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Lethality of microalgae to farmed Atlantic salmon (*Salmo salar*)

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ABSTRACT

Phytoplankton blooms in the Bay of Fundy have been implicated in the deaths of farmed Atlantic salmon (*Salmo salar*). To establish whether or not elevated concentrations of some phytoplankton species can cause harmful effects or mortality, monocultures of three species, *Alexandrium fundyense*, *Ditylum brightwellii*, and *Chaetoceros socialis* were grown in large quantities. Atlantic salmon smolts were exposed to a range of concentrations of each of these cultures for 24 h and, when mortality occurred, an LC50 was calculated (cells DEL id="del2" orig="•"; L^{-1}). *Eucampia zodiacus* was collected by plankton tow and used in bioassays as well. Exposure of Atlantic salmon to *D. brightwellii*, *C. socialis* or *E. zodiacus* at concentrations as high as 1.0×10^6 cells L^{-1}, 4.0×10^6 cells L^{-1} or 9.0×10^5 cells L^{-1}, respectively resulted in no apparent deleterious effect. These concentrations are equal to or greater than concentrations of these species observed in the field. *A. fundyense* was the only species of the 4 examined that was shown to be lethal to Atlantic salmon smolts. The 6 h LC50 was estimated as 7.24×10^5 cells L^{-1} and the 24 h LC50 was estimated as 6.14×10^5 cells L^{-1} indicating effects occur quickly and longer exposures do not change the LC50 estimates significantly. The LC50 concentrations represent cell counts similar to those observed in the field. The no observable (lethal) effect level for *A. fundyense* and Atlantic salmon is estimated as approximately 2.0×10^5 cells L^{-1} over 6 h and 1.0×10^5 cells L^{-1} over 24 h. Cell counts do not reflect the toxicity of individual cultures but may be valuable for aquaculture site management. Chemical analyses of the cultured *A. fundyense* cells in this study show a mean concentration of 33 pg saxitoxin equivalents · $cell^{-1}$.

1. Introduction

Phytoplankton occur naturally throughout the world in both the marine and freshwater environments and it is common for some species to propagate to large concentrations. Impacts to fisheries from harmful algal blooms (HABs) have been observed in various regions of the world (White, 1980; Landsberg, 2002; Kim et al., 2004; Azanza et al., 2005; Doucette et al., 2006; Matsuyama, 2008). Blooms that occur in areas where salmon farming is conducted can result in the health of the caged salmon being compromised (Gowan et al., 1982; Mortensen, 1985; Black et al., 1991). Phytoplankton can affect salmon either through oxygen stress, physical damage to the gills and/or introduced toxins into the fish (Anderson et al., 2001; Tang and Au, 2004). Gill tissue is particularly vulnerable to injury from chemical and biotic agents due to its close contact with the external environment as well as its delicate structure (Bell, 1961).

Mortalities of farmed Atlantic salmon (*Salmo salar*) have been associated with Paralytic Shellfish Poisoning (PSP) toxins in Chile (Fuentes et al., 2008) and the Faroe Islands (Mortensen, 1985). On Canada's Atlantic coast, PSP toxins have been released by algal blooms in Nova Scotia in 2000 (Cembella et al., 2002) and in New Brunswick in 2003 and 2004 (Martin et al., 2006a, 2008a) with coincident economic losses occurring at several aquaculture sites.

Some diatom species (including *Chaetoceros* spp.) possess sharp, highly silicified, even barbed frustules, which have the potential to cause mechanical damage to the delicate fish-gill tissues (Rensel et al., 1989; Horner et al., 1990; Martin et al., 2001). The mode of irritation from many diatoms is the brittle setae from multi-celled chains (Kent et al., 1995; Landsberg, 2002). Other diatoms, such as *Chaetoceros socialis*, can also produce haemolytic substances that can cause gill damage or lesions by physical irritation to the epithelium, resulting in excessive mucus production which consequently leads to asphyxiation.

Although the species *Ditylum brightwellii* has not as yet been documented to have caused biological effects with fish, it co-occurred at high concentrations with a bloom of *Alexandrium fundyense* in the Grand Manan area of the Bay of Fundy when the salmon industry was experiencing losses during September, 2003 (Martin et al., 2008b). In 2002, the aquaculture industry reported loss of fish in the Passamaquoddy Bay region of New Brunswick, Canada when elevated concentrations of *Eucampia zodiacus* cells were observed (Martin et al., 2007).

We herein report the response of Atlantic salmon to exposure to high concentrations of selected local phytoplankton species (*A. fundyense*, *D. brightwellii*, *C. socialis* and *E. zodiacus*) under controlled laboratory conditions.

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2. Methods

2.1. Culture of algae

A. fundyense, *D. brightwellii* and *C. socialis* were isolated (J.L. Martin) from plankton tows taken near the Wolves Islands and in Passamaquoddy Bay, Bay of Fundy, Canada in 2003 (sampling sites shown in Fig. 1). These isolates were grown in aerated filtered seawater (St. Andrews Biological Station). Mass cultures were raised in glass carboys (18 L) at 12 °C and 12:12 L:D (cool white fluorescent lamps at irradiance level 27 μmol m^{-2} s^{-1}) and salinity 28–30 psu. *A. fundyense* was cultured in modified f/2 medium (−Si + Soil Extract); *C. socialis* and *D. brightwellii* were cultured in f/2 medium. When the cultures were in exponential growth phase, two or three cultures were combined.

E. zodiacus was collected by plankton tow from Brandy Cove, St. Andrews, New Brunswick during a significant bloom of this organism in November 2002.

