEPCC (data not listed
15	Fraser River, Vancouver	Rensel et al. (2010)
16	Guemes Channel, Washington	Strain CCMP-1014/NWFSC-504 isolated in 1991

17	Puget Sound, Washington	Rensel (2007)
18	Strait of Georgia, Vancouver	Black et al. (1991)
19	Lummi Bay, Washington	Strain CCMP-314/SPMC117 isolated in 2007

Table 2. Partial list of records from culture records and published literature (Kok et al., 2015).

Mechanism of fish kill by *Heterosigma akashiwo*

As mentioned earlier, there has been a lot of research and evidence that the bloom of *H. akashiwo* can cause massive mortality in farmed fish, but the mechanism by which *H. akashiwo* causes fish mortality is still controversial. Although numerous studies have attempted to explain the mechanism of *H. akashiwo*-induced fish mortality from different perspectives, each explanation is flawed. For example, the study by Twiner (2000) showed that the reactive oxygen species (ROS) produced by *H. akashiwo*, such as superoxide radicals, hydroxyl groups, and hydrogen peroxide, etc., caused fish gill damage, which can lead to a large number of fish Death by suffocation. However, although subsequent studies agreed that the toxin of *H. akashiwo* is caused by the ROS secreted by this species, the species of *H. akashiwo* in the experiment was completely unable to produce hydrogen peroxide at concentrations that could cause death of invertebrates under culture conditions (Twiner et al., 2001; Ling & Trick, 2010).

A study found four neurotoxic components, which corresponded to brevetoxin were inferred from analysis of *Heterosigma* red tide toxins, indicating that *H. akashiwo* produced organo-neurotoxins in water at concentrations that would cause disease in the heart or gills of fish during bloom (Khan et al., 1997). In the same study, however, the authors mentioned that brevetoxin-like organisms were only identified in *H. akashiwo* in this event, rather than the mechanism of action of the toxin explaining the toxicity of this species. Also, the subsequent research proved that the toxin was not brevetoxin (Twiner et al., 2005).

Another explanation for the mechanism by which *H. akashiwo* causes fish kills is that the mucus secreted by the algae coats the fish's gills, causing gill lesions and causing suffocation (Chang et al., 1990). However, the study mentioned that it could not be determined whether the mucus

came from *H. akashiwo* or the fish themselves. The amount of mucus measured was also thought to be unlikely to cause severe disease in the gills of fish. Many people also believe that the toxicity of *H. akashiwo* to fish is caused by the combination of the above-mentioned mechanisms. For example, the mucus in the gills of fish could stimulate *H. akashiwo* to produce more ROS, which damages gills in a critical way (Nakamura et al., 1998). The cause of the mass death of fish could also be inferred to be cell death caused by the combined action of ROS destroying fish cellular immunity and organic toxins changing cellular ion balance (Twiner et al., 2005). Therefore, the mechanism of the massive fish kill caused by *Heterosigma akashiwo* bloom is still controversial and needs to be further studied.

Effects of water environmental factors on the growth of *Heterosigma akashiwo*

According to Grattan et al. (2016), the formation of a harmful algal bloom is usually the result of a combination of specific species' own factors and external environmental factors. Under normal circumstances, different phytoplankton form a relatively stable community structure through competition for resources and individual consumption by other marine organisms, and when environmental factors are suitable for the growth of a particular organism, it will favor the bloom formation of that species.

Temperature, Salinity and Light

Although general phytoplankton are sensitive to the three environmental factors of temperature, light and salinity, *H. akashiwo* has been shown to grow well over a wide range of temperature and salinity, as mentioned earlier, which makes the salinity had little effect on *H. akashiwo* growth. Nevertheless, temperature is still a factor that's as important as light which is greatly affecting the emergence of *H. akashiwo* bloom. According to Shikata et al. (2007), the main supply of *H. akashiwo* blooms in early summer is from cyst populations in bottom sediments of water bodies. Cysts leaving the sediment will germinate within a certain period of time, independent of environmental factors. But it has strong temperature and light requirements in a short time after germination. Shikata et al. showed that post-germinate cysts fail to grow into normal-sized cells and die rapidly in environments below 16°C or in a lack of light, as

demonstrated in Figure 5. Only cells that meet the temperature and light requirements at the time of germination can successfully grow into mature individuals that are less affected by temperature and light, but the mature cysts will still have a higher growth rate with the increase of light intensity and at a high temperature as 25°C (Ono et al., 2000).

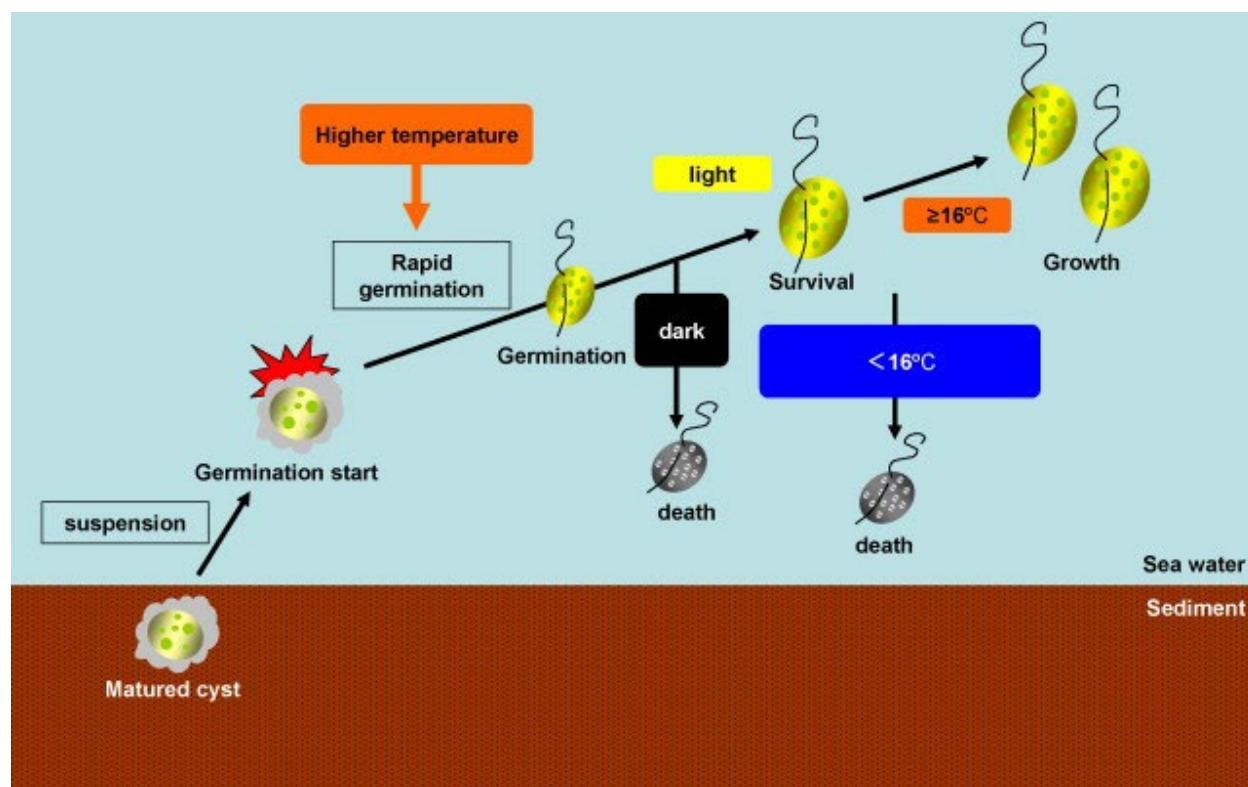


Fig. 5. Schematic diagram of the initial development phase of a *H. akashiwo* red tide (Shikata et al., 2007).

