Fish kills from the harmful alga *Heterosigma akashiwo* in Puget Sound: Recent blooms and review

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Heterosigma bloom (to right) passing southward in Bellingham Channel, North Puget Sound past Deepwater Bay, Site 3 fish farm. Note white spot in each pen from airlift pumps in operation.

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List of Acronyms and abbreviations

CPS	Central Puget Sound
Heterosigma	*Heterosigma akashiwo*
NPS	North Puget Sound
PNW	Pacific Northwest (of the contiguous 48 U.S. states) + British Columbia
SST	Sea surface temperature (satellite derived)
Strait	Strait of Juan de Fuca
the Sound	Puget Sound

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Overview

Heterosigma akashiwo (Raphidophyceae) is a widely-distributed, fish-killing phytoplankton species that may under some conditions kill or injure farmed and wild fish and possibly other aquatic organisms in Puget Sound, Washington state ("the sound") and several other locations around the world.

This report briefly reviews background information on this alga, conditions that allow it to bloom, how it may kill fish and occurrence of past blooms in Puget Sound. The report also summarizes a small portion of the extensive literature regarding nutrients and phytoplankton in Puget Sound. Within this tidally-active region that differs profoundly from most other coastal regions of the U.S., light (or advection and grazing), not nutrient supply, controls phytoplankton production in all areas except for some distal, poorly flushed bays or fjords. Another exception is for short periods near river mouths when freshets create vertical stratification that limits mixing.

It is evident that fish farms do not cause *Heterosigma akashiwo* blooms in marine waters of Western Washington and the data suggests it is unlikely they exacerbate blooms. Rather, there is substantial evidence to the contrary, that *Heterosigma* blooms are large-scale events that initiate from remote cyst germination and are advected by tides, winds and estuarine circulation into other areas including those with fish farms. *Heterosigma* blooms have occurred in Pacific Northwest waters well before net-pen fish farms were installed in Puget Sound. The few fish farms currently operating are clustered in North Puget Sound, Central Puget Sound and Port Angeles Harbor in the Strait of Juan de Fuca. All commercial fish farms are required to locate in areas of naturally high dissolved inorganic nitrogen levels (from Pacific Ocean upwelling) but in this area the industry is relatively small and the effluent accounts for less than 0.1% of the natural background flux of nitrogen from the Pacific Ocean.

Germination of Heterosigma cysts is known to be successful above the 15°C bottom temperature threshold in many locations worldwide. The conditions for development and spread after cyst germination in Puget Sound are not exactly known, but clearly are linked to warm, sunny weather in the May through September period. In North Puget Sound the linkage is also strongly related to annual peak discharge of the Fraser River and resulting vertical stratification of the water column. Fish growers use this knowledge to assess risk and elevate their monitoring when these factors coincide. Data from the large-scale blooms of 2006 and 2007 presented herein to add to the 17 year record of monitoring and demonstrate the importance of the above cited linkages. Blooms can occur in late spring to early fall and biannual blooms have occurred in some previous years.

In North Puget Sound, the brackish Fraser River plume creates suitable conditions for blooms to form near the U.S.-B.C. border or in shallow bays from Bellingham to Samish Bays. These blooms are then advected westerly and southwards into the main channels of North Puget Sound, particularly those associated with the Fraser River plume. Advection is provided by estuarine circulation (the well-known surface outflow toward the Pacific Ocean

via the Strait of Juan de Fuca) that often occurs in fortnightly pulses related to tidal cycle variation as well as fair weather, northerly winds.

On its way through North Puget Sound in late June 2006 a *Heterosigma* bloom killed two million dollars worth of farmed salmon that were being reared in this high tidal energy environment. Another subsequent bloom occurred in May 2007 that followed nearly the same pattern of events. Timing of *Heterosigma* blooms in Northern Puget Sound does not repeat annually as it has in some other areas of the world but is typically a late spring event coinciding with periods of hot weather and basin-wide vertical stratification (from Fraser River discharge). As an exception to the rule, a massive late summer or early fall bloom has occurred in the past in this area (e.g., 1989) when river flows are at annual minimums and in that case neap tides and hot weather appear to be the strongest correlating factors.

A separate bloom occurred in early August 2006 in central Puget Sound that appeared to start in backwaters and spread over much of the basin. Unlike the North Puget Sound blooms of 2006, this bloom was associated with only with highly elevated surface water temperatures as has been observed in the past in this subarea. Blooms in this area are often not unialgal as they are in the late spring North Puget Sound events, similar to a seasonal pattern observed in Korean coastal waters. No fish in the fish farms were killed as the bulk of the bloom remained in the main (central) basin from Seattle northward or backwaters of Kitsap County (west parts of the central basin) and the fish farm area, in a well-mixed channel (Rich Passage) and associated bight (Clam Bay) remained remarkably free of *Heterosigma* cells. Relatively high levels of ammonium were found throughout the sampled surface waters in other areas, possibly from bloom senescence and decay. A subsequent bloom recurred in August 2007 of nearly the same timing and spatial distribution as the 2006 bloom but flood tide waters from the main basin brought increased numbers of *Heterosigma* cells into the fish farming region and about 6% of the farmed fish were lost at commercial fish farms.

From a broader ecosystem and societal viewpoint, *Heterosigma* blooms in Puget Sound may be doing much more damage than killing farmed fish. Wild salmon and other fish as well as invertebrates and plankton may be adversely affected by sublethal effects or killed. The most vulnerable are juvenile fishes, invertebrates and plankton that are restricted to the surface and near-surface waters where the blooms persist. Near-surface migrating fishes such as sockeye salmon that pass through the surface waters of the area in potential bloom periods may also be affected. The extent of past mortality is unknown as these fish typically sink and are rapidly consumed by predators or are transported by the typically strong tides of this region. It is likely that some Central Puget Sound blooms are restricted to the immediate surface waters and are not as harmful to wild fish. However, the June 2006 North Puget Sound bloom extended to 30m depth or more and the subsequent August 2006 bloom was relatively deep too. These depths include those that many larval, juvenile and other fishes typically inhabit and these fishes are not behaviorally or physiologically capable of swimming to the depths necessary to escape the blooms. North Puget Sound areas are more susceptible as the surface ("mixed") brackish layer is often much more extensive and deeper than in central Puget Sound, as a result of massive Fraser River discharge. The author and others including agency staff have observed dead wild fish during previous *Heterosigma*

blooms in North Puget Sound and the Strait of Juan de Fuca while conducting their surveys. Most interestingly, a pen of 400 subadult sablefish (aka black cod, *Anoplopoma fimbria*) held at NOAA's Manchester Laboratory nearby were not killed by the August 2007 bloom while all Atlantic salmon (*Salmo salar*) held in a nearby research pen were lost. These observations provide yet another tantalizing but inexplicable clue about the etiology of fish mortality from *Heterosigma* exposure.

Fish farmers have a working system that adequately allows them to detect, manage and mitigate the blooms to some extent. But if bloom frequency increased, due to climate shift or oscillation, the periodic losses presently incurred could become a major burden. For the broader society, the question of wild fish mortality remains a potentially major problem (to be added to other, larger problems such as global warming, ocean acidification, human overpopulation, etc.). Ultimately, biology is a much more sensitive indicator of the diversity and stability of our coastal oceans than water quality and the occurrence of species like *Heterosigma* could well be an indicator of incipient and undesirable change. Such change is certainly possible if climate change occurs due to global warming or even known regional oscillation of weather patterns become more pronounced.

The report concludes with recommendations to help understand these blooms, their nature and the extent of their effects.

Introduction

The initial purpose of this report was to document what was learned about bloom dynamics during the 2006 *Heterosigma* blooms in Puget Sound in order to further our understanding of the factors responsible for bloom initiation and spread. While interacting with other harmful algal bloom specialists and reviewing a technical report (GEOHAB 2006) that was inaccurate regarding this alga's relationship with fish farms, at least in the Pacific Northwest US, it became clear to me that there was a need to more fully document the history of previous *Heterosigma* blooms in Puget Sound as well as the basic nature of nutrients and phytoplankton dynamics in the area. Additional blooms occurred in 2007 and general observations from those events were included as they mirrored events of the prior year. The report concludes with recommendations for further improvements of monitoring, management, research and modeling of these fish killing algae blooms.

Background

In this background section I discuss a few literature references of local or wider importance necessary to understand *Heterosigma* bloom dynamics in Puget Sound. There is a huge volume of quality literature dealing with this species; no attempt is made to review it all. Smayda (1998) provides an excellent review of the ecophysiology of the species.

The first recorded *Heterosigma* bloom in Puget Sound was at the Lummi Indian Tribal mariculture "Seapond" in Lummi Bay in 1976 (Gaines and Taylor 1986, Table 1). This ~750 acre pond was created in an intertidal embayment of North Puget Sound and its substantial environmental impact (seawater heating, eel grass community and associated invertebrate extirpation) was studied and reported by Rensel (1972). At that time, only a few small net pen farms (both public and private) that reared Pacific salmon were present in other parts of Puget Sound. In south Puget Sound I reported on a fish kill at the Weyerhaeuser Co. net pen project in Henderson Inlet due to the dinoflagellate *Ceratium fusus* (Rensel and Prentice 1979) but *Heterosigma* was not seen in that 1974-1976 project. Later the pens were removed and no commercial fish culture is present in marine waters of South Puget Sound.

Widespread blooms of *Heterosigma* in Puget Sound or nearby waters recur about every 5 to 10 years or more on average but the time series (Table 1) reveals an interesting temporal distribution. Truly massive blooms occurred in sequential years (e.g., 1989-1990 and 2006-2007) separated by a 16 year interval when several blooms occurred, but were limited in distribution or intensity. Fish losses occurred in 1997, but mostly that involved escaped fish as a large set of pens in central Puget Sound was damaged during a towing operation to avoid the threat of a developing bloom. Although cells were often seen in water samples during late spring or early summer in non bloom years, large-scale blooms apparently could not develop because of weather or physical forcing conditions. Fish farmers routinely monitored the water from spring through fall, and sometimes it looked as if a bloom was commencing, but a Pacific cold front and/or some other physical factor would act to attenuate the bloom. With many years of experience, the fish farmers have

Table 1. Summary of documented *Heterosigma akashiwo* blooms in Puget Sound since 1976.

Location	Date	Fish Species	Estimate Loss	Source-Comments
Lummi (Bay) Seapond, N.E. of Bellingham, Wa. Large dike area with small fish enclosure. [1/]	1976, date uncertain	Cultured fish, ostensibly native coho salmon	Unknown. For area description see Rensel (1972)	Jefferson 1976, Gaines and Taylor 1986
Cypress Island, North Puget Sound, 4 commercial fish farms [2/]	September 1989	Atlantic salmon	364,000 subadult fish	First bloom to kill net pen farmed fish
NOAA Manchester Research Laboratory, Central Puget Sound	Peaked near 4 July 1990	Chinook, sockeye, coho, Atlantic salmon	1,910	Rensel/Horner collected cell & CTD vertical profile data
Clam Bay, Orchard Rocks, Fort Ward, three separate fish farms in Central Puget Sound	Peak near 4 July 1990	Atlantic salmon	649,544 subadult fish	Same as above & Rensel 1995
Western Central Puget Sound in Sinclair Inlet, Port Orchard & Brownsville	Mid July 2003	None affected	None known	Rensel 1995
Budd Inlet, Olympia, South Puget Sound	Summer 1993	Unknown	Unknown	Eisner and Newton 1997
Northern Case Inlet in Southern Puget Sound	September 1994	Coho, chum & chinook salmon , marine fish	35 collected, many more observed dead	Hershberger et al. 1997
NOAA Manchester Research Laboratory, Central Puget Sound	July 1997	Coho, chinook and sockeye salmon	737 (100% mortality of coho)	Connell and Jacobs 1999
Clam Bay, Orchard Rocks, Fort Ward, three separate fish farms in Central Puget Sound	July 1997	Atlantic salmon	401,639 (not killed, accidental release while towing pens to avoid a bloom)	Connell and Jacobs 1999
Port Angeles Harbor	August 1997	Atlantic salmon	62,000	Associated with above bloom?
Hood Canal	Mid September 2000	Low D.O. thought to be responsible	Not applicable	Intense surface bloom Connell et al. 2000
North Puget Sound and Strait of Juan de Fuca	Late June & early July 2006	Atlantic salmon Wild salmon smolts and forage fish [3/]	Cypress Island ~140K adult & sub adult fish. Port Angeles 128,000 sub adult fish	NPS-Strait bloom occurred separate timing from the CPS bloom [4/]
Central Puget Sound	early August 2006	None observed	The bloom generally did not affect the fish farming area of CPS but was almost everywhere else. (this report)	
North Puget Sound	May 2007	Atlantic salmon	Cypress Island ~4,000 adults and subadults fish lost.	NPS-Strait bloom occurred separate timing from the CPS bloom and repeated timing of 2006
Central Puget Sound	August 2007	Atlantic salmon	~ 60,000 subadult fish lost	

[1/] A bloom occurred in 1976 at the Manchester NOAA laboratory too, specimen collected by Lee Harrell, identified by Richard Norris, Univ. of Wa. Botany Dept. (R. Horner, pers. comm.)

[2/] Tailfin Inc. fish farm subsequently closed as a result of the bloom. It was near Eagle Harbor and northeast Cypress Island where higher cell concentrations were common as it was nearer the Fraser River plume origin. The entire Strait of Georgia was involved with this same bloom (Taylor and Harrison 1993) that flowed southward into North Puget Sound.

[3/] Kevin Bright, pers. comm. He observed these stressed fish in North Puget Sound during the bloom.

[4/] ~ $2 million in total loss for the entire 2006 NPS and CPS blooms (J. Bielka, AGS manager, Port Angeles WA).

developed routine methods to detect, react and minimize damage from the blooms, although the risk of significant losses still is possible.

Much of the initial work on *Heterosigma* bloom dynamics in the Pacific Northwest was done by Professor Max Taylor, his students and colleagues in British Columbia where fish-killing blooms were noticed before those in Puget Sound. Taylor and Haigh (1993) reported factors associated with prior blooms to the north in British Columbia where fish kills first occurred at fish farms in 1986. Factors such as circulation and vertical stratification are different than Puget Sound and variable among subareas. Taylor and Harrison (2002) report:

"*Cells abruptly appear in the water when the temperature reaches 15°C (usually in June), apparently due to excystment from shallow sediments. Blooms are most extensive in the Strait of Georgia during summers with strong, shallow stratification due to high runoff from the Fraser River, in turn resulting from a higher than normal snow-pack the previous winter. A drop in salinity in English Bay to 15‰ coincides with 15°C in severe bloom years in this area. A prerequisite to strong blooms is that the spring diatom bloom must be over (usually by late May) and the surface water depleted of nitrate*".

In the same work they report: "Blooms of *Heterosigma* in the Strait of Georgia are apparently strongly linked to river runoff and stratification…"

As will be shown later, a similar conceptual model applies to North Puget Sound but the river plume is more deeply mixed which in some cases results in a deeper bloom. Strong river-induced vertical stratification is sometimes not important to midsummer or later Central Puget Sound blooms as discussed herein.

Recent studies from Delaware on the U.S. east coast (D.A. Hutchins pers. comm., K.J. Portune, MS in development) suggests that *Heterosigma* cysts germinate continually throughout the spring and early summer and only "appear" in the water column in sufficient numbers to be visible to microscopic examination when conditions are right for bloom initiation. In many cases authors have pinpointed the threshold of excystment at 15°C, a subsurface temperature that rarely if ever occurs at fish farm sites in Puget Sound but is probable at tentative bloom initiation sites identified herein. Water temperatures are important for bloom initiation but established blooms are often transported seaward through the fast moving channels of North Puget Sound where water temperatures are almost always less than 15°C.

Heterosigma cells in the water column have been observed in various locations of the Pacific Northwest almost every year since at least the 1960s (Horner et al. 1997) which predates salmon or marine fish aquaculture anywhere in the Pacific Northwest. During most years, cell numbers are either very low (< 10^3 cells/L) or too low to cause fish mortality (ca. < 5 to 7.5 x 10^5 cells/L). In British Columbia there have been reports of non harmful blooms of *Heterosigma* too, but to the best of my knowledge, all blooms that exceed the above benchmark at Puget Sound fish farms have resulted in at least some salmon mortality. As mentioned above, basin-wide blooms that occur over much of Puget Sound and adjacent waters are relatively uncommon, occurring approximately every 5 to 10 years or more in 1989, 1990, 2006 and 2007. Many other blooms began (Table 1) but in most cases weather was not suitable and the blooms were restricted in time and space.

Monetary losses for fish farmers can be large during the major blooms, but not disastrously so and growers have insurance that covers losses beyond some deductable threshold of loss such as 10%. Smaller scale blooms occurred in other years (Table 1) and probably other years that were not observed as fish farms either do not exist or are not permitted in other areas. Farms were once installed at Hood Head in North Hood Canal but the area is subject to occurrence of *Chaetoceros* spp. (subgenus Phaeoceros, Rensel et al. 1989) that fish kills both farmed and probably wild fish (Rensel Associates and PTI Environmental Services 1991) and the farm was removed many years ago on this account. These are cryptic events, likely not to be noticed as the diatom kills fish at very low, non-bloom concentrations and cells may be distributed to 50 m or more depth.

Prior to the 1960s *Heterosigma* blooms may have occurred but were not documented or possibly even noticed by biologists as there was no routine monitoring of phytoplankton or marine fish farming and as stated above, wild fish kills are difficult to detect in most temperate waters. A more extensive review of bloom history in Puget Sound is provided in Anderson et al. (2001, available online) for *Heterosigma* blooms in Puget Sound.

Rensel (1995) advanced a conceptual model of bloom development for Puget Sound and adjacent waters (after major blooms in 1990 and 1991) that factored in weather, tidal conditions and timing relative to snow pack melt and river flow (see appendix A). This model was a local adaptation of the work of Max Taylor and his colleagues in British Columbia mentioned above. The importance of temperature or salinity-induced vertical stratification in bloom formation was highlighted and even at that time it was apparent that blooms in North Puget Sound probably originate near the U.S.-Canada border and are advected southward. Hydrographic data were limited but the blooms were monospecific and observations from fixed wing aircraft indicated at one point in 1990 the bloom covered most of central Puget Sound from Tacoma to Port Townsend and beyond into the Strait of Juan de Fuca toward the Pacific Ocean. Several reports of dead and dying wild fish including cutthroat trout were received by the Washington Department of Fisheries including several from the Port Townsend area (Dick Allen, WDF, pers. comm. 1991).

