Formation and decline of a *Heterosigma akashiwo* layer in East Sound, Washington, USA

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Abstract

The formation and decline of a surface layer of the toxic raphidophyte *Heterosigma akashiwo* was observed in a shallow, coastal fjord in Washington state, USA. Physical and chemical water column properties and biological rates were simultaneously quantified to estimate the contribution of biotic factors (i.e. rates of cell accumulation and loss) to *H.a.* layer formation. The results suggest that reduced cell mortality, due to reduced protistan grazing pressure in addition to favorable environmental conditions may have contributed to the occurrence of the *H.a.* layer.

Introduction

The flagellate *Heterosigma akashiwo* (Hada) (*H. a.*) is a globally distributed raphidophyte that has been implicated in toxic events, leading to fish kills and aquaculture losses. The mode of toxicity for *H.a.* has not been identified, although several possible mechanisms exist (reviewed in Kempton *et al*. 2008). Blooms of *H.a*. have been observed for several decades around the Pacific (Honjo 1993, Horner *et al*. 1997) and more recently along the USA's Atlantic coast (Kempton *et al.* 2008).

Bloom formation for any alga can only occur if processes that lead to cell accumulation (e.g. growth, aggregation) exceed processes that lead to cell loss (e.g. dilution due to mixing, grazing). Although the factors driving *H.a.* bloom formation are unknown, there are suggestions that blooms coincide with changes in water properties due to run-off (Smayda 1998). Ecophysiological studies identify *H.a.* as particularly tolerant to a wide range of temperatures and salinities with potentially very high growth rates of multiple divisions per day (reviewed in Smayda 1998).

Fewer studies have quantified *H.a.* mortality either in the lab or *in-situ*. Cell lysis due to viral infection has been implicated in rapid declines in *H.a.* abundance (reviewed by Tomary *et al*. 2008). However, grazing by heterotrophic protists typically accounts for the vast majority of phytoplankton mortality in the ocean. Laboratory results show that *H.a.* are either toxic to or not ingested by some protistan predators (Kamiyama & Arima, 2001, Clough & Strom 2005). This suggests that lack of predator-induced mortality may contribute to bloom formation in this, and by implication other, HAB species.

Since HAB events are unpredictable, their study benefits from chance encounters. During a month-long field study a brief occurrence of dense *H.a.* was observed at the surface of a coastal fjord. This occurrence provided the opportunity to quantify the relative contribution of physical, chemical and biological factors to the formation, persistence and dissipation of the event. The results suggest that, in addition to environmental conditions, biological interactions may contribute to the formation of high-density layers of *H. a*.

Methods

Four stations spanning the longitudinal axis of East Sound, Washington, USA were sampled in July 2007. A site map and more methodological details are reported in Menden-Deuer (2008). A SeaBird 19+ CTD with mounted fluorometer recorded water column profiles. Water samples were collected from one to two depths, between 0.5 to 12 m, using a horizontally mounted Niskin bottle. For each sample, size-fractionated Chl *a* (GF/F, 5, 20μm), nutrient concentrations, phytoplankton growth- and heterotrophic protist grazing rates were measured in triplicate (Strom & Frederickson 2008). Photosynthetic parameters were measured through incorporation of ^{14}C at 14 light levels from 0 to 1400 μmol photons m^{-2} s^{-1} at 12ºC. Statistical comparisons were made by 1-way ANOVA.

Results

A surface slick of *Heterosigma akashiwo* extended throughout East Sound on July 16th 2007. This event followed a warming and freshening of surface waters. At the time of peak *H.a.* abundance, temperature was 15.5ºC and salinity had dropped to 27.5PSU (Fig. 1 A&B). Figures 1&2 show data from the southernmost station within the Sound. The conclusions drawn

here are supported by data from all stations. The Chl *a* induced fluorescence signal was initially low and distributed down to 10 m depth. Concurrent with the stratification of the water column, the fluorescence signal became concentrated within the surface when *H.a.* reached peak abundance on July 16th (Fig. 1C). Note that the CTD records only 0.5 m below the surface, suggesting the data are likely underestimates of maximum fluorescence.

Maximum Chl *a* concentration was 17 µg Chl *a* l^{-1} with >90% of the signal in the <20µm size-fraction on July 16th 2007 (Fig. 2). Peak *H.a.* concentration for that sample was 3700 cells/ml at 0.5 m depth. Concentrations 3 days prior (July 13th) and 2 days after peak *H.a.* abundance (July 18th) were 10 to 100 cells/ml at multiple stations and depths. A second peak in fluorescence and Chl *a* on July 23rd also coincided with warmer and slightly fresher water, but *H.a.* was not detectable in those samples.