Nutrients

Nutrients are the most basic elements for phytoplankton to survive, and their concentration and form in water will greatly affect the reproduction and growth of *H. akashiwo* in the environment. Numerous studies have highlighted the important roles of nitrogen, phosphorus, and trace metals in the induction of *H. akashiwo* over-breeding events. Phosphorus is generally used directly by algae in the form of phosphate. However, most coastal regions of the world are phosphorus-limited due to relatively high N:P ratios. Harmful algal blooms in these areas are often due to algae, such as flagellate species, that are able to utilize dissolved organic phosphorus (DOP) in the absence of inorganic phosphorus (Wang et al., 2011). Among these, the requirement of *H. akashiwo* for both organic and inorganic phosphorus is thought to be lower than that of

competing phytoplankton during periods of rapid proliferation of this species. According to Wang et al. (2011), although *H. akashiwo* cannot survive without phosphorus, this species can utilize more forms of dissolved organic phosphorus relative to other compared red tide algae (eight forms in total). Furthermore, under phosphorus-limiting conditions, *H. akashiwo* was also observed to possess a 2.5-times higher alkaline phosphatase activity (APA) which hydrolyzes organic compounds for utilizing DOP (Wang & Liang, 2015).

In addition to its more efficient use of phosphorus, *H. akashiwo* was found to have the ability to efficiently utilize three common nitrogen sources in the ocean, namely NH_4^+ , NO_3^- and urea. Among them, NH_4^+ and NO_3^- have been emphasized in some studies to provide higher growth levels of *H. akashiwo* than urea in high light environments; while in the low light environment, NO_3^- provided a lower cell growth rate than the other two (Chang & Page, 1995; Wood & Flynn, 1995). Besides light, other environmental factors may also affect the preference of *H. akashiwo* for different N sources. For example, 17°C was introduced as the dividing line between the preference of *H. akashiwo* for two nitrogen sources, NH_4^+ and NO_3^- . NH_4^+ can support higher *H. akashiwo* growth rates in environments above 17°C, while *H. akashiwo* seems to prefer higher growth rates in environments where NO_3^- is the main nitrogen source in environments with the temperature lower than 17°C (Herndon & Cochlan, 2007). Although the above studies show that *H. akashiwo* appears to be highly sensitive to nitrogen sources, cells of this species have been shown to respond to changes in environmental nitrogen nutrients by rapidly regulating the expression of nitrate reductase (Coyne, 2010). Therefore, the efficient utilization of organic phosphorus and various nitrogen sources by *H. akashiwo* can help this species to gain an advantage in interspecific competition in the relatively nutrient-deficient sea area.

The growth of *H. akashiwo* is also affected by the deficiencies of specific trace metals. According to Fuse (1987), the growth of *H. akashiwo* in response to changes in four common trace metal concentrations was explored and compared with five other typical red tide organisms. Unlike most algae, lower ferric ion concentrations do not affect *H. akashiwo* growth rates too negatively, but only delay their blooms according to the degree of deficiency, as shown in Figure 6. Furthermore, low copper ion concentrations have similar effects as iron ion concentrations, but only at very low levels, such as 10^{-8} to 0M, as shown in Figure 7. In contrast, severe deficiency

of Zinc ions (concentrations below $10^{-7.1}M$) significantly reduces the overall population of *H. akashiwo*, as shown in Figure 8. The concentration of manganese ions seemed to have no effect on the growth of *H. akashiwo*, as shown in Figure 9.

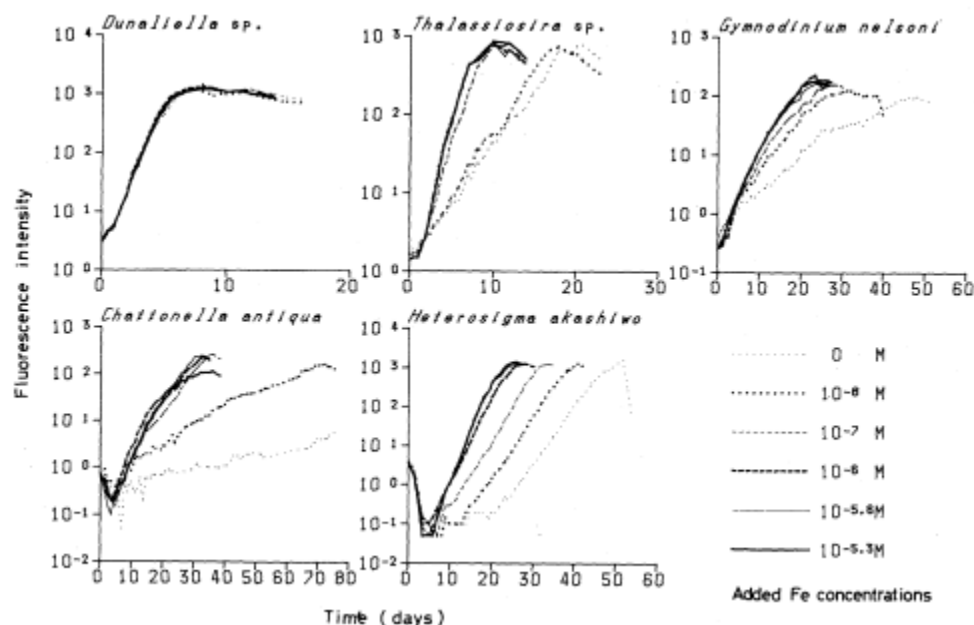


Figure 6. Effects of Ferric Ion Concentrations on Growth.