Some of us believed that there may have been some internal clock or cyst germination mechanism that causes blooms to occur interannually within a tight time frame of early July and September most every year similar to what Li and Smayda (2000) reported for the East Coast U.S. waters of Narraganset Bay. The coincidence of bloom timing for the first several blooms in Puget Sound seemed strikingly similar on both coasts. But subsequently experience in Puget Sound has indicated the importance of physical forcing factors as discussed herein, i.e., weather conditions are paramount for both North and Central Puget Sound blooms and likely Hood Canal too. There are no commercial fish farms in Southern Puget Sound and thus no extensive database for that area, but there have been blooms of *Heterosigma* there, specifically in eutrophic Budd Inlet near Olympia (Eisner and Newton, 1997). The Squaxin Island fish farm several miles north used in the spring for releasing smolts (Rensel et al. 1988) has been infrequently affected in the past by plankton-related fish kills, but no definitive analysis of the causative species has been conducted.

Connell and Jacobs (1997) further documented blooms and contributed to bloom initiation conceptual modeling and reported on localized bloom of *Heterosigma* in the

western portion of central Puget Sound. This bloom was associated with much above average water temperature and reduced salinity of about 2 psu at the surface versus subsurface during the bloom initiation period. The bloom reportedly was first observed near the east side of Bainbridge Island and spread quickly to > 30,000 km^2 area and had mostly subsided after about a week and was gone after two weeks. Later the same author and others (Connell et al. 2000) reported a dense bloom of *Heterosigma* from mid September in Southern Hood Canal. Many wild fish died, but the authors concluded this was due to low dissolved oxygen at that time, not the alga. Although very dense, the bloom may have been restricted to the immediate surface waters. Literature was cited (Hard et al. 1999) that salmonids may actively avoid *Heterosigma* blooms but there is scant evidence of that for species cited (chinook salmon). In the appropriate weather and hydrographic conditions, blooms of *Heterosigma* may concentrate on the immediate surface (to several cm deep) where adult salmonids are not to be found except occasionally they must imbibe air to maintain their buoyancy. Juvenile salmonids including cutthroat trout, chums, pinks and subyearling chinook are, however, often found in spring or early summer in shallow depths and nearshore where algae may be concentrated from winds and bathymetric influences.

As discussed below, *Heterosigma* blooms in Puget Sound can be separated into late spring- early summer blooms associated with riverine-induced vertical stratification versus midsummer to fall blooms that correlate with hot weather-induced vertical stratification. The former are always unialgal or very nearly so, the latter are sometimes mixed with other species, typically dinoflagellates (with some notable exceptions such as the September 1989 and September 2000 blooms). A similar situation has been reported in Korean coastal waters by Kim et al. (2007) where the seasonal variation is pronounced.

Mechanisms of mortality

Heterosigma is harmful in many different regions of the world including the Pacific Northwest, Chile, New Zealand, and Scotland but is not harmful to fish in broad areas such as the entire coast of China and in other regions, such as near Japan, it is much less harmful than in the above mentioned areas. Previously it was thought not to be harmful to fish on the U.S. east coast, but recent fish kills in eutrophic, shallow marine waters of Delaware have correlated with *Heterosigma* blooms (but apparently not monospecific blooms so there is some chance another species is involved).

Heterosigma does not produce any known, persistent toxin but apparently kills fish through an effect on the gills or a labile neurotoxin, both of which could produce blood hypoxia but neither is well-described or understood. The mechanism(s) of fish killing remain unknown or partially-described at best and confusing. As pointed out by Clough and Strom (2005) the alga:

"*may well have multiple modes of toxicity that affect different types of aquatic organisms in different ways. At present, however, there is no accepted chemical measure of toxin content in this species*".

Indeed, there may be no apparent persistent toxin to be found in tissue of *Heterosigma*-killed fish as pointed out by Rensel and Whyte (2003) and in some cases fish killed by the alga may have been consumed by humans without apparent ill effect. Even the physiological

damage or effects of the alga on fish is poorly understood. I conducted histological examinations of moribund fish from a central Puget Sound site (Fort Ward) during the 1990 bloom (using very rapid dissection and preservation techniques) and found highly damaged epithelial cells of all the sampled subadult or adult Atlantic salmon gills. Epithelial disruption, edema, and several other maladies stereotypically seen when gills are damaged by a chemical or abrasion were noted. Previously Black et al. (reported in 1991) conducted studies with juvenile Chinook salmon that showed mortality in the field to fish flown into an affected area. However, in this case only, rapid sampling and fixing of gill tissue showed no histological damage whatsoever. Larger fish usually die more quickly when the gills are damaged by any mechanism compared to smaller fish due to reduced surface area of gills to body mass ratios, so it is reasonable to assume that detectable damage to the small fish gills would have been observed if that was the primary etiology of fish mortality. Surprisingly, there have not been many other observations of affected fish gill or other organ histology in affected areas worldwide. Others raphidophyte species such as those that kill fish in Japan produce similar effects (Oda, 2007).

There are species specific differences of sensitivity within the salmonids and some marine fishes may be immune to at least some strains of the alga as was the case in blooms in Puget Sound in 2007 and sablefish held at the NOAA Manchester Research facility. In this case, a pen of about 400 sablefish (*Anoplopoma fimbria*) was totally unaffected while Atlantic salmon (*Salmo salar*) in an adjacent pen did not survive (K. Massee, NOAA Manchester Lab., pers. comm.). The pens were within a few meters of each other so the difference was likely not due to differing doses. The implications of this observation are potentially very significant but somewhat mystifying as we have documented mortality of other marine fish such as flatfish and sculpins.

Hydrogen peroxide or some other reactive oxygen species of chemical(s) are produced by the alga that possibly affect the fish gills (Yang et al. 1995, Oda et al. 1997) although more recent work provides evidence that it may not be possible as the amount of hydrogen peroxide was insufficient to cause gill damage (Twiner et al. 2001). Mucus production has also been cited as a possible cause of this alga causing clogging of fish gills and blood-hypoxia from suffocation, but I personally have inspected many fresh, moribund fish during *Heterosigma* blooms and find little or no mucus, compared to fish killed by the diatom *Chaetoceros* (subgenus Phaeoceros) which was the subject of my dissertation and related work (Rensel 1993). The observation that *Heterosigma* may cause copious mucus production by fish gills is possibly an error, rooted in casual observations of fish that have been dead for some short (~5 minutes) or longer period of time. Fish gill mucus cells are holocrine cells and are stimulated to discharge upon fish death when the nervous system shuts down and this reaction is noticeably quicker in warm weather of course. The cells arise from subdermal tissues and are stimulated by irritation or other cues and new ones must regenerate. In the case of fish exposed to harmful algae or simply dense blooms of non harmful algae these cells can be stimulated if exposure is rapid and intense such that the fish may suffocate as gas exchange cannot be performed as rapidly as normal at the gills. The mucus does not prevent gas exchange, it merely slows it down proportionate to its

thickness. Affected fish may be observed "coughing" which is a means to expel excess mucus.

The possibility of a brevetoxin cannot be ruled out (Khan et al. 1997, Ono et al. 2000, Haque and Onoue 2002) but the evidence is not compelling because the detected levels are low or undetectable and *Heterosigma* killed fish have been consumed by humans in the past with no apparent ill effects. It is possible the toxin(s) are highly labile so that detection is difficult.

No one has yet killed fish in the laboratory with clean (axenic, i.e., bacteria-free monospecific) cultures of *Heterosigma* except possibly at extremely high concentrations that are unrealistic compared to that which is seen to kill fish in the field. Thus a bacterial or viral cofactor or interaction seems possible or even likely in my opinion. My colleagues and I at the University of Washington conducted numerous bioassays during the early 1990s that involved laboratory exposure of Atlantic salmon (*Salmo salar*) to axenic (bacteria-free) *Heterosigma* cultures grown under a variety of nutrient conditions but not one of hundreds of Atlantic salmon died or even showed signs of stress (K. Banse, F. Taub, R. Horner, J. Postel, R.A. Cattolico, J. Rensel, unpublished data, University of Washington). At the same time in British Columbia, cultures in the laboratory of others were killing fish, but they were not axenic. We did not report our findings in the peer-reviewed literature but it was later reported from other similar observations by Carrasquero-Verde (1999).

Other explanations for *Heterosigma*-caused fish mortality are possible too, including elevated levels of DOC (Smayda 1997), low levels of iron that stimulate exoenzyme production by the alga and light-activated haemolysin (Trick et al. 2007) and complex combinations of nitrogen-rich nutrients (Herndon and Cochlan 2006)

The stage of cell development or population phase may be important in altering the cells from harmless to harmful. As discussed below, in the 2006 North Puget Sound bloom smaller, non-motile cells seemed to produce higher rates of mortality at pen sites. This is a condition long known in British Columbia by growers and interested scientists (Nicky Haigh, pers. comm. on SoundHAB listserve). She also reports that fish mortality may be more common at night, than during the daylight but the reason(s) is unknown.

Some authors and agency personnel have previously consider *Heterosigma* to be a fish killer of farmed fish only. This is unlikely for the Sound. Hershberger et al. (1997) recorded losses of various sizes of wild fish in Case Inlet, south Puget Sound as a result of a *Heterosigma* bloom. There were many reports of dead fish during the 1990 bloom including several in Port Townsend Bay. In other prior or subsequent blooms in Puget Sound wild fish losses likely occurred too but in temperate areas, wild and farmed fish, both salmonids and marine fish, tend to sink to the bottom when killed by *Heterosigma* blooms or other causes such as a bacterial septicemia. As a result, only a few dead fish will be observed on the surface or shorelines and those sinking to the sea bottom are likely preyed on by crabs, birds or other predators. For decades it was thought that the alga did not kill fish on the east coast either, but we now know this is apparently untrue, as fish in a eutrophic estuary in Delaware have been killed by *Heterosigma* blooms.

Keppler et al. (2005) documented sublethal, adverse effects of *Heterosigma* on oyster physiology on the U.S. Atlantic coast that shows that harmfulness is not restricted to fish.

Some blooms have been found to be distributed very near the surface, others such as the one discussed herein, were found throughout the upper water column to depths of 30 m or more. Cough and Strom (2005) conducted laboratory assays to demonstrated mortality of large ciliates (zooplankton) that fed on *Heterosigma*. These are often a major component of coastal microzooplankton communities. No dissolved, active fraction was found but mortality was linked to ingestion. The cells' ability to kill zooplankton is perhaps an important element in forming and maintaining some blooms. However, in Puget Sound, all recorded blooms to date have been relatively short-lived events of just a few days to less than a week in any one area, before tides and winds push the bloom into other areas or the blooms dissipate with weather changes.

Cell counts in excess of 5×10^5 to 7×10^5 cells per liter are thought to be likely to lead to fish death, although farmers know that "toxicity" of the cells varies greatly (Rensel and Whyte 2003). It is known, however, that much lower concentrations of cells, as low as $3x\ 10^5$ cells per liter can cause mortality in some cases and is more often a problem at night when a bloom is transported through a fish farm (N. Haigh, B.C. Harmful Algae Monitoring Program, email July 10, 2006).

Cell development as evidenced by shape and size is thought to be related to degree of harmfulness, for example smaller cells, that are often irregularly shaped, are thought to be more toxic than larger cells as many farmers in British Columbia have expressed (E. Black, Canadian DFO, email, July 11, 2006). In recent Puget Sound blooms discussed herein, a significant portion of the cells were small, non-motile cells that could have been cysts or precursor of cysts. I personally have never seen these in culture, but suggest that they could be induced in the laboratory by cold shocking cultures which is what may be occurring in North Puget Sound as the blooms move from warm northern and shallow waters to relatively cool, deep channel waters near the San Juan Islands and into the Strait of Juan de Fuca.

The literature suggests that *Heterosigma* is mostly surface oriented (e.g., < 10 m depth) but experience with this bloom in 2006 and a few prior blooms in Canada shows this is not always the case. Much of the literature on *Heterosigma* comes from Japan or the U.S. east coast and is therefore not necessarily applicable to the Northwest.

Fish Farms as Possible Causes of Blooms

Production of ammonium and smaller amounts of urea by salmon farms would seem a possible link to initiation or exacerbation of *Heterosigma* blooms and may in fact be so in some other areas of the world. Certainly fish farms located in vertically stratified, poorly flushed bays or inlets with low surface nutrient levels during the growing season are prime candidates to exacerbate blooms of any microflagellate or dinoflagellate species that may be present. However, all evidence in Puget Sound is to the contrary. This topic has been raised before, for example by Taylor and Harrison (1993):

"Taylor et al. (1994) and Taylor and Horner (1994) concluded that there was no evidence for links between HABs and salmon aquaculture in B.C. waters. The sites usually chosen for salmon farming are deep and the large tidal range flushes the surface waters well."

However, the reasons are more numerous and persuasive for Puget Sound. All commercial fish farms in the Sound are required to locate in areas replete in nitrate nitrogen (SAIC 1986, Rensel Associates and PTI 1991, Washington Dept. of Natural Resource lease requirements) that are relatively well and flushed. This simply means that there is typically plenty of nitrogen available in these locations (e.g., Mackas and Harrison 1997) and other factors such as light, horizontal and vertical advection of cells or other factors are more likely to limit microalgal productivity of any kind. Measurements of nitrate during the 2006 bloom in North Puget Sound indicate about 4.0 µM during the peak of the bloom and fish mortality, as discussed below which is much less than normal concentrations, but high relative to the needs of *Heterosigma* cells.

Salmonids excrete mostly ammonium and low levels of urea (Brett and Zala 1976) and it is possible that ammonium could fuel blooms of *Heterosigma* or other algae if nitrate and other sources of N are locally depleted. I personally collected ten years of dissolved inorganic nitrogen measurements and current meter data to calculate the flux up and downstream of numerous fish farms in Puget Sound. Slightly elevated ammonium can be measured in the pens (1 or 2 µM) but by 30 m downstream the concentrations are diminished to 0.1 to 0.2 uM total ammonium in most cases but dissolved inorganic nitrogen (DIN) is about one uM , or ~ 5% of the normal background levels (Fig 1).

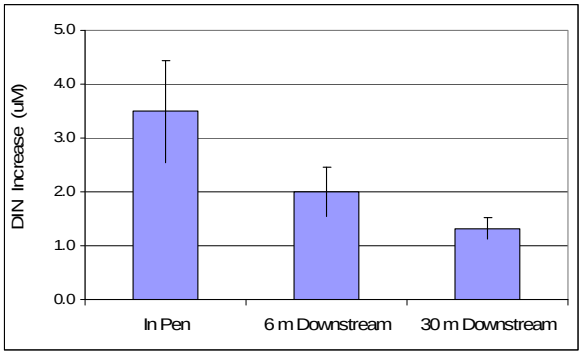


Figure 1. Mean and SD of ten separate DIN measurements at Puget Sound fish farms performed in September during the 1980s and 1990s (Rensel 1991 and unpublished manuscript).

Heterosigma had a minor, non-statistically elevated preference for ammonia over nitrate and urea in studies by Herndon and Cochlan (2007) on the US west coast. Urea and ammonium affinity was greater than that for nitrate in other studies on the US east coast (Johns and Glibert 2007). There are not extensive measurements of background N concentrations during *Heterosigma* blooms in Puget Sound except for the few I have collected or that of Connell and Jacobs (1999) and a few coincidental measurements made during routine monitoring by the Washington Department of Ecology. Thus it is possible that some localized enhancement of the blooms occurs by fish farms, but the evidence to date suggests not. Moreover, fish are not fed prior to and during blooms so as to limit oxygen demand for digestion particularly when the gills may be damaged. Their DIN excretory products are therefore diminished by a factor of ~3x when unfed and at all times they produce very little urea (e.g., Brett and Zala 1976). As blooms initiate at other locations, the nitrogen discharge of these pens is not a factor in blooms initiation. Figure 2 gives some perspective on the relative importance of fish farm nitrogen versus that from oceanic upwelling and other sources.

The nets, floats and lines that compose a net pen farm hold a rich assortment of colonizing organisms (> 100 species of invertebrates and macrophytes, Rensel and Forster 2007) and no doubt a large population of bacteria that allows for nitrification (see Rensel unpublished reports to Washington Dept. of Natural Resources 1986-1995). While increases in nitrate and ammonium levels are seen a few meters downstream of farms a few meters, the increases are very small and all the observed ambient concentrations were always well above the ½ saturation constants of uptake or growth for virtually all types of phytoplankton and in a range likely to be not limiting to *Heterosigma* .

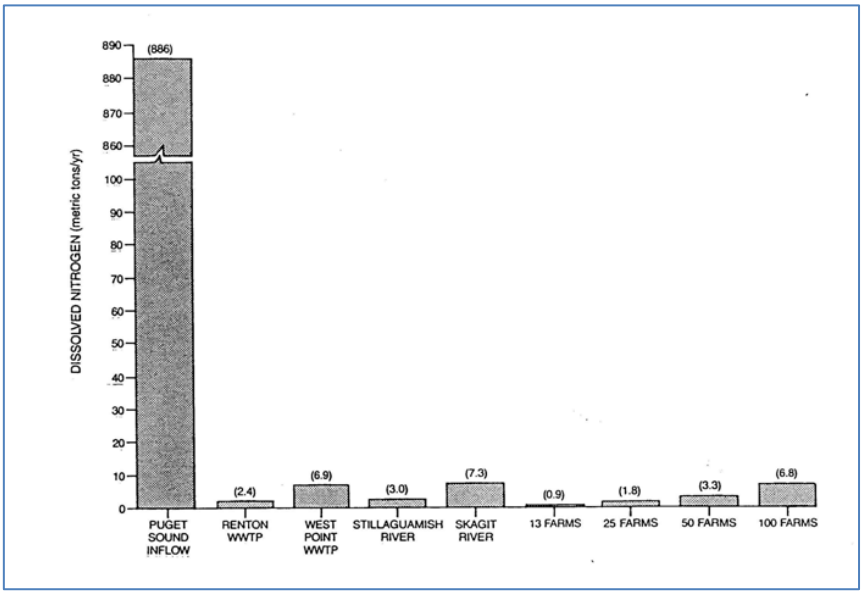


Figure 2. Calculated dissolved nitrogen loading from oceanic inflow versus selected rivers, treatment plants and different levels of fish farm development (13 to 100 farms) from WDF 1991. Presently there are eight farms in Puget Sound but two are smolt production sites with smaller fish only.