The concentration of cells (cells L^{-1}) was then determined by counting sub-samples using a Palmer–Maloney chamber (Guillard and Sieracki, 2005). Appropriate volumes of algal culture were combined with filtered seawater in glass aquaria to produce 5 concentrations ranging from 1.0×10^5 to 2.0×0^6 cells L^{-1}.

While *E. zodiacus* was not isolated and cultured in bulk, it represented close to 99% of the total phytoplankton collected in November of 2002 when the bioassays were conducted.

2.2. Bioassays

Salmon smolts (150–380 g), provided by Stolt Sea Farms Inc., were maintained in dechlorinated municipal (Town of St. Andrews) water for several days after arrival then gradually transferred to full seawater over a period of 5 days. These fish were selected from stocks being transferred to cage sites and are therefore considered representative of local populations of cultured fish. The fish were held at ambient water temperature (7–13 °C), salinity (~30 psu) and natural photoperiod (L:D~11:13 h) at the St. Andrews Biological Station for 4 to 10 weeks until 1 week prior to exposure. The fish were fed a commercial salmon diet, pelleted food (Skretting, St. Andrews, NB), delivered by automatic feeders at the rate recommended by the feed manufacturer.

For the one week prior to testing, the salmon were held at 12 ± 1 °C. Bioassays were conducted with all four algal species and salmon to determine whether or not the algal cells were lethal to salmon and to bracket the lethal threshold. In these range-finding studies, salmon (n = 5) were exposed to either a high (equal to or greater than environmental levels) or low concentration of the alga or a control (no algae). Algal cultures were mixed with filtered seawater in 200 L glass aquaria to produce the targeted concentrations. The concentration of cells (cells L^{-1}) was then determined by counting sub-samples using a Palmer–Maloney chamber (Guillard and Sieracki, 2005). The salmon were placed in 150 L of water/algal mixtures and held for 24 h. The nominal exposure concentrations for range-finding tests were as follows: *A. fundyense*, 1.0×10^5 cells L^{-1} and 2.0×10^6 cells L^{-1}; *D. brightwellii*, 1.0×10^5 cells L^{-1} and 1.0×10^6 cells L^{-1}; *C. socialis*, 1.0×10^5 chains of cells L^{-1} and 4.0×10^6 chains of cells L^{-1} or *E. zodiacus*, 1.0×10^5 chains of cells L^{-1} and 9.0×10^5 chains of cells L^{-1}. During these tests water temperature was maintained at 12 ± 1 °C. Aeration was provided to each exposure tank and dissolved oxygen (DO) was monitored at 1, 3, 6, 12 and 24 h with an OxyGuard Handy Gamma DO meter. The range-finding bioassays were repeated 2 times for a total of 3 bioassays for each species.

As *A. fundyense* proved lethal to Atlantic salmon at the concentrations tested, definitive lethality tests (n = 5) were conducted with this alga. In each bioassay fish were exposed to one of 5 (nominal) concentrations ranging from 1.0×10^5 cells L^{-1} to 2.0×10^6 cells L^{-1} of *A. fundyense* or an algae-free control in 200 L glass aquaria filled to 150 L with water/algal culture mixtures. Lethality tests were of 24 h duration, water temperature was maintained at 12 ± 1 °C and salinity was 30 psu.

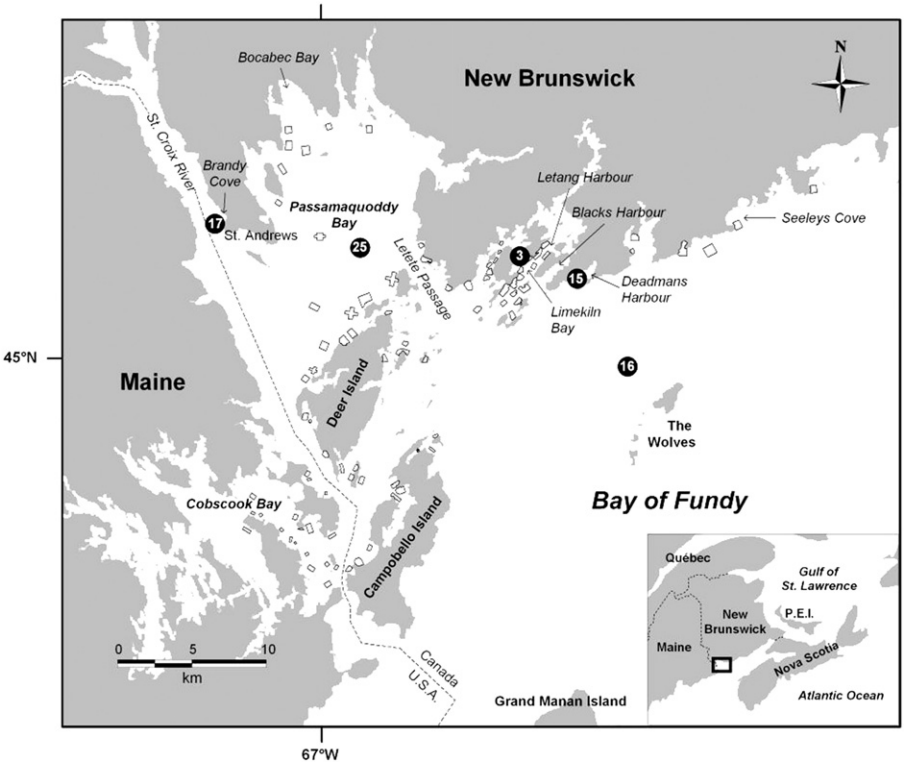

Fig. 1. Map of Fundy Isles area of southwest New Brunswick. Numbered circles are regular sampling sites from Fisheries and Oceans Canada's phytoplankton monitoring program and are the sites where algal samples were collected.