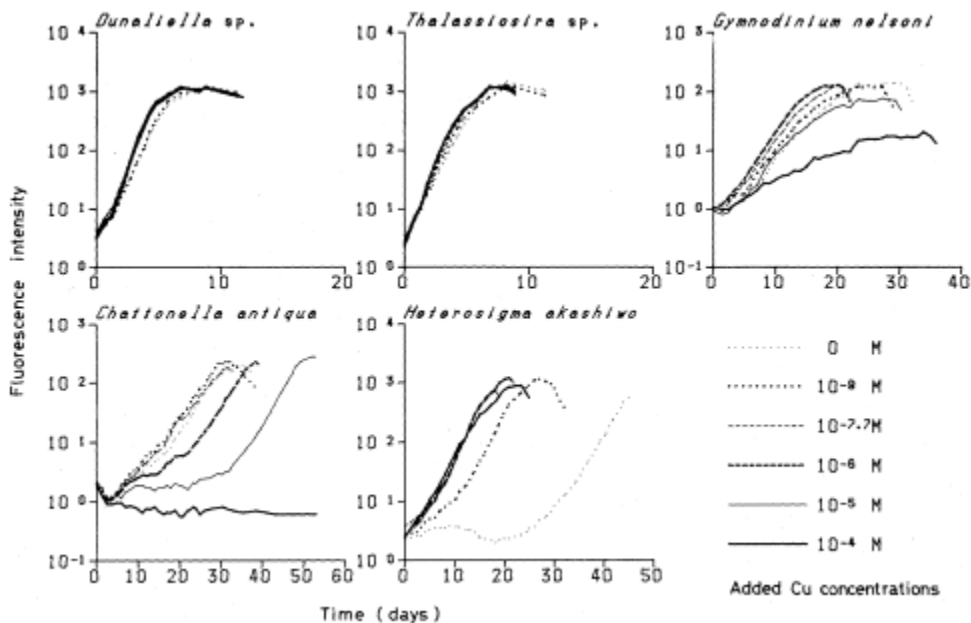


Figure 7. Effects of Cupric Ion Concentrations on Growth.

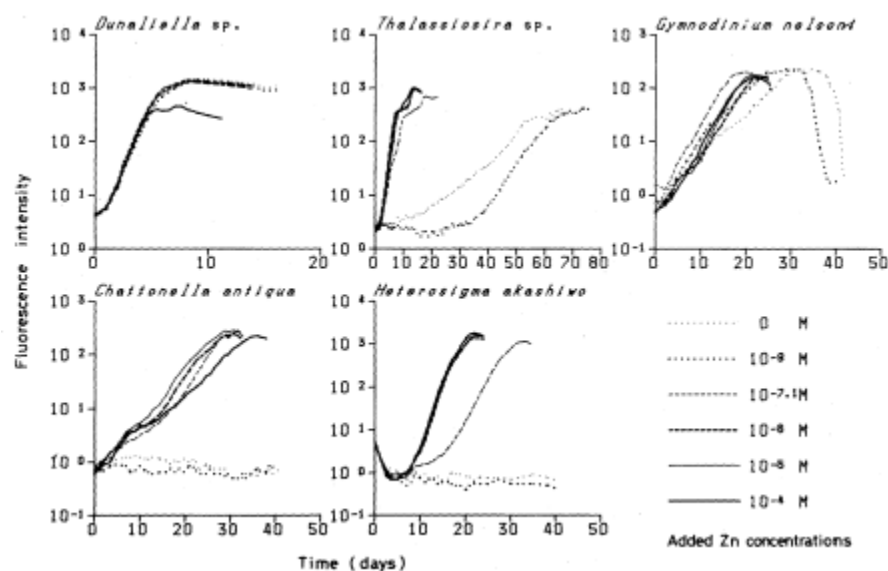


Figure 8. Effects of Zinc Ion Concentrations on Growth.

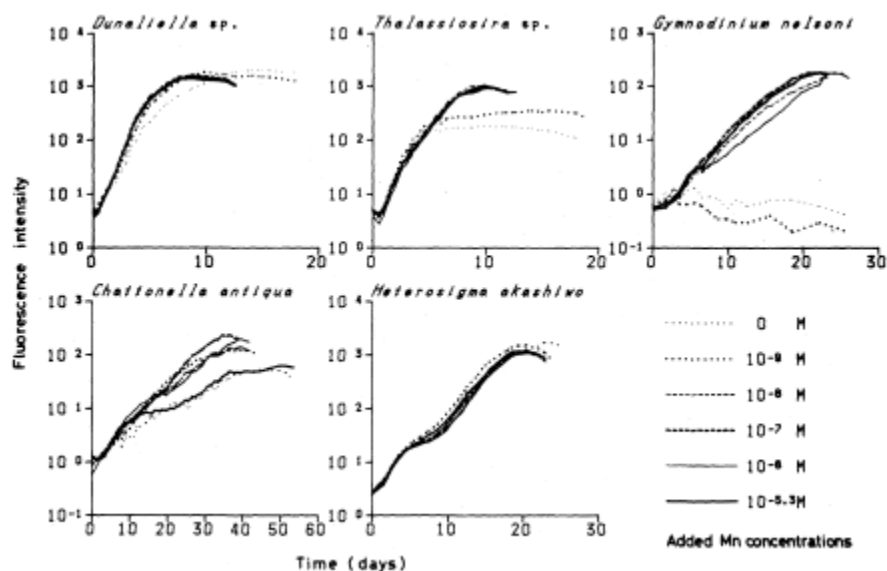


Figure 9. Effects of Manganese Ion Concentrations on Growth.

Results

H. akashiwo in the Salish Sea

The study by Esenkulova et al. (2021) provides up-to-date spatial and temporal patterns of several taxa of HABs, including *H. akashiwo*, in the Strait of Georgia (SoG) and the association of each HAB with environmental parameters. The study provided one to three monthly samplings of 92 sites in the region from February to October, from 2015 to 2018 for a total of 36

months (Figure 10). Environmental parameters include surface water temperature, salinity, stratification, and Secchi depth. Measured nutrients included nitrates, phosphates, and silicates. Phytoplankton cell concentration changes, including *H. akashiwo*, were also measured and recorded. Although the study did not cover the entire Salish Sea, the sampling sites were done in the region that covers more than 70% of the Salish Sea, as well as including all occurrences of *H. akashiwo* blooms in the Salish Sea during the study period. Therefore, the results may be used as an indicator regarding how the growth of *H. akashiwo* is affected by environmental factors and nutrition in the Salish Sea. Although the presence of *H. akashiwo* was found in water bodies every year from May to September, blooms of this HA species have only occurred in 2018 among the four years of sampling.