Recent experimental work by the Washington State Department of Ecology (Newton and Van Voorhis 2003) indicated that some stations in the main basin of Puget Sound were potentially nutrient sensitive at times during the spring. This was attributed to diatom-bloom-caused nitrogen drawdown of the surface waters in the selected areas. The interpretation was from a bottle experiment where 30 µM ammonium was added to seawater from a number of locations in or around the fringes of the main basin of Puget Sound. It should be noted that the level of enrichment was huge relative to any probable source and the authors of the study stated that the bottle experiments did not allow for the process of zooplankton grazing which could be a major control to phytoplankton production. It should also be noted that managers have long known that there is considerable temporal and spatial variation in nutrient sensitivity of Puget Sound and that some subareas have to be protected from all anthropogenic discharges. Also, some of the few test areas were highly vertically stratified from nearby river run off, which can create a nutrient sensitive situation that dissipates when seasonally peak river discharge declines. None of the findings repudiate the basic concept that if naturally occurring levels of DIN are above some moderate threshold (highly variable depending on algal species but in all cases < 10 µg at/L N) then no amount of additional nitrogen will lead to additional productivity by the phytoplankton in that area as long as some other important nutrient is not limiting (e.g.,

silica for diatoms, which is replete in Puget Sound). Degree of nutrient depletion of surface waters in combination with dissolved oxygen depletion have been the basis of salmon net pen "zoning" for over 20 years (e.g., SAIC 1986) and subsequent evaluations of Puget Sound nutrient sensitivity (Rensel Associates and PTI 1991 and subsequent Dept. of Ecology publications). It is arguable that a day or two of nutrient limitation in a typical river mouth area of Puget Sound with shallow surface layer would have little or no effect on fast vertically migrating flagellates like *Heterosigma,* as the alga is able to rapidly (1 m/h) migrate to obtain nutrients from deepwater even if the surface waters are nutrient depleted.

Blooms of *Heterosigma* can be treated and flocculated to the bottom with small amounts of phosphatic clay slurry (Sengco and Anderson 2004). The technique has been tried on a pilot scale at a fish farm with no apparent adverse environmental effect (Rensel and Anderson 2004). The clay causes the cells to sink and may kill them too, and since there is no persistent toxin, it is not a matter of transferring toxicity from one component of the food web (pelagic) to another (benthic). Nevertheless, the method has never been tested on a large scale during a fish-killing *Heterosigma* bloom. It is of course impractical to treat entire areas of Puget Sound with clay, the technique was envisioned for use only in the cages.

Prior Blooms, Initiation Sites and Transport

The first major *Heterosigma* bloom that killed aquaculture fish in Puget Sound was in 1989 and another bloom followed in 1990 (Rensel 1995 see appendix B). These were both major events that caused relatively large losses of farmed, net-pen fish as methods were not available to mitigate the effects of the blooms. At the time local scientists were relatively poorly prepared or funded to document such large blooms. Some hydrographic data were collected but much of the evidence was anecdotal such as visual observations from floatplanes that I made with State Fish and Wildlife staff or the verbal communications from fish farm staff.

During the 1990 bloom dead cutthroat trout and other wild fish were reported to the State agencies from several areas of central Puget Sound (Fig. 3) including Port Townsend Bay. No fish tissue samples were collected or analyzed except for gill histology samples I collected rapidly from moribund fish at the central Puget Sound sites.

No major blooms occurred in the following interim until the present except the localized 1997 bloom in CPS discussed herein and reported by Connell and Jacobs (1999) and the year 2000 bloom in Hood Canal where no fish farms exist (Connell et al. 2000). Many other *Heterosigma* blooms undoubtedly have occurred in Puget Sound in disparate locations or before humans started tracking such occurrences.

Some misunderstanding of bloom origination and cause has surrounded *Heterosigma* blooms in Puget Sound and probably elsewhere. As farmed fish have sometimes been killed by these events in Puget Sound or elsewhere, some have assumed that bloom commencement or embellishment must be linked with fish farms (e.g., GEOHAB 2006). Having worked with these blooms and the fish farming industry in Puget Sound for nearly 20 years, interested scientists and local fish farmers know with certainty that blooms originate in areas remote from the fish farm sites and are advected by wind and tidally driven currents into farm sites. Fish farms are the stakeholders most interested in harmful algae in many areas as they are at risk and the fact that blooms are discovered by fish farmers relates to their routine monitoring programs. Evidence from the most recent blooms is presented below and once again confirms these findings as does the literature mentioned above.

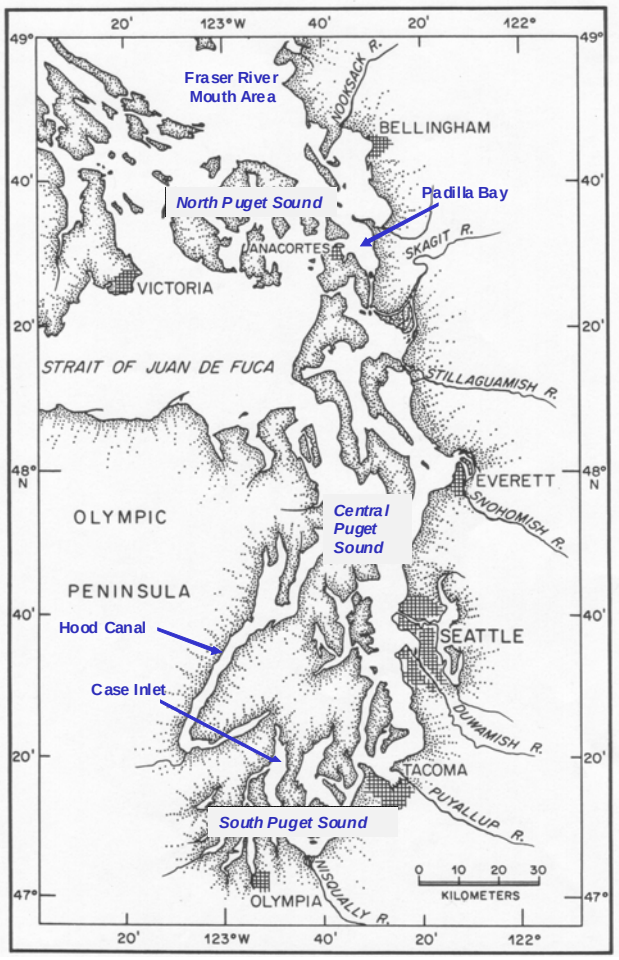


Figure 3. Vicinity map of Puget Sound showing subareas and place names referred to in text.

Heterosigma blooms in Puget Sound may be divided into two spatial areas that often have differing temporal patterns and causal factors: North Puget Sound (i.e,. inland marine waters of Western Washington north of Admiralty Inlet including the Strait of Juan de Fuca) and Central Puget Sound (i.e., inland marine waters of Western Washington south of the Strait of Juan de Fuca including areas from Admiralty Inlet to the Tacoma Narrows.

North Puget Sound

The primary factor associated with North Puget Sound blooms is the Fraser River plume which is most extensive in late spring and early summer when the mountain snowpack is most rapidly melting. The Fraser River is by far the largest river entering the coastal waterways of Puget Sound and the Strait of Georgia and has a pronounced effect on the physics and biology of the region as documented (see Thomson 1981 for an overview). The Fraser River plume initiates near the river mouth and flows into the Southern Strait of Georgia, marked as "probable bloom initiation area 1" in Figure 4.

As it flows out of the river mouth it partially mixes with seawater and forms a stable brackish surface layer that allows a very productive phytoplankton production area that otherwise might be mixed out of the photic zone into deeper waters. During fair weather, the plume is advected southward with the fair-weather north winds. Most of the flow eventually flows out through the Strait of Juan de Fuca to the Pacific Ocean fueling a classical estuarine circulation.

While I believe the most likely *Heterosigma* bloom initiation area for North Puget Sound blooms is in soft sediments of areas near the Fraser River mouth (labeled bloom initiation area 1 in Fig. 4), I cannot exclude other potential areas to the south such as Bellingham Bay to Padilla Bay (labeled as bloom origination area 2 in Fig. 4) where *Heterosigma* blooms are sometimes prevalent.

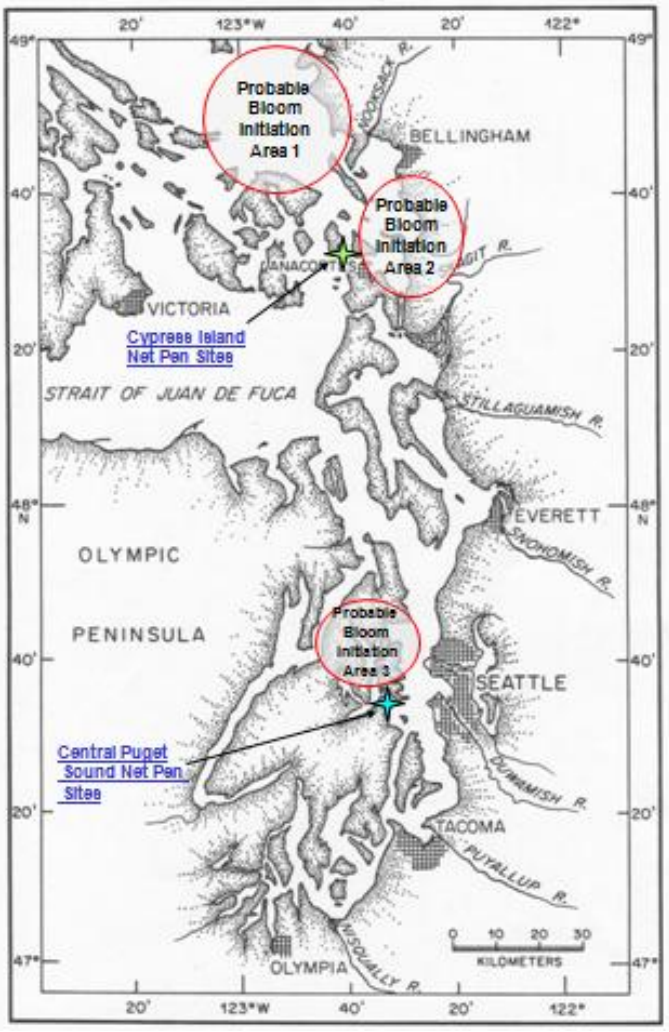


Figure 4. Vicinity map of probable or possible bloom initiation areas for *Heterosigma* in North and Central Puget Sound in relation to clusters of fish farms. (Port Angeles Harbor and north Skagit Bay net pens not shown).

Without more observation and research, it is difficult to differentiate between these two areas and it is academic to fish farmers who are more concerned with waters nearer to the North Puget Sound fish farming sites. The innermost waters of East Sound, a sheltered and isolated bay at Orcas Island in the San Juan Islands, appear to have early summer blooms of *Heterosigma* on an annual basis. But it is unlikely that a bloom could emerge intact due to the strong tidal mixing in surrounding channels and Straits and the very long entry way to the bay. Individual cells from there could, however, survive transport out to other areas to possibly initiate a bloom but the aerial observations suggest that it is not a likely source of blooms in north Puget Sound where blooms appear to move down large, relatively less-well-mixed channels such as Rosario Strait from the U.S.-Canada border.

Fish farmers routinely sample and conduct aerial surveys of waters of north Puget Sound, central Puget Sound and areas near Port Angeles in May or early June particularly if the weather is very warm and the Fraser River flow is large. They prepare maps of estimated bloom distribution based on visual observations from planes. *Heterosigma* blooms have a characteristic purplish-red coloration that is distinctively obvious to an experienced observer and as discussed herein, many blooms are nearly monospecific which makes visual detection from airplanes easier. The aerial observations are then ground truthed with water samples for cell counts using a microscope if the risks appear moderate or high and as blooms approach a fish farming area.

Central Puget Sound

A similar set of circumstances is involved in controlling blooms of *Heterosigma* in central Puget Sound. In this area the three fish farms are clustered on the west side of Puget Sound west of Seattle and south of Bainbridge Island (Fig. 4). This area has no rivers and only a few small streams so freshwater runoff and subsequent surface vertical stratification is typically not experienced to the degree seen in North Puget Sound or even other parts of central Puget Sound. It does, however, experience relatively strong vertical stratification later in the summer when the weather is unusually hot. At such times, surface waters of the shallow backwater bays and inlets experience temperatures in excess of 18°C.

Nearly two decades of monitoring in the backwater to the west of these farms in areas such as Port Orchard, Sinclair Inlet and all marine waters on the west side of Bainbridge Island indicate that blooms in central Puget Sound originate there. These areas include relatively calm, sheltered areas with mud and silt bottoms suitable as cyst beds and initial bloom development. Shallow bays on the east side of Bainbridge Island especially Blakely Harbor are also apparent bloom initiation areas as they are often the first areas to be observed with bloom conditions and have silty mud bottoms suitable as cyst beds for the alga. Conversely, the fish farm areas discussed above have mostly fine sand to cobble bottom sediments, so it is unlikely that cysts are either deposited or survive for any time period beneath or adjacent to the farm sites. Initial blooms are never seen adjacent to the fish farms at any location in Puget Sound and in all cases appear to be advected by currents, winds and circulation patterns into the fish farm areas as discussed in the next section.

June 2006 Bloom Chronology of Events

The late June 2006 and early July *Heterosigma* bloom was monitored by American Gold Seafoods staff and myself and is the principal focus of this report. I assisted by collecting CTD casts throughout areas near Cypress Island, performing cell counts and performing related tasks. Kevin Bright of American Gold Seafoods prepared a chronology of events that was modified and presented below. I summarize this information in brief as follows:

North Puget Sound and Cypress Island

Increasing cell counts of *Heterosigma* at the Cypress Island fish farms were noted beginning on Sunday June 25th which invoked a high risk level of awareness and response. By June 28th significant levels of fish morality were occurring at the Cypress Island fish farm

sites. Aerial flights and ground surveys were mounted on June 26th and all available evidence suggests that this bloom, as was the case for prior blooms in North Puget Sound, was advected into the fish farm area from areas much further to the north or from the shallow bays to the east (Bellingham, Samish, Padilla Bays). Figure 5 is the record of one of the aerial surveys, showing a survey radius with the focus of the survey circle being the fish farm locations in Deepwater Bay. The brackish-water Fraser River plume is known to be the means by which the cells are maintained in surface waters of this area and the density differences act to reduce mixing out of the surface and photic zones. Tides and winds act together to move the cells southward toward the Strait of Juan de Fuca and the Pacific Ocean (Figs. 6, 7).

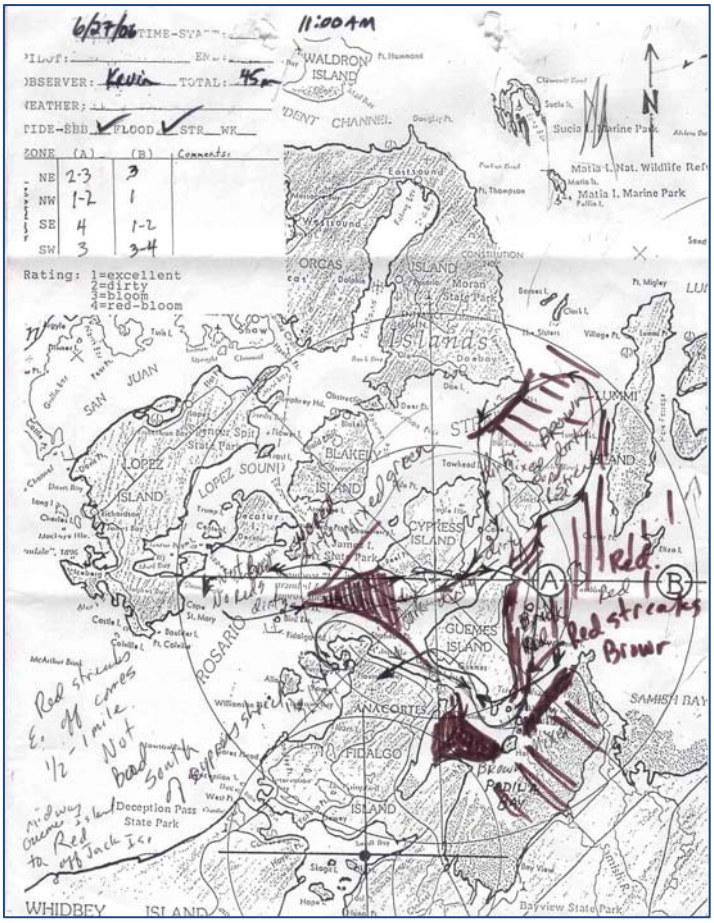


Figure 5. Aerial survey results of June 27th 2006 conducted by Kevin Bright, AGS Biologist. Note that the survey was limited to the areas within the survey circle and part of the Eastern Strait of Juan de Fuca, i.e., no observations were recorded from further North.

Because of time and hired airplane costs, surveys are not conducted all over the North Puget Sound, but in the past the general trends shown in Figures 6 and 7 are known to be more or less accurate.

Samples taken from shallow bays and passages some distance from Cypress Island indicated peak cell counts of 3 to 4 million cells/L which is 3 or 4 times the highest values seen at the fish farm at any time during this bloom. The evidence also includes maps of bloom distribution prepared by an experienced observer (K. Bright) who flew in a small plane over the area periodically during the pertinent time period. *Heterosigma* blooms in the Pacific Northwest exhibit a characteristic color that can be differentiated from blooms of other algae. This has been repeatedly verified by coupling aerial surveys with on the water collection and analysis of samples. Spread of the bloom from north to south and possibly east to west is the typical scenario that has often been seen in the past in this area as previously reported. Cell counts varied as shown in Appendix A but by Friday evening, June 30th there appeared to be a decline in cell counts and by Sunday, July 2nd the farm site and

adjacent usual "hot spots" were experiencing much lower numbers of cells. As in the past, this bloom was an intense but short lived event, lasting approximately a week throughout the NPS region. Tides and circulation in this area are very intensive and the blooms are clearly subject to rapid transport and some degree of vertical mixing despite the river-induced vertical stratification.

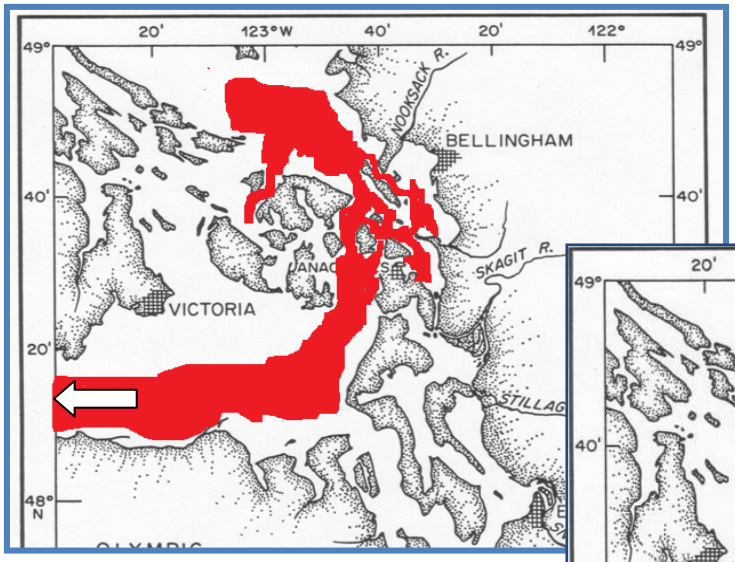


Figure 6. General vicinity of the bloom in the early stages in late June 2006 in red, inferred from survey vessel and aerial survey maps.

