

# **Toxic Algae and Phycotoxin Testing during the Right Whale Mortality Event, 2017**

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## Abstract

Scarratt, M., Michaud, S., Starr, M., Couture, J.-Y., and Blais, M. 2017. Toxic Algae and Phycotoxin Testing during the North Atlantic Right Whale Mortality Event, 2017. Can. Manuscr. Rep. Fish. Aquat. Sci. 3128: iii + 8 p.

Following reports of several carcasses of North Atlantic Right Whale in the Gulf of St. Lawrence in early summer 2017, plankton samples from the affected area were tested for the presence of known toxic species and of *saxitoxin* and its derivatives (paralytic shellfish poisoning - PSP). Phytoplankton cells of *Alexandrium tamarense* and *A. ostenfeldii* (PSP producers), of *Dinophysis acuminata*, *D. norvegica*, and *Prorocentrum minimum* (Diarrhetic shellfish toxins producers), and *Pseudo-nitzschia seriata* (Amnesic shellfish toxin producer), were present but only in low abundances at some stations and with no evidence of a major bloom. Zooplankton samples tested negative for PSP toxins, except for trace concentrations at 8 stations out of 32. These results, coupled with the absence of mortalities of other marine species, suggest phycotoxins are unlikely to have been a cause of this event.

## Résumé

Scarratt, M., Michaud, S., Starr, M., Couture, J.-Y., and Blais, M. 2017. Toxic Algae and Phycotoxin Testing during the North Atlantic Right Whale Mortality Event, 2017. Can. Manuscr. Rep. Fish. Aquat. Sci. 3128: iii + 8 p.

Suite au signalement de plusieurs carcasses de baleine noire de l'Atlantique Nord dans le golfe du Saint-Laurent au début de l'été 2017, des échantillons de plancton issus du secteur touché ont été analysés afin de déceler la présence d'espèces toxiques connues et celles de *saxitoxine* et ses dérivés (intoxication par phycotoxine paralysante – IPP). Des cellules de phytoplancton d'*Alexandrium tamarense* et *A. ostenfeldii* (producteurs d'IPP), de *Dinophysis acuminata*, *D. norvegica* et *Prorocentrum minimum* (producteurs de toxines diarrhétiques) et de *Pseudo-nitzschia seriata* (producteur de phycotoxine amnestique) étaient présentes, mais seulement en faible abondance à certaines stations et sans preuve de prolifération majeure. Les échantillons de zooplancton analysés ne contenaient pas de toxines IPP, à l'exception de concentrations à l'état de traces relevées à huit des 32 stations. Ces résultats, combinés à l'absence de mortalité chez d'autres espèces marines, suggèrent qu'il est peu probable que les phycotoxines aient été la cause de cet événement.

## Context

The presence of phycotoxins in the marine food web, produced by various toxic phytoplankton species, has previously been shown to be a cause of mortality in cetaceans and other marine mammals (Flewelling et al. 2005; Lefebvre et al. 2010; Starr et al. 2017). Sub-lethal exposure to phycotoxins may also contribute to the accidental deaths of marine mammals by modifying their behaviour or mobility, rendering them more vulnerable to entanglement in fishing gear or collision with ships, and possibly affecting their reproduction. Phycotoxins enter the cetacean food web via vectors such as the copepod *Calanus finmarchicus* (Leandro et al., 2010). The North Atlantic Right Whale (NARW) *Eubalaena glacialis* in the Gulf of Maine has been shown to be vulnerable to chronic sub-lethal phycotoxin intoxication by Paralytic Shellfish Poisoning (PSP) via its copepod prey (Durbin et al., 2002). The Southern Right whale *Eubalaena australis* in Argentina has been shown to be exposed to the powerful phycotoxin domoic acid, responsible for Amnesic Shellfish Poisoning (ASP) while feeding in their calving ground (D'Agostino et al., 2017).

Reports of unusual numbers of dead NARW in the Gulf of St. Lawrence in June and July 2017 prompted efforts by DFO and partners to recover and sample the carcasses and to determine the causes of the event. The results of these investigations have been reported by Daoust et al., 2017. From the outset, questions were raised as to whether these mortalities might be linked to blooms of toxic phytoplankton which are known to occur in the region and which have been implicated in marine mammal mortalities in the past (Starr et al., 2017). To address this possibility, we proceeded to consolidate any available sources of samples to determine if phycotoxins could have played a role in this mortality event. The present report focusses on the results of phytoplankton and zooplankton sampling in the area where the whale carcasses were found.

## Methods

### Sample collection:

To complement the toxin testing of tissues from the dead NARW already undertaken by DFO-Gulf Region (Daoust et al., 2017), we tested the potential food for those marine mammals. Samples of phytoplankton and zooplankton from the area where the carcasses were found were obtained via two sources (Fig. 1).