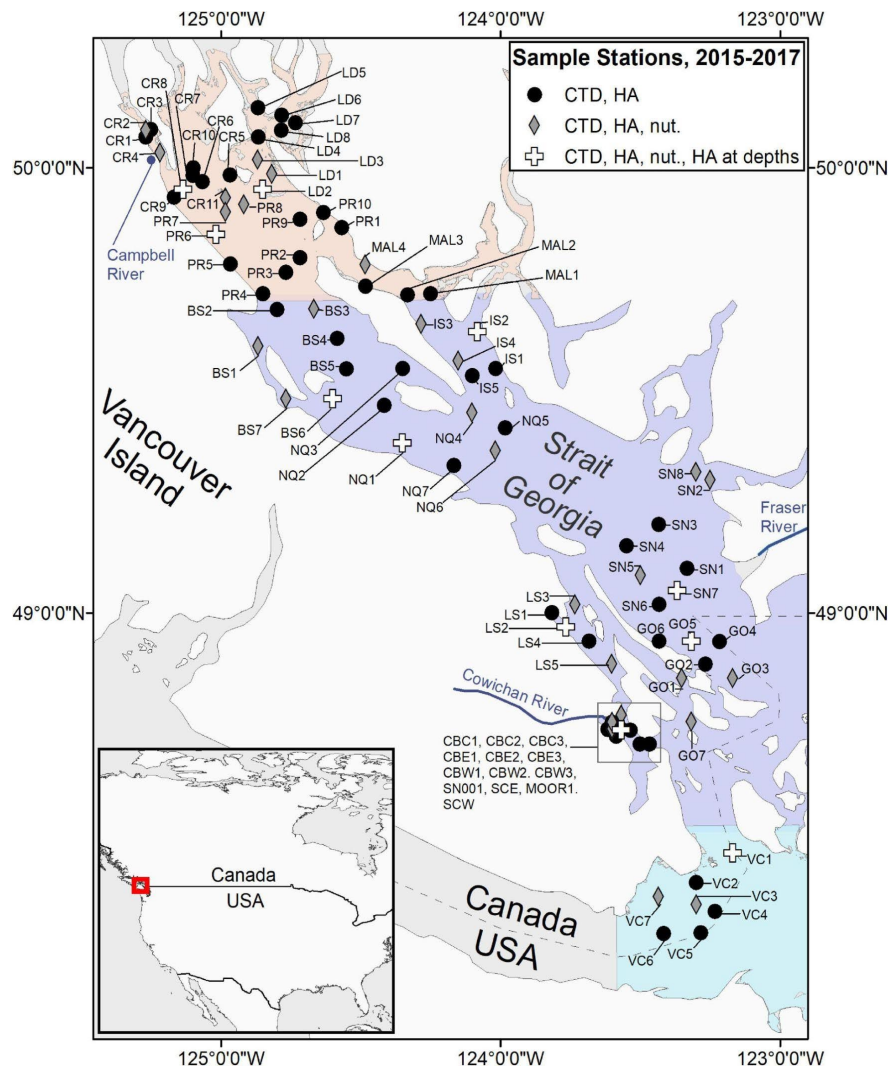


Figure 10. Map of all the sampling locations in the study by Esenkulova et al. (2021).

According to Esenkulova et al. (2021), each environmental parameter exhibited distinct seasonal cycles during the four-year sampling period. The monthly average surface water temperatures of the sampling months are generally between 8.4 and 17.5 degrees Celsius, where the lowest was in February each year, rising over time and peaking in July-August before continuing to decline until October of the year when sampling ended. It is worth noting that the temperature rises in the Strait of Juan de Fuca on the southern side of city Victoria, is far less than that of other northern regions in summer. In 2015, the only sample year in this region when the maximum summer temperature was even below 15°C (Figure 11A). As mentioned earlier, the temperature within a certain range only provides basic conditions for the growth of *H. akashiwo* but does not seem to greatly promote the occurrence of *H. akashiwo* blooms. In addition, under the premise of meeting the growth requirements of *H. akashiwo*, higher temperature is likely to reduce the probability of *H. akashiwo* bloom. Esenkulova et al. (2021) revealed that temperature during *Heterosigma* blooms in the Salish Sea appears inversely related ($r = -0.131$), which also agrees with another study by Brown et al. in 2018.

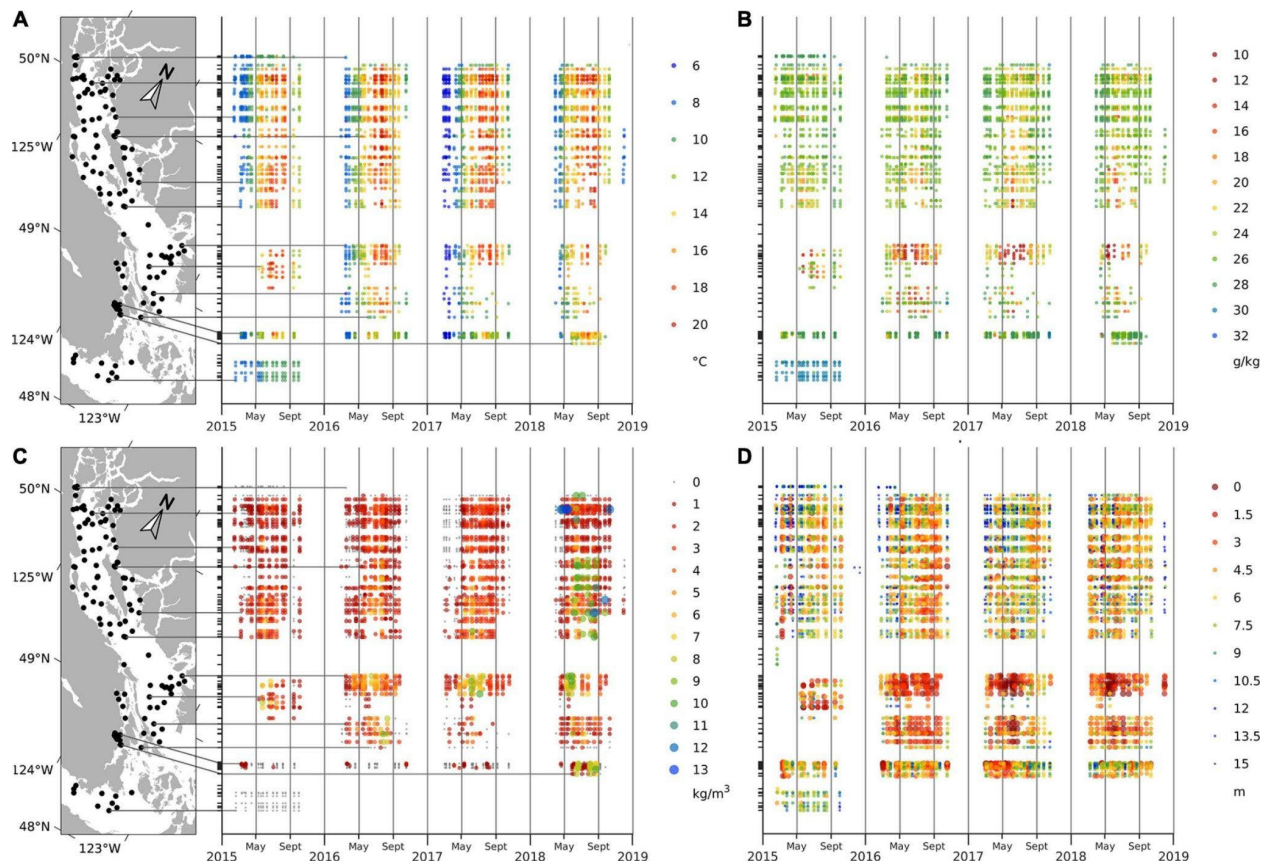


Figure 11. Environmental Parameters in the Strait of Georgia from 2015 to 2018, including surface water temperature (A), salinity at 1m(B), stratification parameter(C), and Secchi depth (D) (Esenkulova et al., 2021).