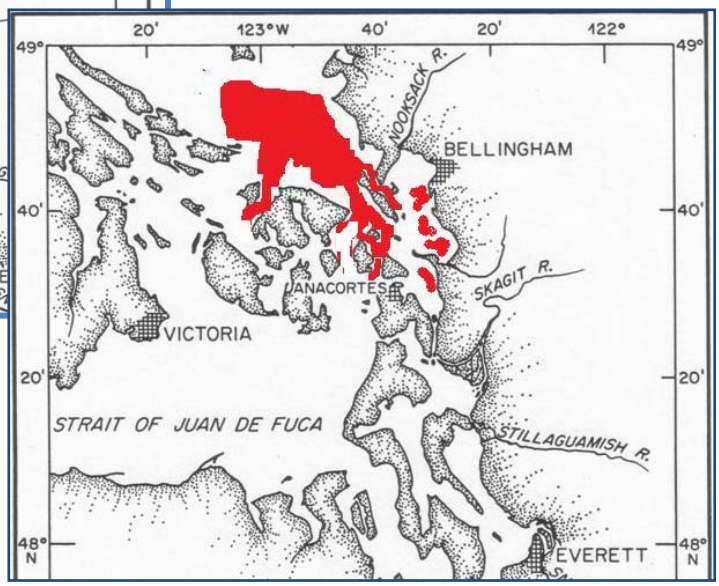


Figure 7. General vicinity of the bloom during later stages as it flowed with surface waters out the Strait of Juan de Fuca and into the Pacific Ocean.

During this bloom samples collected near Cypress Island showed a distinct bimodal size distribution of *Heterosigma* cells. Relatively large numbers of small cells (i.e., ~ 10 um or less) were mixed with larger, more typical cells (i.e., ~ 20 to 25 um). An ocular micrometer was not available at the time so these sizes represent estimates only, but clearly there were two size classes. Typically *Heterosigma* cells seen in PNW waters are motile and relatively similar in size and shape as discussed herein. British Columbia growers have suggested that the smaller cells are more often associated with increased rates of fish mortality. The reason for this is unknown. Phytoplankton including other microflagellates, dinoflagellates and some diatoms that produce toxins do so under a variety of growth stages and environmental stressors, so it is not possible to generalize. Deficiency or abundance of nutrients (nitrogen or phosphorus or micronutrients) is sometimes a factor in toxin production of some other fish-killing species. It may just be a coincidence, but Washington Dept. of Ecology surface water data from Admiralty Inlet was depleted of nitrate and other forms of DIN to below detection limits a week prior to the truly massive 1990 bloom (Admiralty Inlet station). That area is usually rich with DIN and *Heterosigma* is a strong

vertical migrating alga that can prosper when many other phytoplankton especially diatoms are unable to survive. A caution with the use of this observation because the Department of Ecology's DIN measurements at that time may have been influenced by a relatively high detection limit. This came to light after our comment in Hershberger et al. (1997) about no detectable nitrogen occurring in the (surface) water of the area during a large, prior bloom (1990 CPS bloom).

Figure 8. Deepwater Bay at Cypress Island, actually more of a bight than a bay, is subject to very strong tidal currents and has large natural populations of invertebrates, fish and seabirds (Rensel and Forster 2007).

During this bloom dead fish from natural populations were reported in small numbers from North of Cypress Island to shoreline areas as far west as Freshwater Bay in the Strait of Juan de Fuca (SoundHAB list serve entries). It is worth repeating that dead marine and salmonid fishes typically sink in our temperate marine waters, which makes observation of effects very difficult and typically only a few dead fish are observed in these blooms. Dead fish will rapidly be preyed on by scavengers such as crabs and certain fishes and dispersed by tides and currents therefore not always notice on the surface.

Port Angeles Harbor, Western Strait of Juan de Fuca and Pacific Ocean

A few cells first appeared on Thursday, June 29th and by Sunday July 2nd cell counts were high and fish were dying. The timing was just a few days later than the North Puget Sound bloom and there was a noticeable bloom on the U.S. side of the Strait during this time period too. By July 5th cell counts began diminishing concurrent with a weak low pressure weather front movement into the area. The following day the weather continued to be less suitable for bloom maintenance with greatly reduced air temperature, clouds and thunderstorms. Cell counts at 1 to 10 m were low but at 35 m fairly high concentrations (470,000 cells per liter) remained. The remaining cells soon dissipated. It is of interest to note that blooms of *Heterosigma* were spotted off the coast of Washington shortly after this time period, by Brian Bill of NOAA-NWFSC who collected samples. The sequence of timing suggests that the bloom was an extension of the initial North Puget Sound bloom, but it is difficult to prove that exactly.

Other Areas

The actual timing and extent of the 2006 or 2007 blooms in other areas remote from the fish farms is not fully known except for other observations cited herein and from some

general observations from airplane flights in the areas nearer the farms or on transit lines between the subareas as one plane did all the observations. These maps are retained and may actually be a valuable research tool for those interested in the timing and spread of the blooms. For example, satellite imagery from similar time periods could be compared to the maps to begin testing the use of such images for management and risk assessment.

Factors Associated with Late June 2006 *Heterosigma* Bloom

Here I review some of the conditions that preceded and accompanied the bloom late June-July 2006. This is done to add to the body of knowledge regarding conditions that may initiate or sustain blooms, as they occur relatively rarely and without recording the data there is no means to modify conceptual or numerical models for bloom prediction in the future. The sites initially at risk in the 2006 bloom were Sites 1, 2 and 3 at Deepwater Bay at Cypress Island in North Puget Sound near the San Juan Islands.

Snowpack and River Discharge

The late June *Heterosigma* bloom commenced in Northern Puget Sound associated with the major freshwater source for the entire area, the Fraser River plume. River discharge of this very large river peaks this time of year and has a profound influence on surface and near-surface water quality and algal growing conditions throughout much of North Puget Sound and even the Strait of Juan de Fuca. In previous Puget Sound *Heterosigma* blooms where water quality profiles were collected a reduced salinity surface layer coincided with or persisted through the bloom (Rensel 1995, Connell et al. 1997). For other blooms, no data are readily available.

Although snow pack from the winter of 2005-2006 was among the largest in several decades, river discharge was below median long term historical levels during the later periods of June 2006 (Fig. 9) having peaked a bit earlier in the month and in late May and at a level exceeding the maximum median discharge.

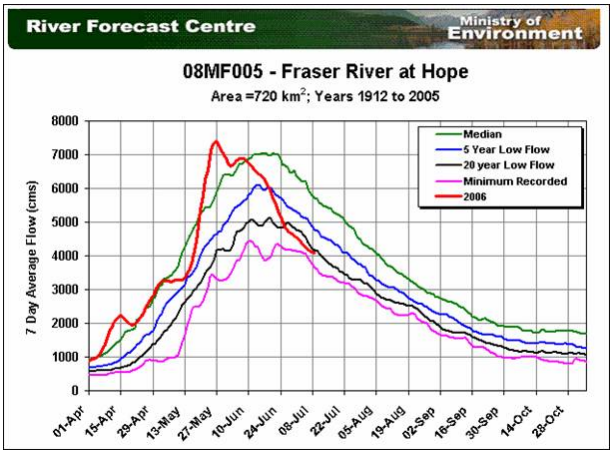


Figure 9. Recent discharge flow data from the Fraser River at Hope, B.C. versus 2006 flow in red.

However, freshwater does not flow out of North Puget Sound into the Strait in a linear fashion, but rather pools up during larger tides and pulses out during neap tidal series, as discussed in the next paragraph**.** Figure 9 indicates that river discharge is typically greater in late spring and early summer, peaking near June 15th and 2006 was an exception with a much earlier run off peak. As discussed

below, we measured relatively low salinity to relatively great depth over broad areas of North Puget Sound that indicates the effects of rivers were pronounced despite the timing records of total discharge.

Data from a concurrent University of Washington PRISM research cruise (J. Newton, UW APL unpublished data) in late June suggests that the Eastern Strait of Juan de Fuca was not experiencing a significant reduction of surface salinity which is evidence that the Fraser River water may have been "pooling" near the Lummi Island to North Cypress Island areas and associated backwaters. So either the river plume waters had not reached those areas at that point or mixing was sufficient to attenuate the signal.

Estuary to ocean exchange in South Georgia Strait, north Puget Sound and the Strait is modulated by tidal mixing and wind forcing that increases export of freshwater during neap versus spring tidal periods (Griffin and LeBlond 1990). Monthly or bimonthly pulses of relatively warm fresh water have been documented traveling seaward from the western entry of the Strait of Juan de Fuca (Hickey et al. 1991). This is the opposite of what many would think, but the relationship is well established. Rensel et al. (2007) reported a mid Strait of Juan de Fuca cold water anomaly that persist throughout most of the year that may or may not have been related to this circulation pattern as it persisted in all tidal phases (suggesting it was not related) but measurably increased during spring tides (when freshwater export was reduced, hence vertical mixing possibly enhanced due to decreased density differences).

Water Temperature

Elevated water temperature has generally been associated with some dinoflagellate and flagellate blooms in Puget Sound. In the past literature and grey literature from our area and elsewhere notes that temperatures of ~15°C are associated either with cyst germination or the growth and spread of vegetative (live, normal) *Heterosigma* cells. However, in the PNW these conclusions were largely based on observations during blooms and not experimental evidence or monitoring of cyst beds. Studies of growth of *Heterosigma* in the laboratory show that maximum growth occurs at 25°C but toxicity was very low (Ono et al. 2000). The same workers found the highest toxicity at 20°C, a temperature that is encountered in back bays and shallows of Puget Sound but is never encountered or even approached at Cypress Island at the net pen site in Deepwater Bay.

Surface to near surface water temperatures during the June-July 2006 bloom at the Cypress Island net pen site were in the range of about 11.5 to 13.5°C but further north with an increase in vertically-stratification, temperatures were consistently nearer to 13.8°C both in my vertical profiles and data records collected by Kevin Bright. We can only speculate about water temperatures further north in the U.S.-Canada border areas, but most likely it was warmer there as typically seen in SST images in summer. In summary, water temperatures were suboptimal for growth but did not prevent harmful effects at the fish farm site during this bloom. Reduced water temperature at the fish farm sites could be causing cyst germination which in turn could have some effect on toxicity to fish.

Air Temperature

June weather in Western Washington is historically relatively cool and cloudy, with occasional showers while in contrast, July is often hot and sunny. In 2006 prior to and during to the fish kill in June we experienced unusually sunny days with temperature in the 80 to 90°F range. Figures 10, 11 and 12 are air temperature records for Seattle-Tacoma Airport (central Puget Sound), Bellingham Airport (North Puget Sound) and Port Angeles Airport (Strait of Juan de Fuca) during the subject period. Note that high and low air temperatures exceeded long term period of record average highs and lows for each hourly observation. This was particularly true for Seattle Tacoma airport and for Bellingham Airport where temperatures exceed the daily averages by up to 20°F but less so for Port Angeles.

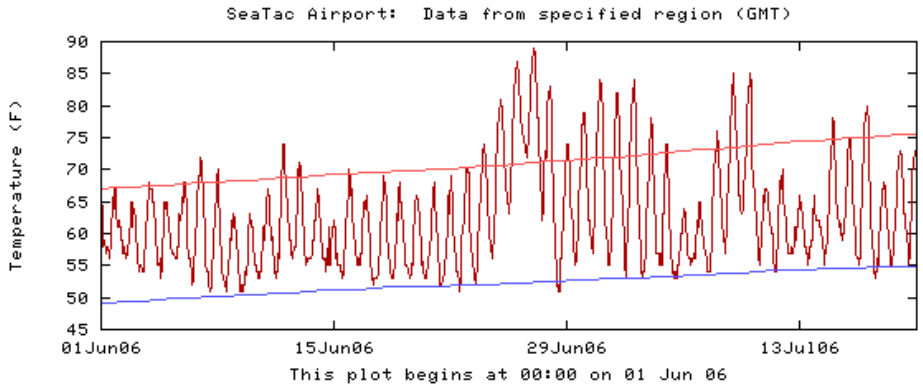


Figure 10. Mean high and low air temperatures compared to hourly air temperatures at Seattle-Tacoma Airport during June and early July 2006.

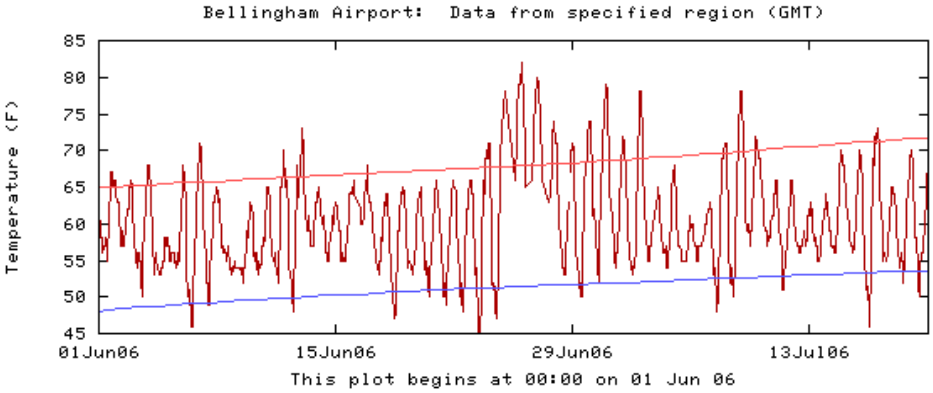


Figure 11. Mean high and low air temperatures compared to hourly air temperatures at Bellingham airport during June and early July 2006.

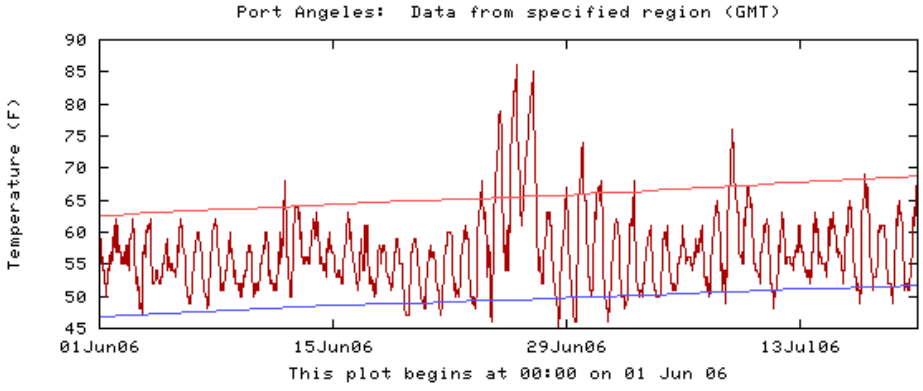


Figure 12. Mean high and low air temperatures compared to hourly air emperatures at Port Angeles Airport during June and early July 2006.

Precipitation far exceeded normal levels in early June 2006 as shown in Figure 13 but the effects of this on bloom formation and spread are unknown and not likely to be pronounced compared to the Fraser River discharge. Although it could have influenced conditions in bays that had *Heterosigma* cysts emerging at that time from soft sediments.

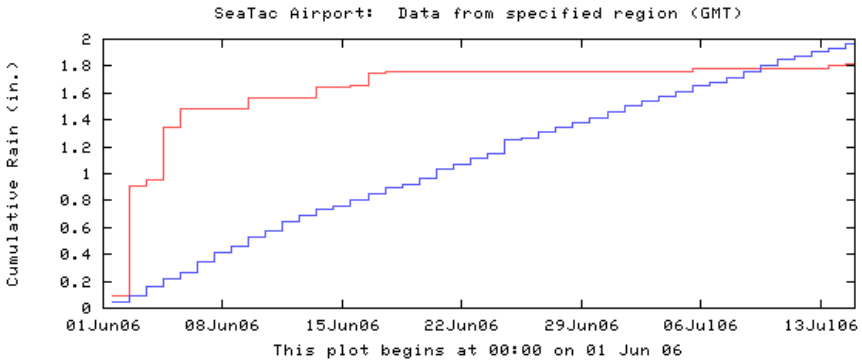


Figure 13. Mean high daily precipitation for period of record compared to hourly precipitation at Seattle-Tacoma Airport during the subject period.

Tides

In the past in Western Washington the onset of *Heterosigma* blooms or even increased background concentrations of cells has often coincided with neap (minimal) tidal exchanges. During the June 2006 bloom the bloom was killing farmed fish at Cypress Island during a period of declining tidal amplitude (Fig. 14). However, the bloom appeared to have formed in areas further to the north including the southern Strait of Georgia during the prior minus tide series.

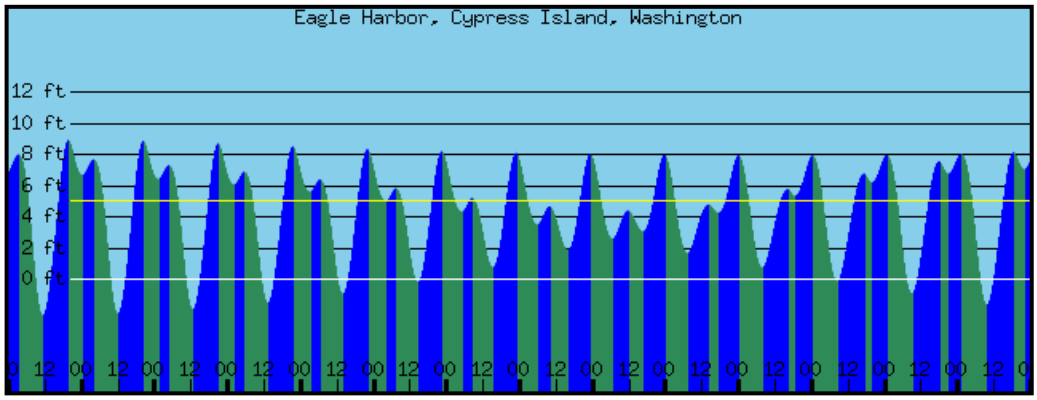


Figure 14. Eagle Harbor, Cypress Island tidal plots from June 25th to July 8th, 2006.

Horizontal lines mark mean sea level and zero datum (mean lower low water).