Nutrients were plentiful with nitrate and phosphate concentrations above 5 and 1 µM respectively, even within the *H.a.* layer. Silicic acid concentrations were above 30 µM.

Photosynthetic efficiency did not vary significantly over the course of the *H.a.* event; maximum photosynthetic rate (3 to 5 µgC (µg Chl h)$^{-1}$) decreased significantly over time, with intermediate values during peak *H.a.* abundance (data not shown).

Before the *H.a.* event, heterotrophic protist grazing on the >20µm size-fraction proceeded at 0.3-0.7 d^{-1} and was significantly higher than grazing on the <20µm size-fraction, which was not significantly different from zero (Fig. 3). On the day of the *H.a.* event, no grazing was measurable on either size-fraction. After the HAB layer had disappeared, grazing was measured in both size-fractions.

Discussion

The timing of the *Heterosigma akashiwo* layer is clearly evident in the change of water column properties, time series of Chl *a* concentrations and contribution of the small size-fractions to the overall signal. The sudden appearance of *H.a.* constituted a 4-fold increase in Chl *a* concentration and coincided with warmer and fresher water. These water column properties are frequently observed in conjunction with *H.a.* blooms, although in British Columbia, conditions are typically more extreme with surface salinities as low as 15PSU (Smayda 1997). Such low salinities would be unlikely in East Sound due to frequent tidally driven influx from the Strait of Juan de Fuca.

In combination, increased Chl *a* concentrations and changes in water column properties may be considered useful indicators for *H.a.* alerts. However, microscopic analysis is still required for verification. Following the disappearance of the *H.a.* slick, another surface layer of high fluorescence was observed. Water column properties were again warmer and fresher, although less extreme than during the *H.a.* event (July 23rd). In those samples *H.a.* was not detected. Therefore, it needs to be determined if observations of "warmer and fresher than typical" conditions apply in a relative or absolute sense. Honjo (1993) showed that *H.a.* blooms occurred over wide temperature and salinity ranges (15-30ºC, 20-30PSU). The broad physiological tolerance and observed differences in salinities associated with *H.a.* blooms even in close proximity to each other (e.g. coastal Washington & British Columbia) suggests that environmental conditions can serve as an indicator in a relative sense at best. Thus, environmental conditions may be a promoter of *H.a.* blooms but, due to the species' wide tolerance range, are not a predictor.

Our study would have benefited from hydrographic measurements, as the tidal exchange in East Sound can be considerable. Such data could reveal whether advection brought *H.a.* into the Sound or dissipated the bloom, as is suggested by changing water column properties. Moreover, *in-situ* observations of *H.a.* behavior may have revealed whether this HAB was due to a behavioral aggregation of *H.a.* to low salinity surface waters, as has been observed in the laboratory (Bearon & Grünbaum 2006).

The biological rate measurements performed provide insight into the biologically mediated rates of cell accumulation and loss. Although these measurements cannot be used to predict plankton population dynamics between sampling dates because of the potential of sampling different water masses, they are useful in assessing net rates of biomass accumulation or loss.

Measured photosynthesis parameters for *H.a.* dominated communities were not significantly higher than for other phytoplankton communities sampled within a few days before or after the HAB event. Although very high growth rates have

been measured for *H.a.*, for our field samples photosynthetic potential alone cannot explain the accumulation of cells at the surface.

In contrast, there was little to no measurable grazing impact on the smaller, *H.a.* containing size-fraction, before or during the HAB event. Thus, there was no measurable, grazer-induced reduction in phytoplankton biomass, which suggests that accumulation of cells would be possible. Lack of measurable grazing on any size-fraction during the HAB event suggests the intriguing hypothesis that presence of this toxic alga may broadly affect trophic interactions within the microbial foodweb.

HAB toxicity to or avoidance of HABs by grazers has consequences for plankton population dynamics, including decreased mortality for the toxic species and increased grazing pressure on competitors. Clearly, the interplay between toxic prey and grazing rate is crucial to the evolution of these organisms and holds the potential to affect the frequency and magnitude of HABs. Therefore, biological drivers, including grazing need to be considered when assessing the potential of HAB formation.