The salinity of the Salish Sea waters is generally lowest in summer and higher in spring and fall. The monthly average salinity at a depth of 1m is 23.3 - 27.4g/kg. The salinity in this area also showed substantial spatial differences: The Strait of Juan de Fuca also exhibits significantly higher salinity than other regions; summer salinity is substantially lower in coastal areas of the Great Vancouver than in the northern waters of the Strait of Georgia (Figure 11B). Appropriate salinity was also not found to have any promoting effect on the growth of *H. akashiwo* under the premise of satisfying the growth (Esenkulova et al., 2021; Brown et al., 2018).

Stratification is highest in summer and lower in spring and fall. Notably, in 2018, the year in which the only *H. akashiwo* blooms occurred during the sampling period, the summer stratification of 2018 on the northern side of SoG was significantly higher than that of the other three years (Figure 11C). Subsequent data analysis by Esenkulova et al. (2021) showed a strong positive correlation between *H. akashiwo* and stratification ($r = 0.237$). The study by Brown et al. (2018) of *H. akashiwo* blooms that occurred between 2001 and 2007 also demonstrated a strong correlation with water column stability. In addition, stratification and water column stability in this region were proposed to be affected by Fraser River discharge.

The inflow of the Fraser River into a turbid plume also results in the relatively shallow Secchi depth in the Salish Sea. Secchi depth, a measure of how deeply light penetrates water, is lowest in summer and higher in spring and fall due to the relatively large discharge of the Fraser River in summer. For the same reason, the Secchi depth on the south side of SoG where the estuary is located is generally smaller than that on the north side of SoG (Figure 11D).

Esenkulova et al. (2021) conducted surface and 20-m depth measurements of nitrate, phosphate, and silicate concentration in the SoG region from February to October each year from 2015 to 2018. The results showed that the concentration of each nutrient in summer was significantly lower than that in spring and fall. In addition, the concentrations of the three nutrients at 20 m were all slightly higher than those on the surface during the same period. The biggest difference between summer and other times is nitrate and phosphate. Nitrate and phosphate are almost 0

μM at the surface in summer in most of the SoG. Silicate has a slightly higher concentration than the other two (approximately 10-30 μM at the surface in summer). Combined with the region's relatively low nitrate and phosphate average concentrations in summer, Esenkulova et al. (2021) also provided further considerably low correlations between *H. akashiwo* taxa concentrations and the concentration of the two nutrients ($r = -0.008$ for nitrate and $r = 0.035$ for phosphate). In contrast, the cellular concentration of *H. akashiwo* was found to have a relatively strong positive correlation with the silicate concentration ($r = 0.227$). For visualization, the specific correlations of *H. akashiwo* with various environmental factors and nutrients in summer are provided in Table 3.

Environmental Drivers and Nutrients	Correlations (r) between each and <i>H. akashiwo</i>
Temperature	-0.131
Salinity	-0.178
Stratification	0.237
Secchi Depth	-0.110
Nitrate	-0.008
Phosphate	-0.035
Silicate	0.227

Table 3. Pearson Product-Moment Correlations (r) examining inter-annual relationships between mean *H. akashiwo* concentrations and factors during summer (June, July, August) ($n = 259$), in the Strait of Georgia from 2015 to 2018 (Esenkulova et al., 2021).

To conclude, Table 3 intuitively shows that with statistically significant values, the summer *H. akashiwo* concentration in the SoG region is positively correlated with stratification and silicate concentration, while its concentration is negatively correlated with temperature, salinity and Secchi depth.

Discussion

By choosing the Strait of Georgia, which occupies a major area of the Salish Sea, as the study site, the relationship between the growth of *H. akashiwo* in the Salish Sea and various hydrological factors and nutrition was explored. As mentioned earlier, *H. akashiwo* is highly adaptable to a wide range of temperature and salinity (high growth rates can be maintained in the range of 10 - 40 ppt at 14 - 30°C), provided that the minimum requirements for the germination and growth of *H. akashiwo* cysts were met. While the temperature and salinity of the Salish Sea are in this range from each May to September, making these two in the Salish Sea not the main factors that promoted the increasing occurrence of its bloom in recent years. Although not significantly affecting the reproductive rate of *H. akashiwo*, high temperature years in the interannual variability may lead to earlier occurrence of its blooms during the year (Riche et al., 2014). Therefore, the influence of temperature should be considered when estimating the occurrence time of *H. akashiwo* bloom.

Although nitrate and phosphate are essential nutrients for the growth of *H. akashiwo*, their negative correlation with *H. akashiwo* in the Salish Sea, as revealed by Esenkulova et al. (2021), has a high probability of proving that the Salish Sea has provided sufficient nutrients of nitrate and phosphate for the growth of *H. akashiwo*. Therefore, the emission of phosphate and nitrate is not considered to trigger the development of its bloom even if there is a minimal summer concentration of nitrate and phosphate in the surface seawater. The reason for this may be because the diurnal vertical migration behavior of *H. akashiwo* enables it to obtain nutrients for growth in deep seawater, where the nutrients are relatively more prosperous than in the surface.

Research in the Strait of Georgia revealed that the most important factors affecting the increasing occurrence of *H. akashiwo* blooms in the Salish Sea were stratification and silicate concentration, both of which were positively correlated with the cellular concentration of *H. akashiwo* in summer. According to historical data, most areas of the Salish Sea where *H. akashiwo* blooms often occur can generally reach the temperature that is sufficient for *H. akashiwo* cysts to germinate in May of each year. The freshwater of the Fraser River provided a low-salt environment and strong stratification suitable for the growth of *H. akashiwo* in the

coastal area of the central SoG near the Great Vancouver. Due to the ability of diurnal vertical migration, stratification is more helpful for *H. akashiwo* to meet the nutrients required for its growth in areas with poor surface nutrients. In addition, the freshwater of the Fraser River also brings a large amount of available nutrient into the Salish Sea to support the growth of *H. akashiwo* by carrying a large amount of dissolved silicon (much larger than 50 μM silica in concentration) (Olson et al., 2021). Since the summer flow of the Fraser River can significantly affect the stratification and the silicon content of the Salish Sea near its estuary, the flow of the Fraser River can be potentially seen as an essential indicator of whether *H. akashiwo* blooms will occur. This also corresponds to the view of Taylor (1993) that the *H. akashiwo* blooms occurred in recent years mostly originated in the coastal areas near the Fraser River and spread northward in the strait.

Although *H. akashiwo* was described in the sampling results as one of the four most abundant HAs (> 6%) in the region from 2015 to 2018, its frequency of occurrence (6.3%) was much lower than the other three HA taxa (*R. setigera*: 16.4%, *Dictyocha spp*: 16.0%, *Alexandrium spp*: 15.5%). In general, the annual average abundance of *H. akashiwo* in this area is far less than the other three dominant HA species. However, its bloom is not significantly limited by these three dominant species through competition for resources. According to Esenkulova et al. (2021), although the four dominant species in this area were all observed from May to September, the high abundance of the three HA taxa except *H. akashiwo* all occurred in August, significantly later than May and June when *H. akashiwo* blooms often occur. The occurrence of earlier *H. akashiwo* blooms eschews the concentrated growth of *R. setigera* that typically occurs in late summer when abundance is higher, and growth consumes large amounts of silicate. Although *Dictyocha spp* was abundant throughout the sampling season, its growth was negatively correlated with phosphate and silicate concentrations, which shows that the environmental niches of *Dictyocha spp* and *H. akashiwo* have only a small overlap and are unlikely to experience serious resource competition. In addition, *Alexandrium spp* did not bloom in this area during the sampling period, possibly because of some other constraints. Although the overall abundance is greater than that of *H. akashiwo*, the lack of concentrated mass growth of *Alexandrium spp* is not likely to limit the blooms of *H. akashiwo* in this area by occupying a large number of resources.