As bulk transport of brackish surface water tends to exit North Puget Sound and the Strait during neap periods, tidal amplitude may have as much to do with allowing cells to congregate near the surface as well as be advected south and east out of the two areas, respectively. Whatever the relationship, fish farmers use decreasing tidal amplitude as one measure of increased bloom risk and it has proved to be useful.

It is probable that the effects of river discharge in creating a stable surface layer for *Heterosigma* outweighed the likely bloom-deterring effects of large tidal exchange prior to late June 2006. When the bloom was approaching the fish farming area, the tides were tending toward neap exchanges that would have allowed the bloom to escape some of the effects of tidal mixing that would occur during spring tides. We know this to be true as the salinity probe on the CTD (multiprobe) I used to take vertical profiles of water quality showed a relatively deep, brackish surface layer throughout much of the area surveyed near Cypress Island. Additionally, *Heterosigma* has an amazing attraction to tiny amounts of fresh water when added to tall laboratory columns (Hershberger et al. 1997), which is an important clue to the understanding of its vertical migratory behavior.

Winds

No regional or subarea-specific wind data were acquired for the preparation of this report. In general, winds were typical of what is usually seen for the Cypress Island area in June, with some afternoon breezes that in some cases produced whitecap conditions in open areas such as the Clark Point of north Guemes Island.

In the eastern Strait of Juan de Fuca and Port Angeles Harbor, however, winds are typically strong in the afternoon in spring and summer fueled by the land breeze phenomenon. Yet during this *Heterosigma* bloom they were atypically strong and persistent. Estimated wind speeds of 20 to 30 mph from the west[1] occurred during the bloom period and unlike the normal conditions in the Strait in spring, did not diminish in the evening but continued through the night hours. The fact that the bloom continued during such strong and persistent wind conditions was remarkable as many microflagellate or dinoflagellate blooms are often linked to calm, warm conditions. It is possible that the upper water column by that time (several days after the PRISM cruise data was collected) were sufficiently brackish to restrict mixing to depth by the winds but limited antecedent water quality data, discussed below, suggests that was not the case.

Time of Year

Occurrence of *Heterosigma* vegetative cells in the water column is known to be most prevalent biannually in the summer (Tomas 1980) and during the late June or early July period and again in mid September in decades of sampling conducted in Narraganset Bay in Rhode Island (Li and Smayda 2000). There is no systematic sampling and recording of phytoplankton occurrence in Puget Sound and approaches but from my own observations I believe that blooms do indeed occur more regularly at those times. Dr. Max Taylor at the University of British Columbia observed late spring blooms of *Heterosigma* in English Bay near Vancouver, B.C. most every year for several decades. The most severe blooms in the

[1] Westerly winds near Port Angeles were observed but wind direction is remarkably divergent in the Strait during spring, with a divergence in direction often occurring near Port Angeles. Easterly winds in the outer Strait would have enhanced bloom transport to the ocean.

Puget Sound in the past have always been near the 1st of July or in mid September[2]. The most likely forcing factor is water temperature that drives cyst germination, but other co-factors may contribute. It is reasonable to assume that the year after a basin-wide or larger *Heterosigma* bloom there would be many more viable cysts, which increases the likelihood of a bloom in sequential years. This is what we have seen in Puget Sound in 1989-1990 and 2006-2007. Thus there is an increased risk of blooms in the area in 2008, but as in all years, the physical forcing of vertical stratification, water temperature, tidal amplitude and hot weather periods dictate if the cells can survive and bloom.

Water Quality Sampling

Principal measurement of interest included chlorophyll *a* (a measure of phytoplankton density via pigment concentration), water temperature, salinity, dissolved oxygen, dissolved inorganic nitrogen and turbidity. In my work, many of the profiles I collected were accompanied by collection of cell counts. A Hydrolab 4a multiprobe with a variety of probes and integrated Turner SCUFA *in vivo* chlorophyll sensor with 100 m cable on a continuous reel was used to record vertical profiles at a number of locations around Cypress Island and approaches on June 28th, 29th and 30th. Calibration of the Hydrolab was conducted for all the available probes as per previously cited protocols and the chlorophyll sensor was separately calibrated by concurrently measuring and collecting water samples with a "Scotty" 2L water bottle sampler for laboratory extraction and analysis using standard protocols. Water from different depths with varying chlorophyll *a* readings that spanned the range from low (about 2 ug/L) to high (about 15 ug/L) was used and a correction factor was derived with very low (0.1 ug/L) standard deviation from the mean.

Figures 15 to 18 show the distribution of chlorophyll a measurements relative to depth. Normally we would expect a general trend of more cells near the surface, particularly during the daylight hours when these measurements were collected. The farm site (Figure 15) however shows no linear trend line (the dashed line) because algal biomass was more or less equally distributed at all measured depths.

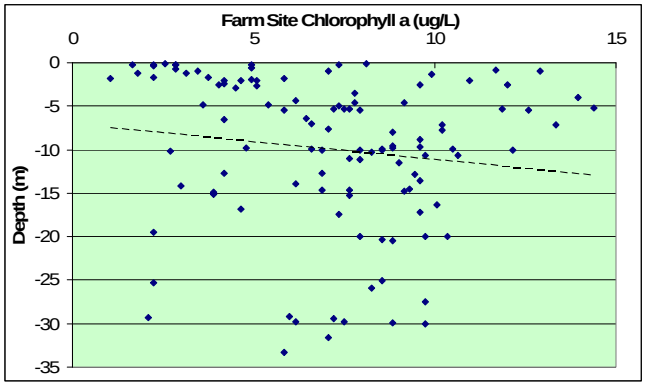


Figure 15. Algal density versus depth from vertical profiles at the farm site in Deepwater Bay.

[2] A *Heterosigma* bloom occurred in early May 2007 in north Puget Sound during an especially warm period but after killing just a few fish at the Cypress Island site it dissipated. As usual, most of the bloom was concentrated in areas north of Cypress Island and not adjacent to the fish farms.

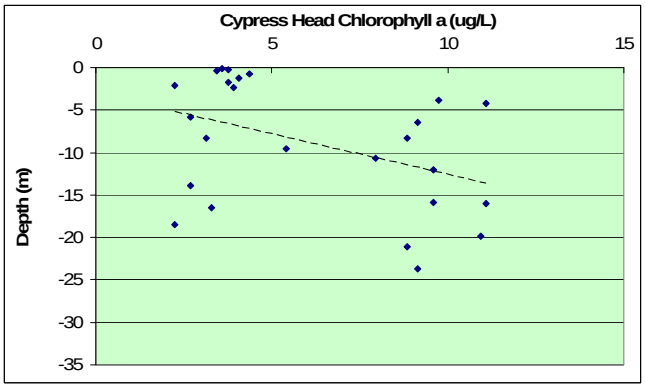


Figure 16. Algal density versus depth from vertical profiles near Cypress Head about 2 m north of the farm site in a well mixed area.

North of the farm site in Bellingham Channel (Fig. 16), the few available data also show the unexpected general increase in algal density with increasing depth. South of the farm site at the entry to Bellingham Channel (Fig. 17), a more clear trend of declining chlorophyll concentration with depth, similar to the far north end of the channel (Fig. 18).

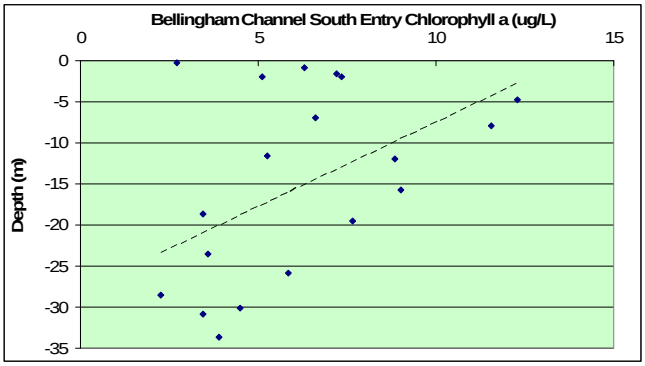


Figure 17. Algal density versus depth from vertical profiles about 2 km south of the farm site near the entry to Bellingham Channel and nearer Anacortes, Washington.

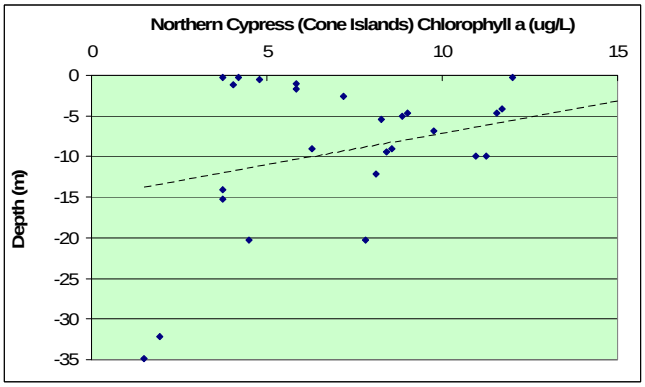


Figure 18. Algal density versus depth for vertical profiles near the north end of Bellingham Channel about 10 km north of the farm site.

NPS surface waters had reduced salinity from the normal 28.5 to ~26 psu or less and the mixed layer extended to relatively great depths of 30 to 50 m or more in many areas. For comparison, full strength seawater in the North Pacific near Washington State is ~32 psu or more. Interestingly, there is evidence that the optimum salinity for *Heterosigma* growth is ~ 25 psu (Haque and Onoue 2002) or very near the salinity observed in areas just north of Cypress Island during the June-July 2006 bloom. Of course *Heterosigma* grows faster than this in warmer waters (> 1 d^{-1}), but how the growth relates to harmfulness to fish is unknown.

An example of the range of salinity recorded during the bloom is shown in Figure 19. Note the reduced surface salinity to about 15m depth from the station near the north end of Cypress Island. This same water ebbs through Bellingham Passage to the farm site but is mixed by turbulence when flowing around headlands and over irregular bathymetry. A relatively large concentration of *Heterosigma* cells was found in this sample (both from in vivo fluorescence and cell counts) at 5 m depth especially. Note too how salinity changed within a few hours at fish farm Site 1 from 27.2 to 28 psu. This illustrates the dynamic nature of these waters and the patchiness of conditions that make it difficult to generalize.

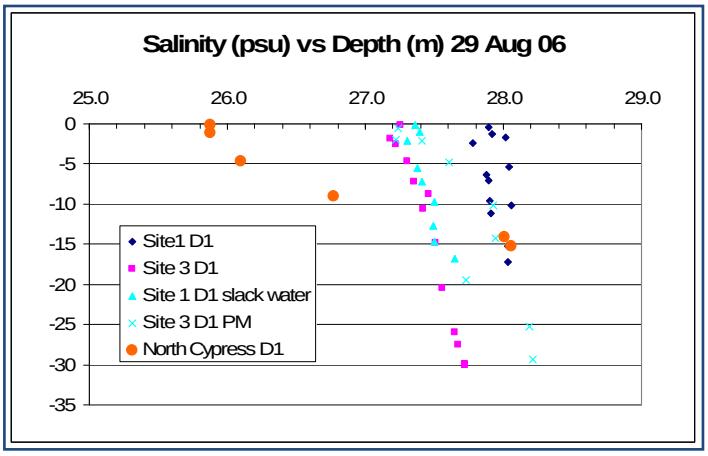


Figure 19. Salinity versus depth for 29 June 2006 near Cypress Island at fish farm sites 1 and 3 (both in Deepwater Bay) compared to conditions near the north end of the island by the Cone Islands.

Some nutrient data were collected, including nitrogen samples during the peak of fish mortality in Deepwater Bay at Site 3. Unfortunately, the laboratory that analyzed the data did not conduct nitrite or ammonium analysis, but the nitrate results are of interest. Figure 20 shows these data compared to chlorophyll *a* and water temperature profiles. For reference, the ½ saturation constant for *Heterosigma* growth in saturating light conditions is between ~ 1 and 2 uM for nitrate or ammonium and there apparently is a slight preference for the latter (Herndon and Cochlan 2007, Johns and Glibert 2007).

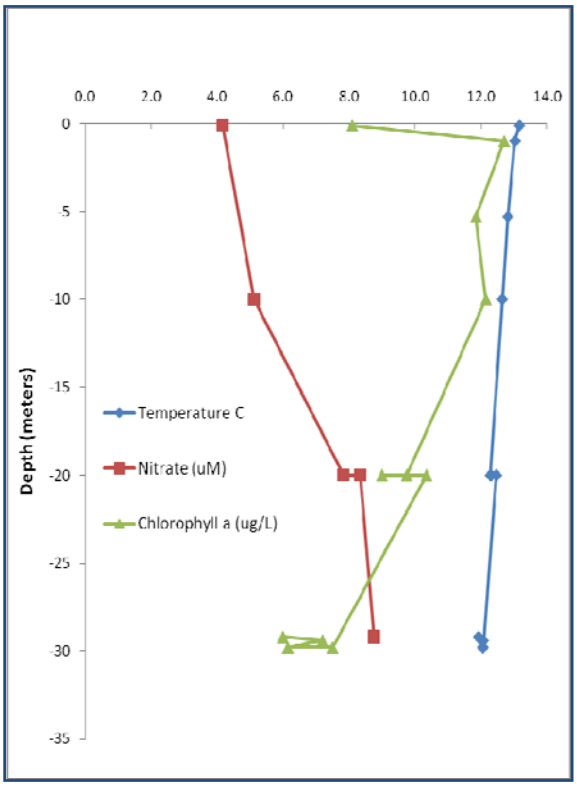
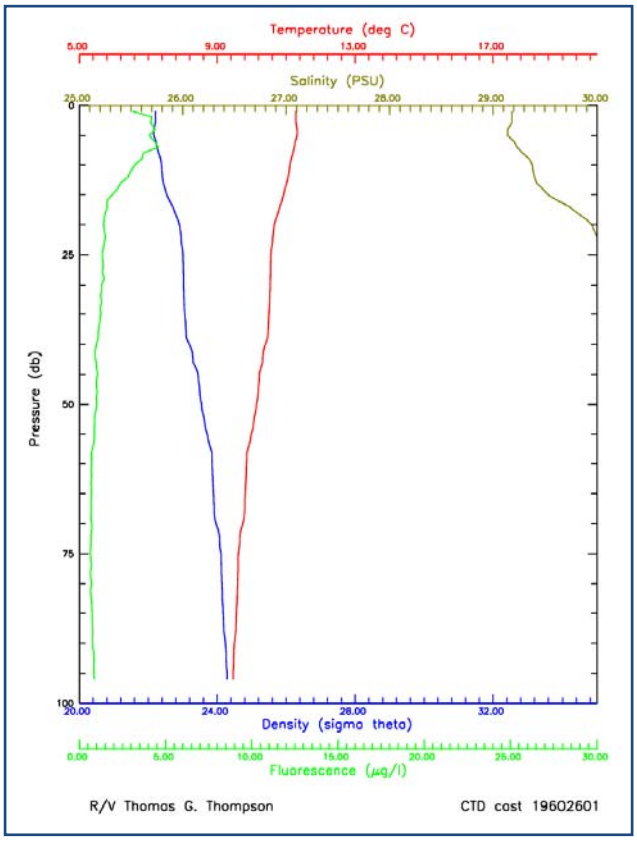


Figure 20. Nitrate, *in vivo* chlorophyll *a*, and water temperature for Deepwater Bay on the west side of net pen Site 3 on 29 June 2006.

Thus *Heterosigma* cells at the peak of the fish kill were subject to over twice the calculated ½ saturation rate concentration, which means they may not have been growth

limited (if there was some ammonium or urea available) or very marginally growth limited by N supply (if not). Note as discussed in this report, these levels are much lower than normal for the fish farm sites which is a short term product of the Fraser River plume that was dominant throughout the area at this time. As an aside, in Figure 20 note relatively high algal abundance with only modest decline to depth of 30m.

Data from a University of Washington cruise of the oceanography vessel RV Thompson at Station 26 (just south of Deception Pass in the far eastern Strait of Juan de Fuca (herein "the Strait") is shown in Figure 21 from the early morning of June 28, 2006. These data indicate relatively cool waters and the presence of a surface layer of reduced salinity waters. Also the presence of a modest chlorophyll signal in the top 15 meter of water of ~4 ug/L was noted but these are not bloom quantities typical of *Heterosigma*. It is impossible to say, of course, what species those alga(e) might have been but conditions were generally suitable for *Heterosigma* survival, although significantly cooler than is preferred. Observations from fixed wing aircraft confirmed the presence of the bloom in U.S. waters of the eastern Strait but nearer Port Angeles. No surveys were recorded on the Canadian side of the Strait that I am aware of during this bloom.



These data and many others from the PRISM cruise (not shown here for brevity, Fig. 22) as well as aerial surveys in the central Strait, observations in and near Port Angeles Harbor and the central Strait by HAB technicians working for the fish farm suggest that the bloom had not begun moving into the Strait of Juan de Fuca or the Pacific Ocean at this time but that some cells were present, as previously discussed.

Figure 21. Vertical profiles of salinity, chlorophyll, temperature and density from station P26 (see Fig. 22) the eastern Strait of Juan de Fuca, west of Whidbey Island and south of Deception pass from the morning of June 28, 2006.

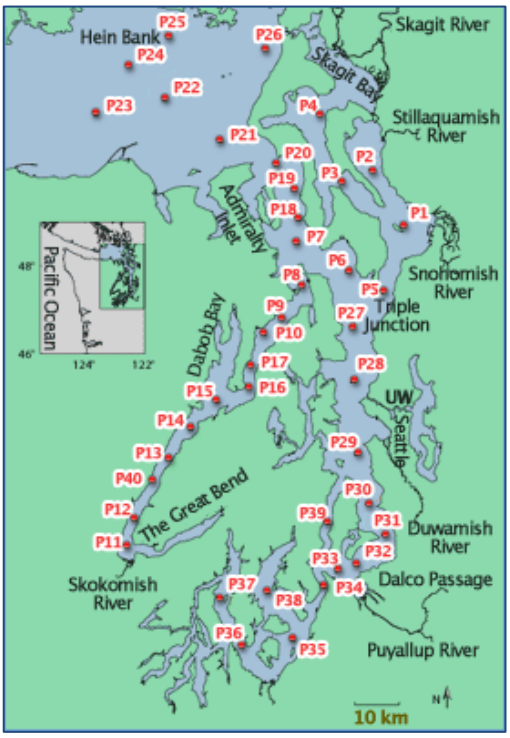


Figure 22. University of Washington research vessel sampling locations. Data from stations P21 though P26 from June 28th, 2006 were inspected for this review.