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References

Bearon R.N., Grünbaum D. & Cattolico R.A. (2006) Mar. Ecol. Prog. Ser. 306, 153-163.
Clough, J., & Strom S. (2005). Aquat. Microb. Ecol. 39, 121-134.
Horner R.A., Garrison D.L., & Plumley F.G. (1997). Limnol. Oceano. 42, 1076-1088.
Kamiyama T. & Arima S. (2001). J. Exp. Mar. Biol. Ecol. 257, 281-296.
Kempton J., Keppler C.J., Lewitus A., Shuler A., & Wilde S. (2008) Harmful Algae 7, 235-240.
Menden-Deuer S. (2008). Mar. Ecol. Prog. Ser. 355, 21-30.
Smayda, T.J. (1998) In: Physiological Ecology of Harmful Algal Blooms, Anderson, D.M. & Cembella A.D., & G.M. Hallegraeff (eds), Springer, Berlin, pp. 113-131
Strom S.L., & Frederickson K.A. (2008) Deep-Sea Res. II 55, 1761-17
Tomaru Y., Shirai Y., & Nagasaki K. (2008). Fish. Sci. 74 701-711.

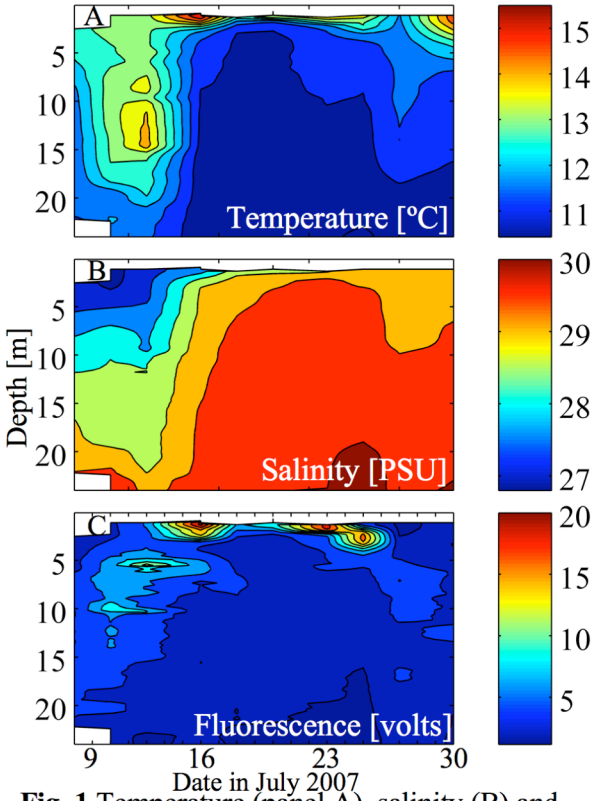

Fig. 1 Temperature (panel A), salinity (B) and fluorescence (C) in the fjord in July 2007.

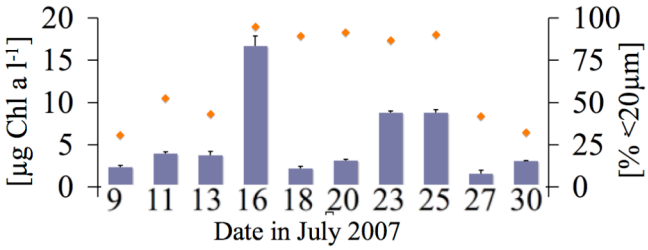

Fig. 2 Total Chl *a* concentration (bars) and %-contribution of the <20µm size-fraction(♦) to total Chl *a*. Error bars in all figures are 1 SD. *H.a.* abundance peaked on July 16th 2007.

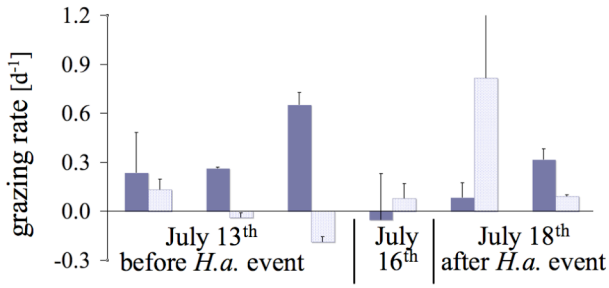

Fig. 3 Grazing rates (d^{-1}) on >20µm (dark) & <20µm (light) size-fractions before (July 13th), during peak (July 16th) and after the H.a. event (July 18th). Each bar represents triplicate measurements of discrete stations.