The long-distance transport and local discharge of metals, organic pollutants, and structural contaminants such as, plastics and fishing gear into the Strait of Georgia have been documented since the 1990s and have continued until present (Johannessen & Macdonald, 2009).

Contaminants affect primarily fish, mammals and birds mainly through toxicity and obstructing breath. This can lead to the result that the local input of nutrients and contaminants may cause changes in the community structure of phytoplankton by affecting the upper level of the food web. However, its effect on the growth of algae, especially *H. akashiwo*, is not much mentioned in the current research. It can be explained that most of the nitrogen and phosphorus for algal growth in the Salish Sea comes from the inflow of Pacific ocean water through the Strait of Juan de Fuca (about 98% nitrogen) (Davis et al., 2014), indicating that nutrient pollution from land in the Salish Sea is not significant.

Based on a review of existing research and evidence, the frequent occurrence of *H. akashiwo* blooms in the Salish Sea over the past few decades appears to be less related to coastal human activities, including urban sewage discharge and agricultural nutrient infiltration, as the main nutrients produced by such activities did not significantly affect the growth of *H. akashiwo* in this region. Changes in Fraser flow are considered to be the dominant factor promoting the occurrence of *H. akashiwo* blooms, but the impact of human activities along the coast on its interannual flow changes is complex and beyond the scope of this study. In addition, climate change and ocean currents can potentially affect when *H. akashiwo* populations begin to develop and alter the species composition and ecosystem structure of phytoplankton blooms, as there are some other HA organisms in the region that are very sensitive to temperature changes. The same speculation was proposed by Mudie et al. (2002), who suggested that the annual increase in HAB over the past five decades may be caused by climate change and increased shipping.

At present, many studies have mentioned that extreme weather and annual warming brought about by global climate change may have led to unstable phytoplankton population structure, mainly affecting cell metabolism, cyst germination, nutrient uptake and photosynthesis rate (Wells et al., 2020). In addition, stratified perturbations caused by climate change can also be detrimental to the upward input of bottom nutrients, which can lead to a competitive advantage for phytoplankton with vertical migration behaviors, such as *H. akashiwo*, which is the focus of

this paper. Although this study illustrates the influence of various factors in the Salish Sea on the formation of *H. akashiwo* blooms from a macro level, there are still many influencing mechanisms that need to be studied further, and some have been mentioned.

Conclusions

In recent years, the frequency of harmful algal blooms has increased year by year, which has caused serious ecological damage worldwide. As the most important fish-killing harmful algae recorded in British Columbia, Canada, *H. akashiwo* has been the focus of attention in this field of research. This paper reviewed the existing records and systematically integrated the biological characteristics of *H. akashiwo*, its world distribution range, its effect on fish lethality, and the influence and limitation of hydrological factors on its growth. Then, the relationship between the growth trend of *H. akashiwo* blooms in the region in recent years and the impact of environmental factors and human activities in the region has been analyzed and summarized through research of a sampling study that occurred in the Salish Sea from 2015 to 2018. The results showed that the occurrence of *H. akashiwo* bloom in the Salish Sea was mainly affected by seawater stratification and silicate content. The timing of its occurrence is dependent on the temperature in the early spring of the year to be suitable to achieve the minimum requirements for cyst germination. The Fraser River is thought to have a more significant impact on the cell amount of *H. akashiwo* summer bloom due to the strong stratification and low salinity near the estuary caused by its large summer flow, as well as carrying a large amount of silicon that could aid the growth of *H. akashiwo*. In contrast, nitrogen and phosphorus emissions from coastal human activities only account for a very small part of the nutrient sources in the Salish Sea, and *H. akashiwo* does not have high requirements for these two nutrients for growth. Therefore, human activities along the coast are so far not considered to be having a great impact on the occurrence of *H. akashiwo* bloom.

References

Admin. (2020, November 23). *Nanaimo water temperature*. WATERTEMPERATURE.NET. Retrieved June 28, 2022, from <https://www.watertemperature.net/canada/nanaimo.html#waterC>

Blanco, E. P., Hagström, J., Salomon, P. S., & Granéli, E. (2013). Detection of *Heterosigma akashiwo* (Hada) using specific RNA probes: Variability of RNA content with environmental conditions. *Harmful Algae*, 24, 80–88.

Branco, S., Menezes, M., Alves-de-Souza, C., Domingos, P., Schramm, M. A., & Proença, L. A. O. (2014). Recurrent blooms of *Heterosigma Akashiwo* (raphidophyceae) in the PIRAQUÊ channel, Rodrigo de Freitas Lagoon, Southeast Brazil. *Brazilian Journal of Biology*, 74(3), 529–537.
<https://doi.org/10.1590/bjb.2014.0074>

Brown, Tamara; Haigh, Nicola; and Johnson, Devan, "Interannual variation of the toxic raphidophyte *Heterosigma akashiwo* in Departure Bay (Nanaimo): data from the harmful algae monitoring program 2001–2017" (2018). Salish Sea Ecosystem Conference. 91. <https://cedar.wvu.edu/ssec/2018ssec/allsessions/91>

Chang, F. H., Anderson, C., & Boustead, N. C. (1990). First record of *aheterosigma*(raphidophyceae) bloom with associated mortality of cage-reared salmon in Big Glory Bay, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 24(4), 461–469. <https://doi.org/10.1080/00288330.1990.9516437>

Chang, F. H., & Page, M. (1995). Influence of light and three nitrogen sources on growth of *heterosigma carterae*(raphidophyceae). *New Zealand Journal of Marine and Freshwater Research*, 29(3), 299–304.
<https://doi.org/10.1080/00288330.1995.9516664>

Coyne, K. J. (2010). Nitrate reductase (NR1) sequence and expression in the harmful alga *heterosigma AKASHIWO* (RAPHIDOPHYCEAE)1. *Journal of Phycology*, 46(1), 135–142.
<https://doi.org/10.1111/j.1529-8817.2009.00781.x>

Crittenden, J. C., & Borchardt, J. H. (2012). *MWh's water treatment principles and design*. John Wiley & Sons.

Cochlan, W. P., Trainer, V. L., Trick, C. G., Wells, M. L., Bill, B. D., & Eberhart, B. T. L. (2013). *Heterosigma akashiwo* in the Salish Sea: defining growth and toxicity leading to fish kills. In *Proceedings of the 15th International Conference on Harmful Algae*.