4It is possible that Heterosigma cells were being transported through the eastern Strait in late June, but tidal and wind mixing may have reduced their concentrations in vertical mixing. I believe this is entirely plausible as there were persistent winds of relatively high velocity during this time period. At the time we were greatly impressed that the bloom managed to continue under such strong winds, but no data except aerial observations were collected from the Strait during the peak of the bloom (~July 2nd) to quantify the extent and intensity of the vertically stratified surface layer. By July 2, 2006 the bloom had commenced in earnest in Port Angeles Harbor and adjacent areas of the Strait of Juan de Fuca. Several days later NOAA HAB field scientists reported observations of the bloom offshore of the Olympic Peninsula in the Pacific Ocean. It may have been a coincidence, but most likely the bloom was riding the reduced salinity surface layer out of the Strait, which is the normal estuarine circulation pattern.

August 2006 Bloom Chronology of Events

A second *Heterosigma* bloom occurred later in the summer, but this one was isolated in Central Puget Sound and was not seen in North Puget Sound or the Strait of Juan de Fuca. It was also not monospecific, but mixed with other phytoplankton cells. It is likely that this bloom was a result of the extremely warm, calm weather that occurred at that time. It is possible that cells from the North Puget Sound bloom were present in this area to initiate the bloom, but regular monitoring during the May through July period did not indicate the presence of cells or any north to south transport, which would have been against the outward surface flow to the Strait. A remarkable feature about this bloom was that the least affected area was the Clam Bay location which is where two major fish farms were operating. Very few fish mortalities occurred, and they may not have been due to *Heterosigma*. At the same time waters both to the east (Sinclair Inlet) and to the west (central Puget Sound main basin and east shore of Bainbridge Island) had significant quantities of *Heterosigma* and other phytoplankton. The bloom extended into North Hood Canal and an indeterminate distance to the south in the main basin.

I made limited observations around Bainbridge Island (backwaters near Bremerton WA and to the north) and in the main basin of Puget Sound all the way to Seattle on August 2nd and 3rd, 2006. I circumnavigated Bainbridge Island, taking water samples and CTD casts at numerous locations. An aerial survey was performed by Kevin Bright on the 3rd of August

too, and the results showed patches of the alga throughout Central Puget Sound with major concentrations east and west of Bainbridge Island but not near the fish farm, similar to other observations by myself and repeated cell counts by fish farm technicians (Figure 23). Concurrently, an *Alexandrium catenella* bloom that had initiated about three weeks before the *Heterosigma* bloom was still in progress. Subsequently, this turned out to be a major PSP event for Puget Sound. The fact that fish farmer technicians clearly identified the fact that there was a *A. catenella* bloom of major magnitude prior to the closure of shellfish beds indicates the potential power of routine monitoring. Some kind of formal or informal link between the fish farmers and the Washington Dept. of Health to aid in early warning is discussed in the recommendations section below. (see inset news item, this page)

Closure of area shellfish beds prompts worries over health

By Curt Woodward The Associated Press August 26, 2006:

"OLYMPIA - The worst red tide in perhaps a decade has shut down shellfish beds all along Puget Sound and prompted serious public-health worries, state officials said Wednesday. The state Health Department said the newest round of closures means virtually the entire shoreline from Everett south to the Nisqually River just north of Olympia is off-limits for shellfish harvesting. The eastern Kitsap Peninsula also has been affected, along with areas near Port Gamble, Port Ludlow and along the Strait of Juan de Fuca, said Frank Cox, a Health Department marine-biotoxin coordinator. 'I don't think we've ever had anything quite to this scale," Cox said Wednesday. "I'm concerned if people ignore these warnings, we could wind up with people with illness, if not worse."

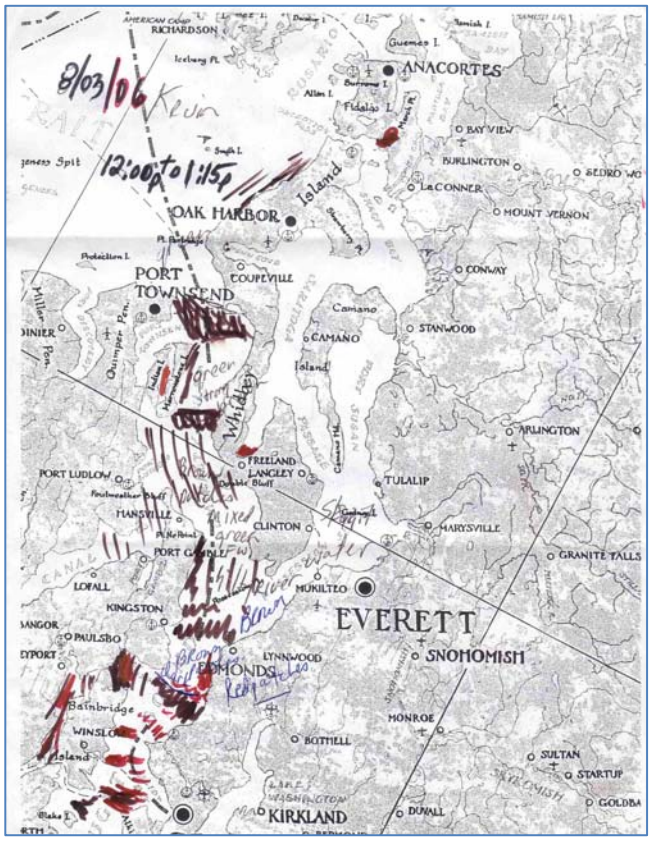
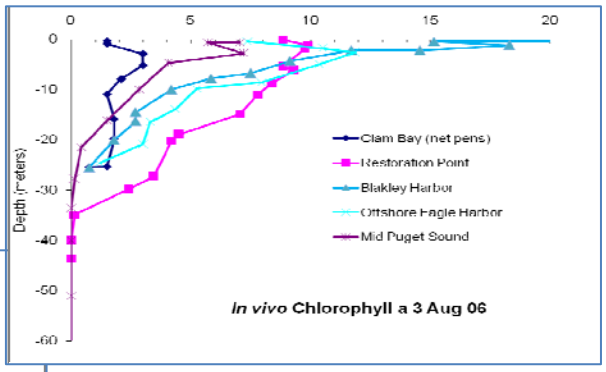


Figure 23. Aerial survey results of August 3, 2006 from Central Puget Sound conducted by Kevin Bright, AGS biologist.

Extensive cell count and bloom distribution data were collected during this bloom by the fish growers and I collected vertical profiles of water quality as in the June bloom in NPS. The bloom was generally more restricted to near surface layers than the June 2006 NPS bloom (Figure 24) and as it was not unialgal, no conclusions about vertical distribution can be made (although the fish growers collected and recorded such data).

Figure 24 . In vivo chlorophyll a profiles from 3 August 2006 in central Puget Sound.



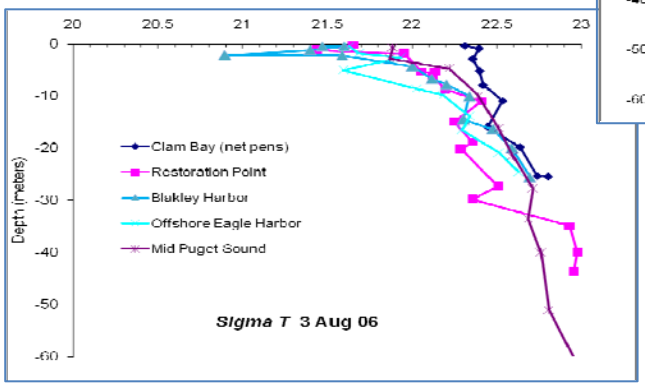


Figure 25. Sigma T (density) of same stations shown in Figure 23.

Figure 25 shows sigma T density vertical profiles for the same CTD casts discussed above. These data indicate that the fish farm site (Clam Bay in this case) was much less vertically stratified than other stations around Bainbridge Island or in the main basin of Puget Sound. Surface water temperatures were 1 to 3°C cooler and slightly more saline than the other stations, probably as a result of vertical mixing in adjacent channel areas of Rich Passage. Similar conditions recurred throughout the August 2006 bloom which may have protected the fish farm area, although in the past this has not always been the case. Connell and Jacobs (1999) report significant water temperature and salinity layering in the same area during the July 1997 bloom. In discussions with Dr. Connell and looking at river data, there was a very unusual and large mid-summer discharge of the distant Skagit River that exceeded 20,000 cfs just before the bloom[3]. Even the density differences created by the temperature layering could have created a highly suitable bloom condition even without the effects of salinity induced vertical stratification but it was likely that both were pronounced.

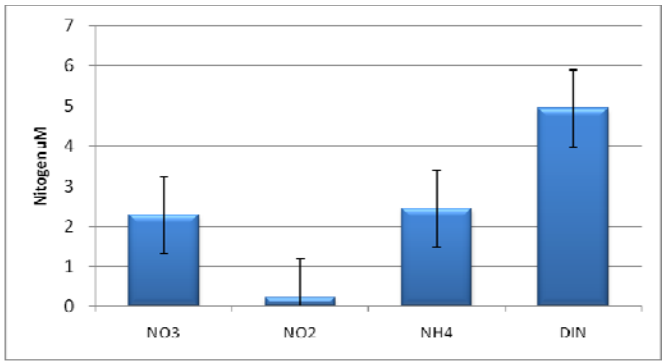


Figure 26. Dissolved inorganic nitrogen (DIN) mean and standard deviation and components of nitrate, nitrite and ammonium from 5 locations shown in Figure 23 and 24, August 2006.

[3] I detected probable systematic error in the typical and bloom period profiles (figure 3) of that publication but the relative difference (drop in salinity and increase in temperature) were undoubtedly correct. Profiles of salinity from several stations in CPS collected by the Washington Department of Ecology about the same time period from their routine monitoring program show > 25.9 psu at the surface in all cases, typically ~ 27 psu.

Skagit River peak discharge has been attributed as the primary cause of *Alexandrium catenella* bloom spread to CPS by Nishitani et al. (1988).

During the August 2006 period I collected vertical profile samples for DIN analysis (University of Washington Routine Chem. Lab.) for the same stations discussed above. Figure 26 shows that DIN averaged about 5 µM with about equal amounts of nitrate and ammonium. The averages for nitrate would have been higher, but one station (confluence of Port Orchard and Sinclair Inlet) was in a very strong tidal mixing area and mixing was in progress during sample collection. Ammonium was, however, similar there despite the active mixing.

Connell and Jacobs (1999) reported a significant drop of nitrate (no other N species reported) for the 1997 bloom in central Puget Sound to 1.4 µM for several days from the normal levels of about 15 µM. They attributed this drawdown to the bloom, not to a prior diatom bloom as had been stated as a bloom requirement by Max Taylor and his colleagues in British Columbia. As discussed above, nitrate was undetectable in surface waters of Admiralty Inlet about a week prior to the 1990 *Heterosigma* bloom and fish kill, but detection limits were high for the available agency data. Cumulatively, these data do indicate that dissolved nitrogen concentrations usually are much less than normal during *Heterosigma* blooms for at least short periods. Urea was not measured in any case, but with regard to fish farm effects, salmonids produce very little urea compared to ammonium (Brett and Zala 1976).

Figure 27. Aerial photograph of *Heterosigma* bloom front in central Puget Sound near Elliot Bay (Seattle).

Management of Farmed Fish

As a scientist with some experience in harmful bloom studies and fish farming, I offer the following observations about how fish farmers managed their fish during the bloom. I restrict my observations to the Cypress Island site only, where I was present for several days during the peak of the June 2006 bloom.

American Gold Seafood LLC (AGS) staff had anticipated the late June 2006 bloom, as cells had been seen in the water but the first clue was that the fish seemed to behave unusually. AGS immediately increased its normal sampling frequency on Sunday June 25th when it became apparent that a few cells were present in the water. Sampling by boat in adjacent regions and aerial surveys were carried out over the following 10 days to monitor the bloom's vertical and horizontal distribution so that appropriate management measurements could be taken.

In the past towing of the cages away from the blooms has been successful both here and internationally (Anderson et al. 2001) and is considered one of the most effective measures to reduce fish loss due to algal blooms. But in 1997 the towing caused pen damage and escapes of fish from one site so at present towing is not allowed in Washington State. To be effective there needs to be a refuge area that will remain bloom free. No such area exists near Cypress Island although for central Puget Sound pens the Colvos Passage area west of Vashon Island has filled this need in the past. Strong currents and vertical mixing apparently reduce or diminish the concentration of algal cells in surface waters there.

Fish farmers were challenged with several tasks during the 2006 blooms including attempts to mitigate the effects of the bloom on living fish and the need to quickly remove dead fish from the net pens at the same time. For the former, fish feeding was suspended early on after the onset of the bloom to reduce digestive demand for oxygen that could be in short supply due to damaged gills. Cell counts were taken from discrete water samples from several depths to judge if upwelling of water would be warranted. If deep water (>10 to 12 m) had lower than surface concentrations of the alga, diesel compressors were started to pump large volumes of air to depth to create an "airlift" of deepwater to the surface that would displace the alga-rich surface waters (Fig. 28). There are potential drawbacks to this method, such as the creation of vertical convection cells of circulating water that some authors have argued exist at some times. But in many cases upwelling that is coupled with some sort of vertical profiling of algal cells seems to be a useful mitigation method.

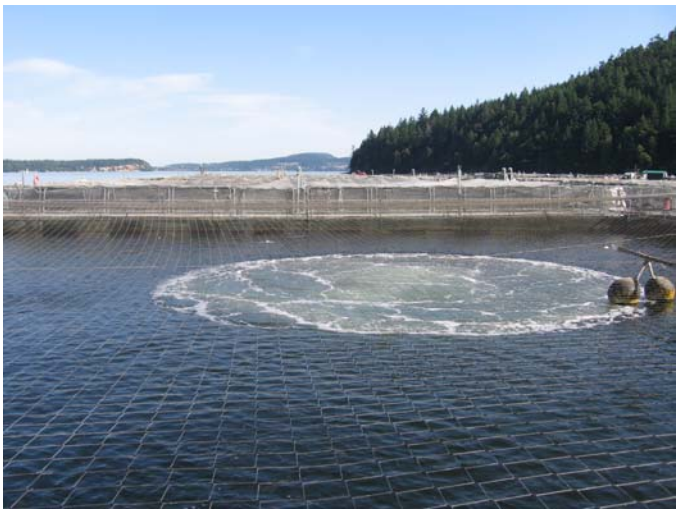


Figure 28. Upwelling water inside a pen a minute after a compressor and airlift assembly was turned on.

As plankton may be patchy in their vertical distribution too, a cell count conduced 10 or 20 minutes in the past may not be indicative of conditions that exist at a later time. To deal with this during daylight hours, farm managers watch the color of the upwelling water to judge real time cell concentrations. I conducted a few lateral sweeps with my fluorometer-equipped CTD to verify that upwelling water was indeed lower in algal concentrations and in one case, where it was not, the farm manager had already noted this

visually (with no cue from me whatsoever) and shut down the system. Visual observation systems are useful but do not offer any means of operation at night and often fish mortality in British Columbia increases at night (N. Haigh, Harmful Algae Monitoring Program, email July 10, 2006) so the availability of an *in situ* fluorometer for unialgal bloom monitoring is recommended.

After a few minutes the extent of the upwelling covered at least ½ of the surface area of the cages. Managers visually inspect the color of the water to determine if deep water is better or worse than surface water and if upwelling should be continued.

Cell Shapes, Sizes and Motility Relative to Fish Mortality

During this bloom technicians and managers noticed that there were different types of *Heterosigma* cells as previously noted by researchers and fish farmers in British Columbia. These included non-motile (sessile) cells that did not move but otherwise appeared healthy in all other aspects. On one occasion during the peak of the bloom at Cypress Island we observed one such cell actually dividing (asexual reproduction) into two daughter cells. It is possible that some or all of the non-motile cells were "cyst, or non-motile round cells" as noted by Connell et al. 1997 towards the end of the 1997 bloom in CPS. However, Han et al. (2002) report such resting cells as:

"completely immobile, although both flagella remained attached. *Heterosigma* resting cells did not require a maturation period before successful activation to the vegetative state could occur. Cell division and motility were impacted sequentially during both the induction and activation phases of resting cell development".

Figure 29. Strands of distinctive purplish-red *Heterosigma akashiwo* as visible from a small plane while flying over backwaters on the east side of Guemes Island, Puget Sound (Photo by K. Bright).

Recommendations

The following recommendations are made to assist in regional understanding of *Heterosigma* bloom dynamics, fish mortality, monitoring and mitigation.

Wild Fish As discussed above, we know very little about the effects of *Heterosigma* blooms on wild fish. It is clear that wild fish are killed in some cases by the alga and that some blooms are not restricted to the immediate surface water and again, the fish sink when killed in our cool temperate waters, thus being less visible to detection. A simple review of fish ecology and distribution of Puget Sound using the extensive data developed by University of Washington, state agency and NOAA workers would identify where juvenile or migrating fish would be expected during the risk periods and surveys with video drop cameras or camera sleds could be mounted during periods when the farmed fish are being killed. In this manner the farmed fish have been and continue to be a "canary in a mineshaft". Agency and public interest as well as research funding for adequate *Heterosigma* research work could well pivot on this issue. Few are concerned about aquaculture fish in the Pacific Northwest; many are interested in the fate of wild fish.

Etiology of Fish Mortality Several researchers in North America remain interested in *Heterosigma* and the mystery of how it kills fish or other aquatic organisms. I believe there is enough evidence to suggest that bacteria or some other interacting factor in non-axenic populations of the alga may be the key to understanding the cause of fish mortality. There are a number of likely explanations discussed above, but so many are potentially viable and may possibly co-occur that to probe them all adequately would require a massive research effort. Whatever the ultimate cause(s), I believe that we have hardly begun to link field and laboratory approaches to helping unlock the mystery. While the basics of bloom initiation location, transport and timing are better understood now than 20 years ago, we have not begun to tap existing resources to more explicitly characterize these blooms and the cause of fish death, as noted below. For example, side by side exposures of different species of fish with sampling of fish tissue upon death or distress would also aid in this effort. To date, such work has been very limited and pathology work very incomplete in my opinion. The fish gill histology studies and results are very limited in scope and conflicting in conclusions. There also has been a very limited attempt to collect and isolate toxins or causative chemicals from actual fish kills in the field in North America. With a algal species that seems to "lose" its harmfulness when isolated in axenic cultures, this seems like an obvious approach that could easily yield results. Insight may be gained from other simple laboratory approaches, such as treating field samples of cells that have freshly killed fish with antibiotics or antiviral compounds and compare results of fish bioassays of untreated samples to see if harmfulness can be manipulated. We know that cultures held for long periods in the laboratory are not harmful to fish at reasonable concentrations, but how they lose their harmfulness is perhaps a valid way to assess the etiology of fish mortality. Another example would be to separate small, non motile cells from larger, swimming cells and test for differences in harmfulness. This could be done through established methods that utilize the alga's response to very small amounts of freshwater (Hershberger et al. 1997) or by other methods.