1. **Phytoplankton** samples from the regular harmful algae monitoring program (Programme de monitoring des algues nuisibles PMAN) of Maurice Lamontagne Institute (MLI). This program provided weekly phytoplankton samples during almost the entire ice-free season from several coastal stations around the Gaspé Peninsula and the Québec North Shore. Among them, we tested samples from Mont-Louis, Gaspé, Gascons and Sept-Îles stations (Figure 1, black dots).
2. **Phytoplankton** and **zooplankton** samples from the annual Atlantic Zonal Monitoring Program (AZMP)/mackerel survey mission. A scientific team aboard the research vessel CCGS *Teleost* had passed through the region of the Magdalen Shallows in early- to mid-June, roughly simultaneous with the time the first mortalities are presumed to have occurred. During this survey, preserved phytoplankton samples were collected at regular AZMP stations (Fig. 1, red dots, transect names indicated), including twice at the Shediac Valley buoy station. Zooplankton samples from 32 stations of the mackerel survey grid

(Fig. 1, blue stars), covering essentially the entire area west of the Magdalen Islands and north of Prince Edward Island (PEI) as far as Gaspé/Chaleur Bay were also collected.

### Sample treatment:

#### Phytoplankton

Phytoplankton samples were analysed by light microscopy for the presence of known toxic species. Cells of the following toxin producers present in the samples were identified to the species level with confidence and cellular counts were performed:

- As known producers of Paralytic Shellfish Poisoning (PSP), cells of *Alexandrium tamarense* and *Alexandrium ostenfeldii* were found.
- As a known producer of Amnesic Shellfish Poisoning (ASP), cells of *Pseudo-nitzschia seriata* were found.
- As known producers of Diarrhetic Shellfish Poisoning (DSP), cells of *Dinophysis acuminata*, *D. norvegica* and *Prorocentrum minimum* were found.

#### Zooplankton

We looked for the presence of saxitoxin, a potent neurotoxin, and some of its derivatives that constitute the paralytic shellfish toxin family in the zooplankton samples. The toxins were measured using an Enzyme-Linked Immunosorbant Assay (ELISA) method (Abraxis Inc.). This assay has proven to be sensitive, reliable, and rapid in similar situations in the past (Starr et al. 2017).

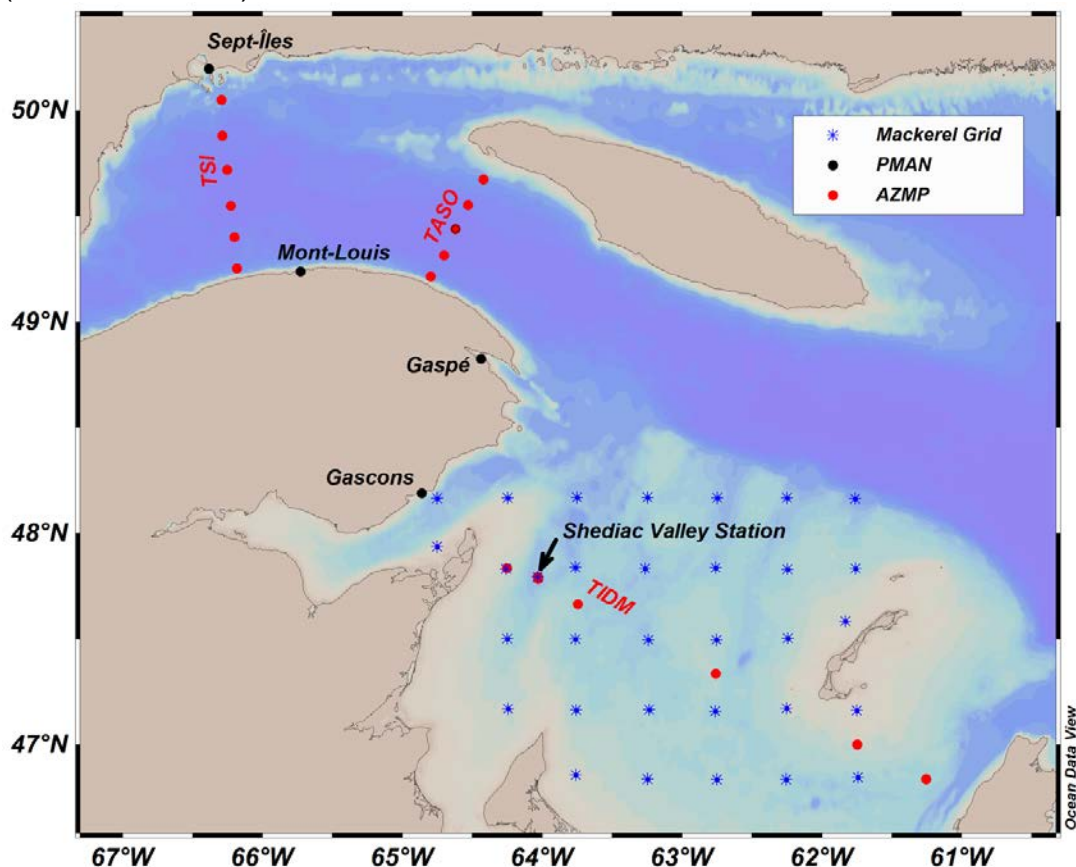


Figure. 1: Map of sampling stations

## Results and Discussion:

### Phytoplankton samples:

Results of identification and counts of phytoplankton cells in samples from late May, June and early July revealed the presence of toxic algae cells but in relatively low abundance and with no evidence of notable blooms in progress or in decline (Table 1).