Davis, K. A., Banas, N. S., Giddings, S. N., Siedlecki, S. A., MacCready, P., Lessard, E. J., Kudela, R. M., & Hickey, B. M. (2014). Estuary-enhanced upwelling of marine nutrients fuels coastal productivity in the U.S. pacific northwest. *Journal of Geophysical Research: Oceans*, 119(12), 8778–8799.
<https://doi.org/10.1002/2014jc010248>

Engesmo, A., Eikrem, W., Seoane, S., Smith, K., Edvardsen, B., Hofgaard, A., & Tomas, C. R. (2016). New insights into the morphology and phylogeny of *heterosigma akashiwo* (raphidophyceae), with the description of *heterosigma minor* sp. nov. *Phycologia*, 55(3), 279–294. <https://doi.org/10.2216/15-115.1>

- Esenkulova, S., Suchy, K. D., Pawlowicz, R., Costa, M., & Pearsall, I. A. (2021). Harmful algae and oceanographic conditions in the Strait of Georgia, Canada based on Citizen Science Monitoring. *Frontiers in Marine Science*, 8. <https://doi.org/10.3389/fmars.2021.725092>
- Fuse, H. (1987). Effects of trace metals on growth of red tide phytoplankton and their accumulation of metal. *Agricultural and Biological Chemistry*, 51(4), 987–992. <https://doi.org/10.1080/00021369.1987.10868147>
- Grattan, L. M., Holobaugh, S., & Morris, J. G. (2016). Harmful algal blooms and public health. *Harmful Algae*, 57, 2–8. <https://doi.org/10.1016/j.hal.2016.05.003>
- Haque S. M., Onoue Y.. Effects of salinity on growth and toxin production of a noxious phytoflagellate, *Heterosigma akashiwo* (Raphidophyceae), *Bot. Mar.*, 2002, vol. 45 (pg. 356-363)
- Hara, Y., & Chihara, M. (1987). Morphology, ultrastructure and taxonomy of the Raphidophycean alga heterosigma akashiwo. *The Botanical Magazine Tokyo*, 100(2), 151–163. <https://doi.org/10.1007/bf02488320>
- Herndon, J., & Cochlan, W. P. (2007). Nitrogen utilization by the raphidophyte *Heterosigma Akashiwo*: Growth and uptake kinetics in laboratory cultures. *Harmful Algae*, 6(2), 260–270. <https://doi.org/10.1016/j.hal.2006.08.006>
- Honjo T., Smayda T. J., Shimizu Y. Y.. Overview on bloom dynamics and physiological ecology of *Heterosigma akashiwo*, *Toxic Phytoplankton Blooms in the Sea.*, 1993 AmsterdamElsevier(pg. 33-41)
- Horner, R. A., Garrison, D. L., & Plumley, F. G. (1997). Harmful algal blooms and red tide problems on the U.S. West Coast. *Limnology and Oceanography*, 42(5part2), 1076–1088. https://doi.org/10.4319/lo.1997.42.5_part_2.1076
- Huffman, Jason (6 June 2018). "Algal bloom kills half of Grieg's salmon at two British Columbia farms". Undercurrent News. Retrieved 2018-06-08.
- Interannual variation of the toxic raphidophyte Heterosigma Akashiwo in ...* (n.d.). Retrieved June 16, 2022, from https://www.researchgate.net/profile/Tamara-N-V-Brown/publication/343084829_Interannual_variation_of_the_toxic_raphidophyte_Heterosigma_akashiwo_in_Departure_Bay_Nanaimo_data_from_the_harmful_algae_monitoring_program_2001-2017/links/5f1618fc92851c1eff2205ec/Interannual-variation-of-the-toxic-raphidophyte-Heterosigma-akashiwo-in-Departure-Bay-Nanaimo-data-from-the-harmful-algae-monitoring-program-2001-2017.pdf
- Jack Rensel, J. E., Haigh, N., & Tynan, T. J. (2010). Fraser River Sockeye Salmon Marine Survival Decline and harmful blooms of *Heterosigma Akashiwo*. *Harmful Algae*, 10(1), 98–115. <https://doi.org/10.1016/j.hal.2010.07.005>

- Jeong, H. J., Lim, A. S., Franks, P. J. S., Lee, K. H., Kim, J. H., Kang, N. S., Lee, M. J., Jang, S. H., Lee, S. Y., Yoon, E. Y., Park, J. Y., Yoo, Y. D., Seong, K. A., Kwon, J. E., & Jang, T. Y. (2015). A hierarchy of conceptual models of red-tide generation: Nutrition, behavior, and Biological Interactions. *Harmful Algae*, 47, 97–115. <https://doi.org/10.1016/j.hal.2015.06.004>
- Jeong, H. J., Seong, K. A., Kang, N. S., Yoo, Y. D., Nam, S. W., Park, J. Y., Shin, W. G., Glibert, P. M., & Johns, D. (2010). Feeding by raphidophytes on the cyanobacterium *Synechococcus* sp.. *Aquatic Microbial Ecology*, 58, 181–195. <https://doi.org/10.3354/ame01354>
- Johannessen, S. C., & Macdonald, R. W. (2009). Effects of local and global change on an Inland Sea: The Strait of Georgia, British Columbia, Canada. *Climate Research*, 40, 1–21. <https://doi.org/10.3354/cr00819>
- Khan, S., Arakawa, O., & Onoue, Y. (1997). Neurotoxins in a toxic red tide of *Heterosigma Akashiwo* (raphidophyceae) in Kagoshima Bay, Japan. *Aquaculture Research*, 28(1), 9–14. <https://doi.org/10.1046/j.1365-2109.1997.t01-1-00823.x>
- Kok, J. W., Yeo, D. C., & Leong, S. C. (2015). Growth and physiological responses of a tropical toxic marine Microgalga *Heterosigma akashiwo* (Heterokontophyta: Raphidophyceae) from Singapore waters to varying nitrogen sources and light conditions. *Ocean Science Journal*, 50(3), 491–508. <https://doi.org/10.1007/s12601-015-0045-x>
- Leftley, J. (1993). Toxic phytoplankton blooms in the sea. *Journal of Experimental Marine Biology and Ecology*, 174(2), 279–280. [https://doi.org/10.1016/0022-0981\(93\)90023-h](https://doi.org/10.1016/0022-0981(93)90023-h)
- Ling, C., & Trick, C. G. (2010). Expression and standardized measurement of hemolytic activity in *Heterosigma Akashiwo*. *Harmful Algae*, 9(5), 522–529. <https://doi.org/10.1016/j.hal.2010.04.004>
- Map of the Salish Sea & Surrounding Basin*. Maps by Stefan Freelan. (n.d.). Retrieved June 24, 2022, from <https://maps.stefanfreelan.com/salishsea/>
- Martinez, R., Orive, E., Laza-Martinez, A., & Seoane, S. (2010). Growth response of six strains of *Heterosigma akashiwo* to varying temperature, salinity and irradiance conditions. *Journal of Plankton Research*, 32(4), 529–538. <https://doi.org/10.1093/plankt/fbp135>
- Mudie, P. J., Rochon, A., & Levac, E. (2002). Palynological Records of red tide-producing species in Canada: Past trends and implications for the future. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 180(1-3), 159–186. [https://doi.org/10.1016/s0031-0182\(01\)00427-8](https://doi.org/10.1016/s0031-0182(01)00427-8)