Solutions may not come from Asian researchers, as *Heterosigma* is not an important fish-killing species in China and Japan (although it does so in New Zealand, Chile, and Scotland). Much of the North American work on this species is in the laboratories of Drs. Trick, Cochlan,

Glibert, Cattolico and Strom. There is a need for government funding for these laboratories to continue this difficult but promising work.

Figure 30. Atlantic salmon with secondary lamellae of gills visible after having been killed during a *Heterosigma* bloom.

Modeling: the existing conceptual model of bloom initiation and spread could relatively easily be parameterized and modeled if support were available. The repeating nature of these blooms suggests some significant physical forcing factors, as discussed above, and the occurrence during clear weather would allow the use of satellite sea surface temperature and chlorophyll *a* sensor data to map blooms and correlate distribution with existing and developing hydrodynamic models, routine and special monitoring and other forms of remote or in situ monitoring. As discussed herein, the conceptual model of bloom development and spread is rooted in years of experience and provides relatively accurate guidance for fish growers, but is not adequate for further scientific and Puget Sound wide use at present.

Response to Blooms**:** It is clear that a very rapid response to blooms is necessary to derive useful information and data. This involves having people and equipment ready to go on a moment's notice during the bloom risk period. Awareness about the blooms, both with agencies and the public should also be heightened. In the first documented blooms there was more of a response from state and federal agencies, but little or no state agency interest has been shown in recent years and NOAA-NWFSC staff are interested, but their funding does not include Heterosigma specifically at present. Some sort of coalition of interested parties could be assembled to delegate tasks and coordinate sampling. The SoundHAB list serve provided by NOAA and WHOI are steps in the right direction in this regard to maximize coordination and minimize expense, although this is a volunteer service I provide.

Remote Sensing Monitoring**:** Presently little use is being made of available remote sensing resources for bloom detection and monitoring in Puget Sound. Both *Heterosigma* and PSP causing *Alexandrium catenella* blooms are closely linked to fair weather patterns as described above, so satellite data could be very useful for detecting onset of blooms even though *A. catenella* does not usually form monospecific blooms in our waters and is usually present in relatively low numbers. For subsurface waters, there is an urgent need for a Puget Sound wide buoy sensing system to vertically profile conditions real time. Presently buoys are being used in Hood Canal, but without source water buoys in the Strait or more

frequent monitoring; water and nutrient budgets for such threatened areas will remain the subject of guesswork and speculation because of the dynamic nature of source waters[4].

Figure 31. Greenish-yellow colored bloom of *Heterosigma* seen in June 2006 in Deepwater Bay and throughout the region. These blooms are sometimes green colored when viewed from a boat, but curiously appear a shade of purple-red when viewed from airplanes.

In the future an integrated monitoring system could house molecular-based HAB detection systems, which are presently operational but require refinement in terms of longevity of operation and reduction of bulk size.

Early warning of all HABs**:** Presently the system that the fish farmers operate is self-sufficient but participating in a regional-wide program might offer additional capabilities that would improve bloom forecasting and response management capability. I believe that NOAA scientists are aware of the importance of having fish farm participation in a regional wide monitoring system. The fact that fish farmers know and routinely sample phytoplankton populations throughout the growing season in three major basins of Puget Sound is potentially a major societal and scientific contribution. No other routine monitoring program in Puget Sound exists or is likely to in the near future. As mentioned herein, in the future there may be molecular-based automated monitoring, but until then it would be helpful to our society to be able to utilize the efforts of the fish farmers who are willing to share their data. The fish farmers are in the business of growing fish, so they are not able to devote the time to quality assurance and distribution or maintenance of their data base. It would seem that this is a role for government. I strongly believe that biology is a better, more sensitive indicator of the health of the environment compared to water quality measurement, a belief rooted in 35 years of experience with both. Ecosystem based management of our coastal waters cannot be accomplished if we have little idea about the status and trends of the species responsible for primary productivity!

Through the SoundHAB list serve, I voluntarily distribute important HAB data acquired by the fish farmers, but more formal linkages may be in order. For example, detection of the massive summer 2006 PSP toxin event in the central basin of Puget Sound was made several weeks before it became evident in shellfish toxicity testing. Such early warning could be

[4] The Strait of Juan de Fuca is highly dynamic, monthly monitoring is inadequate to capture its nature, see review by Rensel 2002 at http://www.wfga.net/SJDF/reports/2001annualrep.pdf

shared with State Department of Health officials in the future once communication channels are made. Similarly, the threat of domoic acid poisoning remains in Puget Sound and observations of *Pseudo-nitzschia spp.* diatom blooms could aid in this effort. There is no direct benefit to the fish growers for such a service but if it was facilitated by government in some fashion it could be a societal benefit. It may be possible to gain more than HAB warnings from the system, this is a topic that should be raised and discussed by responsible agencies.

In this report I have identified likely *Heterosigma* bloom initiation areas, based on where blooms are first noticed. A survey for detection of viable cysts could be conducted to more precisely locate these areas using techniques worked out in Japan (Imai and Itakura 1999).

Fish Farm Bloom Management The performance of fish farm staff and management was exemplary during the 2006 and 2007 bloom event and demonstrated how far the monitoring and mitigation has come in the past 20 years. I offer only a few recommendations to improve the ability to predict and deal with future blooms should they recur. Most of the monitoring is either visual from airplanes or by cell counts. As the blooms are often monospecific in North Puget Sound particularly, use of a chlorophyll sensor probe to monitor algal cell concentrations and could reduce the need to perform cell counts. A chlorophyll *a* to cell count ratio was developed during the 2006 bloom that had little variance over short time scales of days. We performed side-by-side chlorophyll sensor casts along with cell counts in a number of locations during that bloom. Although still preliminary, it appeared that each unit of chlorophyll *a* (in ug/L = ppm) was equivalent to about 100,000 cells/L of the alga (after post-calibration of the unit with laboratory extraction measurement). Regardless of the relationship, it would be useful to be able to rapidly predict where cells are concentrated to avoid pumping up water that might, in some cases, be worse than surface water. Such a probe could also be of use during a harmful *Chaetoceros* event too, although they tend to be mixed in with other species.

Several different manufacturers produce chlorophyll sensors. Some, like the Turner Co. SCUFA unit I use can be used with a multiprobe CTD or alone with a cable connecting it to a personal computer or data logger. A single sensor could be purchased and used at different sites as blooms often do not co-occur at varying areas of Puget Sound at the same time. They are relatively easy to calibrate and hold their calibrations well with use of a solid reference standard. Recently the fish farmers have started recording water quality continuously, and the combination of temperature, salinity and *in vivo* chlorophyll *a* will provide a strong suite of measures to assess risk and variance from normal conditions.

There are molecular tests for *Heterosigma* presence such as the sandwich hybrid assay (e.g., Tyrrell et al. 2002) that has been recently improved and miniaturized (Marin et al.2007). The test takes some equipment and supplies and about an hour to assay 8 samples. Since live, wet mount identification or enumeration with a microscope is relatively easy and quick for an observer with minimal training[5], I see no advantage to a relatively complex test for *Heterosigma* detection in Puget Sound as long as live samples are used, but

[5] Fish growers use 0.1 ml on a wet mount slide which allows them to slow down the live cells to allow easy counts. Sometimes the sample can be chilled slightly that slows down the alga's swimming.

for preserved samples the method has definite advantages (O'Halloran et al. 2006). The assays seem more appropriate for detection of *Heterosigma* in other areas that may be at risk that have mixed assemblages of phytoplankton where other HABs may need to be monitored too. HABs that occur in mixed phytoplankton assemblages in Puget Sound include *A. catenella* (PSP) and *Pseudo-nitzschia* spp. (ASP). Undoubtedly other HAB species will eventually bloom in Puget Sound, e.g., *Cochlodinium polykrikoides* and a fish killing *Chrysochromulina* (possibly *C. polylepis*) have killed fish in British Columbia in recent years.

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Appendix A. Report on June-July 2006 Heterosigma Bloom - Cypress Island and Port Angeles

Prepared By Kevin Bright, American Gold Seafoods, July 20, 2006

Cypress Island Sites Chronology

Monday, June 26th – Cypress sites see first signs of Heterosigma. Evening shift employee, Svein Wiese Hansen, observed some "jumpy" behavior in fish stocks and performed a plankton count prior to doing the evening feed. Cell counts are approximately 150,000 (150K) cells per liter of Heterosigma at 4:30 PM. Kevin Bright and Site Manager, Bill Clark, were notified by phone and a decision is made to not feed the fish this evening. Counts were zero earlier in the day and this sample was taken on a flood tide which typically brings clear water from the south end of Cypress Island. Heterosigma counts go down as flood tide continues into the evening. Plankton counts later that night are in the 20K to 30K cells/liter range.

Tuesday, June 27th – Fish still off feed from previous night. - Kevin Bright takes plankton flight (11: 00 AM) looking at immediate area around Cypress Island, Guemes Channel and north of Sinclair Island. Red streaks and brown water seen in Fidalgo Bay, Samish Bay and the eastern side of Guemes Island, water on west side of Cypress and to south of Cypress look clean with no strong colors in that sector. Data from cell counts starting at 6:00 AM. All cell counts are from Site 1 location unless otherwise noted. Cell counts start out low in the morning hours but continually climb through out the day. Peak counts are 1.1 million cells per liter at 3:45 PM. Counts go down as flood pushes in cleaner water.

0600 – 80K cells @ 1.5 m (ebb tide)

0930 – 140K cells @ 1.5 m

1100 – 370K @ 1.5 m (tide begins flooding)

510K @ 10.0 m

1545 - 1.1mill @ surface (fish showing some stress signs)

870K @ 10.0 m

1900 – 60K @ surface

50K @ 1.5 m

Wednesday, June 28th – Fish still off feed. Plankton flight (at 10:30am) to look further north at waters during ebb tide. Reddish browns streaks continue on east side of Guemes Island, water north of Sinclair brownish, but no strong reddish browns. Water further north toward Clark and Barnes Island, west side of Lummi appears milky with Fraser River fresh water at surface and patches of reddish/brown color indicating plankton colonies thriving in brackish water. Brownish, brown and red colors appearing in these patches appear to be

dinoflagelate blooms (*Heterosigma*, *Noctiluca* and *Alexandrium*) Water appears stratified. Weather sunny and hot. Northwesterly winds 10 knts.

0600 – 70K cells

0830 – 180K @ 1.5 m

180K @ 10.0 m

1130 – 130K @ 1.5 m (ebbing)

1600 – 150K @ 5 m

1620 – 860K @ 1.0 m (flood tide)

1645 – 300K @ 8.0 m

1710 – 1.8 M @ 1.0 m (fish stressed and some roll overs seen)

1900 – 980K @ 1.0 m

2230 – 110K @ 1.0 m (water cleaned up at end of flood)

As can be seen from the plankton data, Heterosigma counts started out the day low and remained low through out the ebb tide. During the strong ebb tide, reddish/brown bloom water was pulled south down through Guemes Channel and around the north end of Guemes Island. This water was then pushed into the Deepwater Bay area and into the Cypress net pen sites during the flood tide. The start of the flood tide brings water from a south easterly direction toward the farm and into Deepwater Bay. At the end of flood tide, the current velocity strengthens and the water comes from a more southerly direction. Counts peaked at 1.8 million cells per liter around 5:00 PM which was toxic to the fish. It was noted by Bill Clark, Kevin Bright and Dr. Jack Rensel that a large number of the cells of Heterosigma appeared smaller and less motile than previously seen. The "resting" cells were still enumerated in the samples, as they were positively identified as Heterosigma under the microscope. It should also be noted that there were high number of cells even at 10 to 12 meters in depth. As the flood tide progressed and the currents strengthened, cleaner water replaced the bloom water and the cell counts subsided.

Thursday, June 29th

Fish still off feed. Fish showing signs of stress and mortality. Floaters in some pens. Heterosigma counts started out high in the morning at 800K cells per liter. As the day progressed the cell counts peaked again at 1.8 million cells per liter around 6:30 PM. Fish were visibly stressed through out the day, with fish gasping and rolling over in the corners of the pens. Mortality increased significantly in the afternoon with increasing cell counts recorded during the beginning of flood tide. Water in Guemes Channel, Bellingham Channel had high counts in patches and mixed bodies of water. High counts recorded in Guemes Channel (approx., 4.0 million cells/liter) and Clark Point (2.2 million), Bellingham Channel (1.3 million). As the flood strengthened toward the end of the tidal cycle, water temperatures dropped and plankton levels flushed out of Deepwater Bay, being replaced by deeper and clearer water from the south end of Cypress Island. Turn over of the water column occurred rapidly, and was easily visible from the surface and by the behavior of fish stocks. Fish began schooling and circling in pens, their coloration returned to normal.

0700 – 810K @ 1.0 m

510K @ 10.0 m

1000 – 950K @ 1.0 m (SE Cypress Head)

1030 – 250K @ 1.5 m

350K @ 10.0 m

1230 – 1.7M @ 5.0 m (Clark Point 4 miles NE of fish pens)

2.2M @ 10.0 m (Clark Point 4 miles NE of fish pens)

1430 – 750K @ 1.5 m (Site 1)

650K @ 10.0 m (Site 1)

1445 – 1.26M @ surface (Site 3)

1630 – 1.3M @ 1.5 m

320K @ 320K

1830 – 1.8M @ 1.5 m

640K @ 10.0 m

1845 – 1.1M @ 1.5 m (Site 3)

350K @ 10.0 m (Site 3)

2015 – 750K @ 1.5 m

220K @ 10.0 m

2030 – 170K @ 2.0 m (end of flood)

160K @ 10.0 m

Friday, June 30th – Fish still on starve. Fish seemed to be handling plankton levels better today. Significantly less mortality found in pens that had been cleaned out on the previous day by divers. Concentrations at the farm did not reach the peaks of earlier two days. By 9:00 PM water appeared to be clearing up with the flood and fish were visibly better in appearance.

0630 – 360K @ 1.5 m

460K @ 10.0 m

0900 – 900K @ 1.5 m

550K @ 10.0 m

1120 – 1.3M @ 2.0 m

1530 – 800K @ 2.0 m

1800 – 1.0M @ 2.0 m

750K @ 10.0 m

2100 – 800K @ 5.0 m

Saturday, July 1st Plankton counts going down and numbers of motile cells appear to be increasing. Samples from Guemes Channel are still high (2.5 million). Water samples from areas to the immediate north, east and south of farm appear to be diminishing quickly. Farm counts down and fish appear to be doing better. Mort numbers way down.

0950 – 300K @ 1.0 m

1500 – 110K @ 2.0 m

80K @ 10.0 m

1600 – 200K @ 1.0 m

Sunday, July 2nd Fish doing better. Samples clearing up around known hot spots.

0930 – 110K @ 2.0 m

80K @ 10.0 m

1710 - 40K @ 1.5 m

Port Angeles Site Chronology:

Thursday, June 29th First signs of Heterosigma. Plankton counts pick up 10K cells/liter at the surface and 40K cells/liter at 5 meters. Fish feed in the morning, but taken off feed in the afternoon by Site Manager Randy Hodgin. Weather sunny, hot and strong westerly winds blowing through out the day.

Friday, June 30th Fish still off feed. Plankton counts show 40K cells at 1.0 and 5.0 meters and 10K at 10 meters. Sunny, west winds.

Saturday, July 1st Fish off feed. Plankton levels begin climbing through out the day.

0900 – 160K @ 1.0 m

230K @ 5.0 m

130K @ 10 m

1600 - 500K @ 1.0 m

360K @ 5.0 m

180K @ 10 m

Sunday, July 2nd Fish off feed. Plankton starts out at 1.0 million at 6:00 AM and climbs to 2.7 million cells at 15 meters by 12:15 PM. Site manager begins seeing fish mortality by the afternoon of the day in most pens. Airlifts being run on and off through out the day. The same type of resting cells that were seen at Cypress are seen in Port Angeles.

0600 – 1.0M @ 1.0 m

1.3M @ 5.0 m

500K @ 10 m

850K @ 15 m

1215 – 2.0M @ 1.0 m

1.6M @ 5.0 m

2.2M @ 10 m

2.7M @ 15 m

1615 – 1.8M @ 1.0 m

2.3M @ 5.0 m

2.1M @ 10 m

1.6M @ 15 m

1700 – 4.6M @ 1.0 m at Francis Street location south east of farm near shore.

Monday, July 3rd Plankton counts start out high in the morning (2 million at 15 meters) and peak at 3.5 million cells per liter at 1.0 meter by 4:21 Pm. Fish are extremely stressed and rolling over rapidly. Farm is in full mortality clean up mode. The F/V Harvestor is pumping fish into holds assisted by 2 to 3 divers. Weather continues sunny and hot, with strong westerly winds blowing all day (15 to 25 knts). John Bielka and Kevin Bright charter plane and fly along shoreline from Port Angeles eastward to Pt. Townsend, then south down Admiralty Inlet to Rich Pass sites. The entire Harbor of Port Angeles is visibly a coffee color. Water outside of Ediz Hook is brown, and water along shoreline to Pt. Townsend is brick red to brown. Stronger colors near shore then dissipating as you travel north away from shoreline. Discovery Bay and Sequim Bay are reddish/brown color. Bloom extends offshore for several miles in some areas along the Strait. By Pt. Townsend color disappears. Water looked good through Admiralty Inlet except for West Point area was brownish red in a very localized area.

0630 – 1.0M @ 1.0 m

1.5M @ 5.0 m

1.6M @ 10 m

2.0M @ 15 m

1045 – 2.0M @ 1.0 m

1.8M @ 5.0 m

2.1M @ 10 m

900K @ 15 m

1621 – 3.5M @ 1.0 m

2.7M @ 5.0 m

2.1M @ 10 m

1.1M @ 15 m

Tuesday, July 4th Farm counts still high. Weather still sunny after morning fog burns off. Still loosing fish through out the day. Harvestor continues pumping off pens.

1000 – 1.9M @ 1.0 m

2.0M @ 5.0 m

2.0M @ 10 m

3.0M @ 15 m

1330 – 4.4M @ 1.0 m

2.9M @ 5.0 m

1500 – 2.5M @ 1.0 m

2.8M @ 5.0 m

Wednesday, July 5th Counts begin to go down at farm. Mortality rate drops. Weather conditions begin to break down as weak system moves in.

0700 – 400K @ 1.0 m

850K @ 5.0 m

1.0M @ 10 m

800K @ 15 m

1040 – 240K @ 1.0 m

130K @ 5.0 m

140K @ 10 m

140K @ 15 m

1300 - 30K @ 1.0 m

80K @ 5.0 m

70K @ 10 m

210K @ 15 m

1715 – 250K @ 1.0 m

530K @ 5.0 m

920K @ 10 m

850K @ 15 m

Thursday, July 6th Water returns to normal and weather continues to cool down. Farm cell counts return to low levels. Samples in morning show cells are sinking. Low levels at surface (30K), and counts down to 35 meters of depth are nearly 16 times higher (470K)

0700 – 30K @ 1.0 m

20K @ 5.0 m

60K @ 10 m

160K @ 15 m

470K @ 35 m

1330 – 200K @ 1.0 m

180K @ 5.0 m

10K @ 10 m

10 K @ 15 m

Appendix B. Harmful Algal Blooms and Finfish Resources in Puget Sound

Reprinted from: Rensel, J. 1995. Harmful algal blooms and finfish resources in Puget Sound. *In* R.M. Strickland (ed.): Puget Sound Research '98; Proceedings of the Water Quality Action Team. Puget Sound. Olympia, WA, p. 422-429.

(Note: *Heterosigma* species name has reverted to *akashiwo* since this was published).

INTRODUCTION

Several harmful algal blooms (HAB) species have economic and biological importance to finfish resources by causing biotoxin accumulation in the food-web or fish kills. These problems are apparently less prevalent for wild finfish in Puget Sound than in other U.S. coastal areas, but there is uncertainty regarding their significance here. Worldwide at least a dozen genera of microalgae have been involved in mortality of wild or aquaculture fish (Hallegraeff 1991). Several occur in Puget Sound, although only three types have often been implicated in fish kills locally (Table 1). The table shows that little is known regarding exposure concentrations of most microalgae that kill fish; the causes of physiological harm are also poorly documented in several cases. Table 1 should not be considered all inclusive, as there may be other microalgae in Puget Sound that can cause fish kills. Unexplained losses of hatchery-raised and wild fish have been reported from Puget Sound in recent years. For example, an unidentified dinoflagellate was responsible for losses of salmon smolts in a state-operated net pen in Hood Canal during 1993 (R. Horner and J. Rensel, unpublished data). Two types of harmful microalgae problematic in Puget Sound, *Chaetoceros* spp. and *Heterosigma carterae,* are discussed below.

CHAETOCEROS SPP.

The marine diatom genus *Chaetoceros* is separated into 2 subgenera by the presence (*Phaeoceros*) or absence (*Hyalochaete*) of long, partly hollow setae or primary spines that contain chloroplasts. *Phaeoceros* species, such as *C. concavicornis* and *C. convolutus* have more robust setae and frustules than *Hyalochaete* species and the setae are armed with short secondary spines that point toward the distal ends of the primary setae (Cupp, 1943). With few exceptions, the chain-forming *Phaeoceros* species are responsible for mortality of fish, although dense blooms of *Hyalochaete* species were implicated in the poor survival of one group of salmon smolts in Port Angeles Harbor net pens in 1988 (R. Elston and J. Rensel, unpublished data).

The first reported case of *Chaetoceros*-caused fish mortality involved wild lingcod (*Ophiodon elongatus*) that were captured and held temporarily in fishermen's cages in British Columbia (Bell, 1961). Subsequently these diatoms have been involved in occasional losses of net-pen salmon, including a major loss near Cypress Island in 1987 (Rensel et al., 1989; Horner et al., 1990). It has been suggested that harmful *Chaetoceros* setae break off

the cells and enter the gill tissue butt-end first; the apically-pointed secondary spines on the primary setae may then act like barbs on fish hooks (Bell, 1961). This hypothesis and reference is often repeated in the literature but is based on only a few samples of wet-mounted gill tissue. Rensel (1992, 1993a) used scanning electron microscope techniques to show that penetration of the gill tissue by *C. concavicornis* was uncommon. Rather, chains of cells tended to lodge between the secondary lamellae and be present in the surrounding gill mucus. Blood-gas studies showed that affected salmon had severe blood-hypoxia as a result of mucus production during acute exposure or physiological damage to the gills after longer-term exposure. Longer chains of *C. concavicornis* caused significantly lower blood-oxygen partial pressures compared to fish exposed to shorter chains. Longer setae associated with longer chains were apparently more likely to become wedged in the gills and stimulate mucus cell release, lesions and epithelial damage.

Salmon respond to *C. concavicornis* exposure by an immediate and periodic cough response that diminishes slightly in frequency over time (Rensel, 1992, 1993a). This is similar to fish coughing caused by many environmental irritants and chemicals (Heath, 1987). Coughing and mucus production act in concert to help clear the gills of the diatoms. Long strings of mucus were seen trailing from the gills of live fish during the 1987 Cypress Island fish kill.

Short-term laboratory exposure to as few as 10 cells per ml of *C. concavicornis* caused a rapid increase in mucus cell discharge on the gills as well as a severe hypoxia and elevated carbon dioxide content in the blood of Atlantic salmon (Rensel, 1992; 1993a). Long-term exposure to < 5 cells/ml of harmful *Chaetoceros* in net pens has been reported to increase disease and mortality of farmed salmon (Albright et al., 1993), although sampling in this study may not have been frequent or extensive enough to assure that higher concentrations of the diatoms did not occur. Nevertheless, it is clear that low concentrations of these diatoms can kill salmon, hence the term "bloom" is not always appropriate when referring to harmful *Chaetoceros*-caused fish kills.

It is likely that harmful *Chaetoceros* have caused mortalities of wild and hatchery-released fish. South Hood Canal and Dabob Bay/North Hood Canal are areas where these harmful diatoms often occur (Rensel et al., 1989; Rensel Associates and PTI Environmental Services, 1991; J. Postel, unpublished data). During the fall of 1990 a mixed water column in north Hood Canal containing *Chaetoceros concavicornis* to at least 40 m correlated with the otherwise unexplained mortality of migrating adult chinook salmon (Rensel Associates and PTI Environmental Services, 1991). In southern Hood Canal, mortality of juvenile salmon in seawater pumped from a subsurface depth was due to harmful *Chaetoceros*; this suggests the possibility of a recurring problem for wild smolts moving through the area. Harmful *Chaetoceros* often occur in late spring and again in September or October, although they may be present at any time of year in Pacific Northwest waters (R. Horner, unpublished data; Rensel, 1992). Their geographic range and relationship to occasional fish kills in Puget Sound is not well defined.

HETEROSIGMA CARTERAE

This raphidophyte flagellate is known worldwide as a fish killer and has caused significant mortality of net-pen salmon in Puget Sound in 1989 and 1990 after several years of problems in British Columbia waters. *H. carterae* may have been present for at least several decades in Puget Sound before the 1989 and 1990 blooms. Taylor and Haigh (1993) note the species has appeared with great regularity in late spring in the Strait of Georgia since 1967, when surveys were first initiated. Appearance there coincides with a rise in water temperature above 15°C and a decline in surface salinity to less than 15 psu. The 1989 bloom in Puget Sound was apparently restricted to the northern areas of Puget Sound and the San Juan Islands, but the July 1990 bloom involved large areas of northern, central and southern Puget Sound. In 1993, a bloom of *H. carterae* was recorded in Budd Inlet, the southern most extreme of Puget Sound.

Recent laboratory studies suggest that reduced salinity plays an important role in bloom formation of *H. carterae*. By simply adding a small amount of distilled water to the surface of vertical columns containing the alga, large quantities of the cells accumulated near the surface (Hershberger et al., in prep.). The importance of freshwater-induced vertical stratification in field studies of *H. carterae* has been observed by Taylor and Haigh (1993).

Heterosigma blooms apparently require quiescent weather conditions, as was experienced immediately prior to and during the 1989 and 1990 blooms in Puget Sound. Strong vertical stratification of the water column due to freshwater and perhaps solar heating also seems to be a prerequisite. The September 1989 bloom was apparently associated with the Fraser River plume in Northern Puget Sound. The July 1990 bloom occurred in an extremely calm, warm period in early July following a June when Puget Sound lowland precipitation was 51% more than normal (NOAA, 1990). Moreover, routine monitoring in Admiralty Inlet approximately 10 days prior to the 1990 bloom confirmed that the surface layer was warm (near 15°C) and of reduced salinity (19.5 ppt) (Janzen, 1992). This is significant given the mid Puget Sound location of this sampling station, remote from creeks and riverine sources of freshwater. Other regional stations had similar values. Macronutrient content in the surface layer was undetectable, but *H. carterae* is capable of vertical migration, moving up to 1 m/hr to obtain nutrients from subsurface depths.

In mid-July of 1993, a bloom of *H. carterae* occurred in the Port Orchard/Brownsville area east of Bainbridge Island. Antecedent rainfall and quiescent weather conditions were similar to those of the 1990 bloom. The bloom appeared to increase in apparent size for several days but an abrupt weather change, including strong south winds, correlated with the rapid termination of the bloom. Soft bottom sediments of this protected area may be a seed bed for some of the blooms.

The physiological cause of fish mortality from *H. carterae* exposure is unknown despite several laboratory studies that used clones or cultures taken from local fish kills. Histopathology of gills of moribund subadult salmon from net-pen kills usually show major damage to the epithelium and mucus buildup, but not in one case with juvenile fish where a labile icthyotoxin was suspected as the cause of mortality (Black et al., 1991). However, no toxin has been detected in the edible tissues of any affected fish. Although *H. carterae* grows well in culture, most laboratory bioassays here and abroad have yielded few fish deaths even when fish are exposed to very high concentrations of cells or conditioned

medium. Several authors have suggested that the fish-killing mechanism may be similar to that of the related alga *Chattonella antiqua* that occurs in Japan. Superoxide anion radical and hydrogen peroxide produced by the alga reportedly strips the fish gill of mucus and leads to fatal osmoregulatory stress (Tanaka et al., 1994). Yet this does not fit with current knowledge of salmonid physiology because the normal, unstressed condition is to have little or no mucus on the gills (Handy and Eddy 1991). A possible advance regarding laboratory-induced toxin production by *Heterosigma* was reported in 1994, although few details are available at this time (R.A. Cattolico, pers. comm. 1994).

H. carterae blooms may extirpate or outcompete virtually all other algal species in the upper water column (Taylor and Haigh, 1993). Its effect on larval or other fish frequenting shallow depths has not been studied, but there is evidence that wild fish or hatchery-released fish have been harmed. There were several reports of wild fish kills and wild fish acting in a distressed manner in Port Townsend Bay and the Strait of Juan de Fuca during the 1990 bloom. Limited aerial surveys failed to validate the reports (R. Allen, J. Rensel, survey of 12 July 1990), but the surveys were restricted to the Port Townsend area and there was a lag of at least one day between the reports and the surveys. Dead adult chinook salmon were seen by local boaters in Bellingham Channel during the 1989 bloom, but no tissue samples were collected.

More recently, in the fall of 1994, chinook, coho and summer chum salmon as well as several marine fish species were killed by a *Heterosigma* bloom in upper Case Inlet of southern Puget Sound. It is a shallow area where several streams enter Puget Sound and the fish were apparently unable to escape the bloom. The full extent of fish loss was unknown, but numbered at least in the hundreds. State agency and university researchers were not notified of the kill until well past its commencement, reducing the ability to collect useful data.

DETECTION AND MITIGATION OF FISH KILLS AND ALGAL BLOOMS

Detection of wild fish losses due to HABs in Puget Sound is a difficult task. Affected wild salmon most likely sink to the bottom, similar to HAB-killed salmon in net pens. Usually shoreline residents or boaters do not notice fish losses or will notify state agencies too late for adequate data collection during a bloom. In some cases, residents have mistakenly supposed that dead adult salmon are simply spawned-out fish, particularly in areas with creeks and rivers having salmon runs. Fish mortalities can occur in areas with less shoreline development, such as North Hood Canal, resulting in fewer people noticing dead fish. Migrating adult salmon do not necessarily travel in large schools (e.g., chinook salmon), so an occasional dead fish does not seem significant. A partial solution to these detection problems is public and government agency education and resource agency planning prior to the occurrence of HABs.

Reducing non-point and direct discharges of nitrogen into nutrient-limited marine waters may be viewed as one means of prevention or mitigation of some types of HABs. A review of several HAB case histories worldwide suggests that some, but certainly not all HABs, are associated with increased coastal eutrophication (Rensel Associates and PTI Environmental Services, 1991). The connection may be more related to rates of tidal flushing and vertical

stratification in specific areas than to characteristics of a given HAB species. For example, nutrient discharge is an important factor controlling the extent of *Heterosigma* blooms in the Seto Inland Sea of Japan (Honjo 1993), but there is no evidence of this in the main basins of Puget Sound. Yet, poorly-flushed and nutrient-sensitive areas of Puget Sound have minimal nitrogen supply rates in clement weather that may limit algal production (Yake, 1981). Therefore HABs can be stimulated by increased nitrogen loading. Rapid urbanization, poor agricultural or logging practices and municipal discharges have in some cases resulted in very large increases of nitrate loading to Pacific Northwest streams and rivers (e.g., Smith et al., 1987; Harr and Fredriksen, 1988), but few nutrient discharge data have been analyzed with regard to eutrophication or HAB trends in nutrient-sensitive areas of Puget Sound.

Monitoring and experimental data suggest that very low or undetectable concentrations of nitrogen in surface and subsurface waters of nutrient-sensitive areas of Puget Sound have limited the geographic range or activity of paralytic shellfish poisoning (PSP) caused by the dinoflagellate *Alexandrium catenella* (Rensel, 1993b). Salmonid smolts and other phytophagous finfish may be at risk from saxitoxins accumulated by zooplankton that have consumed *A*. *catenella*. This has been demonstrated in the laboratory (Erickson, 1988), but not as of yet in Puget Sound. It follows that phytophagous fish in these areas are at risk if PSP activity increases due to increased nitrogen loading. With regard to the occurrence of harmful *Chaetoceros*, however, there are no data suggesting an association with nutrient discharge or specific type of area, nutrient-sensitive or otherwise. For example, these diatoms occur in both seasonally nitrogen-limited South Hood Canal and nitrogen-replete North Hood Canal.

Harmful *Chaetoceros* are difficult for fish farmers to detect because a lethal concentration of cells may have no effect on easily measured parameters such as water transparency, dissolved oxygen, pH, etc. The vertical and horizontal distribution of cells is difficult to ascertain without extensive and time-consuming microscopic cell counts. Automated cell counting techniques are not possible due to the large size and spines of these diatoms. A simple means to detect the presence of harmful *Chaetoceros* at fish farms is microscopic examination of wet mounts from gill scrapings. Other techniques such as histopathology are slow, expensive, and not appropriate for routine detection on the gills because the diatom cells occur in the interlamellar spaces and on the epidermis and may be washed away during the extensive tissue preparations that are required.

Blooms of harmful microflagellates or dinoflagellates that occur in high concentrations are often easier for fish culturists to identify and manage because visual surveys from boats or airplanes can be used to define their geographic limits. This facilitates towing of net pens to unaffected areas such as in 1990 when pens were towed from Rich Passage into Colvos Passage. These blooms are often restricted to near surface depths allowing for the pumping of algal-free deep water into the pens equipped with surrounding skirts; onshore or upland facilities may have water intakes at several depths to avoid HABs. Because *Heterosigma* blooms occur in surface waters during clear weather, sea-surface temperature and chlorophyll *a* sensing satellites that are scheduled to be launched may be useful to track and predict the distribution of these blooms.

RESEARCH NEEDS

There is a need to investigate wild fish kills involving HABs more completely and expeditiously and to document accompanying hydrographic and meteorological conditions. These investigations can form a basis for understanding HAB dynamics, trends and possible connections with land and riverine management practices. Nitrogen loading trends for streams, rivers and municipal discharges should be examined for nutrient-sensitive areas of Puget Sound.

The physiological causes of fish mortality due to *Heterosigma* remain to be discovered. Larvae or juveniles of marine fish and salmonids that utilize shallow embayments during late spring through fall may be at risk to the apparent recent spread of *Heterosigma.* Juvenile and adult salmon and marine fish in Hood Canal and perhaps elsewhere in Puget Sound appear to be at risk to harmful *Chaetoceros* occurrence, but this remains to be quantified. Finally, field studies are recommended to test the importance of saxitoxin accumulation from the dinoflagellate *Alexandrium catenella* to zooplankton and throughout the food web to salmon and other predators.

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Appendix Table 1. Fish-killing phytoplankton species known to be present in Puget Sound; those with an asterisk have caused documented fish losses in Puget Sound. The second column relates the concentration suspected to be harmful to fish and the etiology of harm.

Category and Species	Harmful Concentrations & Etiology	References
DIATOMS		
Chaetoceros concavicornis* and *C. convolutus** and possibly others of the subgenus Phaeoceros such as *C. danicus*	> 2-5 cells/ml for salmonids; depends on chain length. Cells lodge between gill lamellae causing mucus production, irritation & leads to blood-hypoxia or anoxia	Bell, 1961; Rensel, 1992; 1993a Albright, 1993;
DINOFLAGELLATES		
Alexandrium catenella	unknown; acute mortality to farmed fish not well documented or prevalent; chronic, food web problem with wild fish	White, 1980; Mortenson, 1985 (for *A. tamarense*); Erickson, 1988
Ceratium fusus*	unknown; gill irritation, poorly understood	Rensel and Prentice, 1980
Noctiluca scintillans	variable; unionized ammonia causes gill damage and other problems for fish	Okaichi and Nishio, 1976
PRYMNESIOPHYTE FLAGELLATES		
Chrysochromulina polylepis	unknown; causes gill damage and osmoregulatory problems	Estep and MacIntyre, 1989
Phaeocystis pouchetii	unknown; irritant substances and the alga's mucus can clog gills	Gaines and Taylor, 1986 Smayda, 1989
RAPHIDOPHYTE FLAGELLATES		
Heterosigma akishiwo*	probably variable, in most cases >750 to 1,000 cells/ml; cause of fish death unknown, may be similar to *Chattonella*	Black et al., 1991; Taylor and Haigh, 1993; Tanaka et al., 1994
SILICOFLAGELLATES		
Dictyocha speculum	unknown; siliceous skeleton may irritate gills, possible toxin action too.	Larsen and Moestrup, 1989
UNKNOWN ALGAL SPECIES		
Net pen Liver disease*	unknown; chronic losses possibly caused by a microcystin-producing alga.	Kent, 1990
unidentified dinoflagellate*	mortality of delayed-release net-pen salmon in Hood Canal in summer, 1993	R. Horner and J. Rensel, unpublished data