- Nakamura, A., Okamoto, T., Komatsu, N., Ooka, S., Oda, T., Ishimatsu, A., & Muramatsu, T. (1998). Fish mucus stimulates the generation of superoxide anion by *chattonella marina* and *heterosigma akashiwo*. *Fisheries Science*, 64(6), 866–869. <https://doi.org/10.2331/fishsci.64.866>
- Nygaard, K., & Tobiesen, A. (1993). Bacterivory in algae: A survival strategy during nutrient limitation. *Limnology and Oceanography*, 38(2), 273–279. <https://doi.org/10.4319/lo.1993.38.2.0273>
- Olson, E., Nemcek, N., & Allen, S. (2021). Observations inform improvements in model silicon cycling in a semi-enclosed coastal sea. <https://doi.org/10.5194/egusphere-egu21-10502>
- Ono, K., Khan, S., & Onoue, Y. (2000). Effects of temperature and light intensity on the growth and toxicity of *heterosigma akashiwo* (raphidophyceae). *Aquaculture Research*, 31(5), 427–433. <https://doi.org/10.1046/j.1365-2109.2000.00463.x>
- Pratt, D. M. (1966). Competition between *skeletonema costatum* and *OLISTHODISCUS luteus* in Narragansett Bay and in culture. *Limnology and Oceanography*, 11(4), 447–455. <https://doi.org/10.4319/lo.1966.11.4.0447>
- Riche, O., Johannessen, S. C., & Macdonald, R. W. (2014). Why timing matters in a coastal sea: Trends, variability and tipping points in the Strait of Georgia, Canada. *Journal of Marine Systems*, 131, 36–53. <https://doi.org/10.1016/j.jmarsys.2013.11.003>
- Smayda TJ (1998) Ecophysiology and bloom dynamics of *Heterosigma akashiwo* (Raphidophyceae). In: Anderson DM, Cembella AD, Hallegraeff GM (eds) Physiological ecology of harmful algal blooms. Springer, Heidelberg, pp 113–131
- Smetacek, V., & Zingone, A. (2013). Green and golden seaweed tides on the rise. *Nature*, 504(7478), 84–88. <https://doi.org/10.1038/nature12860>
- Shikata, T., Nagasoe, S., Matsubara, T., Yamasaki, Y., Shimasaki, Y., Oshima, Y., & Honjo, T. (2007). Effects of temperature and light on cyst germination and germinated cell survival of the noxious raphidophyte *Heterosigma Akashiwo*. *Harmful Algae*, 6(5), 700–706. <https://doi.org/10.1016/j.hal.2007.02.008>
- Strom, S. L., Harvey, E. L., Fredrickson, K. A., & Menden-Deuer, S. (2013). Broad salinity tolerance as a refuge from predation in the harmful raphidophyte alga *Heterosigma akashiwo* (Raphidophyceae). *Journal of phycology*, 49(1), 20–31.
- Tang DL, Kawamura H, Hai D-N, Takahashi W (2004) Remote sensing oceanography of a harmful algal bloom off the coast of southeastern Vietnam. *J Geophys Res* 109(C3):C03014. doi: 10.1029/2003JC002045

- Taylor, F. J. R. (1993). The ecology of fish-killing blooms of the chloromonad flagellate *Heterosigma* in the Strait of Georgia and adjacent waters. *Toxic phytoplankton blooms in the sea*, 705-710. Tomas C. R., *Olistodiscus luteus* (Chrysophyceae) I. Effects of salinity and temperature on growth, motility and survival, *J. Phycol.*, 1978, vol. 14 (pg. 309-313)
- Twiner, M. J., Chidiac, P., Dixon, S. J., & Trick, C. G. (2005). Extracellular organic compounds from the ICHTHYOTOXIC red tide alga *heterosigma akashiwo* elevate cytosolic calcium and induce apoptosis in SF9 cells. *Harmful Algae*, 4(4), 789–800. <https://doi.org/10.1016/j.hal.2004.12.006>
- Twiner, M. J., Dixon, S. J., & Trick, C. G. (2001). Toxic effects of *heterosigma akashiwo* do not appear to be mediated by hydrogen peroxide. *Limnology and Oceanography*, 46(6), 1400–1405. <https://doi.org/10.4319/lo.2001.46.6.1400>
- Twiner, M. J. (2000). Possible physiological mechanisms for production of hydrogen peroxide by the ICHTHYOTOXIC flagellate *Heterosigma Akashiwo*. *Journal of Plankton Research*, 22(10), 1961–1975. <https://doi.org/10.1093/plankt/22.10.1961>
- Watanabe, M., Kohata, K., & Kunugi, M. (2008). Phosphate accumulation and metabolism by *Heterosigma Akashiwo* (raphidophyceae) during diel vertical migration in a stratified microcosm¹. *Journal of Phycology*, 24(1), 22–28. <https://doi.org/10.1111/j.1529-8817.1988.tb04452.x>
- Wang, Z.-hui, Liang, Y., & Kang, W. (2011). Utilization of dissolved organic phosphorus by different groups of phytoplankton taxa. *Harmful Algae*, 12, 113–118. <https://doi.org/10.1016/j.hal.2011.09.005>
- Wang, Z.-H., & Liang, Y. (2015). Growth and alkaline phosphatase activity of *Chattonella marina* and *Heterosigma Akashiwo* in response to phosphorus limitation. *Journal of Environmental Sciences*, 28, 1–7. <https://doi.org/10.1016/j.jes.2014.04.015>
- Wells, M. L., Karlson, B., Wulff, A., Kudela, R., Trick, C., Asnaghi, V., Berdalet, E., Cochlan, W., Davidson, K., De Rijcke, M., Dutkiewicz, S., Hallegraeff, G., Flynn, K. J., Legrand, C., Paerl, H., Silke, J., Suikkanen, S., Thompson, P., & Trainer, V. L. (2020). Future HAB science: Directions and challenges in a changing climate. *Harmful Algae*, 91, 101632. <https://doi.org/10.1016/j.hal.2019.101632>
- Wood, G. J., & Flynn, K. J. (1995). Growth of *Heterosigma Carterae* (RAPHIDOPHYCEAE) on nitrate and ammonium at three photon flux densities: Evidence for n stress in nitrate-growing cells¹. *Journal of Phycology*, 31(6), 859–867. <https://doi.org/10.1111/j.0022-3646.1995.00859.x>
- Yamasaki, Y., Shikata, T., Nukata, A., Ichiki, S., Nagasoe, S., Matsubara, T., Shimasaki, Y., Nakao, M., Yamaguchi, K., Oshima, Y., Oda, T., Ito, M., Jenkinson, I. R., Asakawa, M., & Honjo, T. (2009). Extracellular polysaccharide-protein complexes of a harmful alga mediate the allelopathic control it exerts

within the phytoplankton community. *The ISME Journal*, 3(7), 808–817.
<https://doi.org/10.1038/ismej.2009.24>