PSP producers *Alexandrium tamarens* and *A. ostenfeldii* were found in relatively low concentrations at almost stations and transects investigated. *A. tamarens* is the organism responsible for the major 2008 toxicity event in the St. Lawrence Estuary (Starr et al., 2017). During the 2008 event, multispecies mass mortality of marine fauna was observed, including several species of cetaceans. The mass mortality coincided with a peak of abundance of *A. tamarens* of 80000 cells per litre in August 2008. In 2017, from late May to the beginning of July, cell abundance of *A. tamarens* ranged from 20 to 1120 cells per litre (Table 1a). At the Sept-Îles coastal station, the abundance slowly increased from 100 cells per litre at the end of May to 1120 cells per litre at the end of June. At the Gascons coastal station, the abundance ranged from 160 to 940 cells per litre, the maximum being reached in the first week of June. It is well known that the St. Lawrence *A. tamarens* strain is a very powerful producer of paralytic toxins. It has been demonstrated that 1000 cells per liter is sufficient to raise PSP toxins in mussel tissues and trigger mandatory shellfish harvesting closures (Blasco et al., 2003). During the period of concern, the threshold of 1000 cells per litre of *A. tamarens* was reached once at the end of June at Sept-Îles. However, while this level is susceptible to intoxicate shellfish, none of the other sampled stations indicated similar abundances, suggesting that there was not a widespread bloom. The other PSP producer *A. ostenfeldii* was also present in relatively low abundance. Cell concentrations of *A. ostenfeldii* varied from 20 to 660 cells per litre, this maximum value was found at the coastal station Sept-Îles on June 7 (Table 1b). To our knowledge, *A. ostenfeldii* has never been involved in a PSP outbreak in the St. Lawrence Gulf and Estuary.

The Diarrhetic Shellfish Poisoning (DSP)-producing species *Dinophysis acuminata*, *D. norvegica* and *Prorocentrum minimum* were observed in the samples. It should be noted that while these species are known to be toxic, the clinical syndrome observed in humans is not fatal, and to our knowledge the toxins have never been associated with cetacean disease or mortality. The abundance of *D. acuminata*, *D. norvegica* and *P. minimum* reached a maximum of 3420, 6500, and 4480 cells per litre respectively from mid-June to beginning of July (Table 1c, d, e).

Domoic acid (DA) is the phycotoxin responsible for the Amnesic Shellfish Poisoning (ASP). In the study area, the DA producer is the diatom *Pseudo-nitzschia seriata*. Cells of *P. seriata* were present in the 2017 samples. *P. seriata* was present from time to time at Sept-Îles station at a maximum concentration of 1400 cells per litre. Traces of *P. seriata* were found at Mont-Louis and Gascons stations (80 cells per litre). The maximum abundance of *P. seriata* was measured on the Magdalen Islands transect (TIDM) at 1680 cells per litre in mid-June 2017 (Table 1f).



Table 1: Cell abundances of toxic phytoplankton species (cells per litre) in 2017

<b>a) <i>Alexandrium tamarense</i></b>							
day/month	Sept-Îles	Mont-Louis	Gaspé	Gascons	TSI	TASO	TIDM
15/05	100						
1, 2/06	180			160	20		60
7/06	340		20	940			
12/06	360		540	160			
16, 19/06	840	220*	40	840			940
30/06	1120	920*		640			

\*temporary cyst

<b>b) <i>Alexandrium ostenfeldii</i></b>							
day/month	Sept-Îles	Mont-Louis	Gaspé	Gascons	TSI	TASO	TIDM
24/05				40			
1, 2/06	60				20	20	40
7/06	660			400			
12/06	200		160	60			
19/06	240						
26/06	40	120		20			

<b>c) <i>Dinophysis acuminata</i></b>							
day/month	Sept-Îles	Mont-Louis	Gaspé	Gascons	TSI	TASO	TIDM
15, 24/05	80			60			
1, 2/06	140			240	20	40	200
6, 7/06	360		20	1020			
12/06	480		1300	520			
16, 19, 20/06	340	2000	1540	560			240
26, 29, 30/06	120	680	3420	1340			
4/07			260	100			

<b>d) <i>Dinophysis norvegica</i></b>							
day/month	Sept-Îles	Mont-Louis	Gaspé	Gascons	TSI	TASO	TIDM
15, 24/05	20			20			
1, 2, 3/06	100			20	60		200
6, 7/06				220			
12/06	140		140	60			
16, 19, 20/06	200	80	20	40			120
26, 29, 30/06			280	140			
4/07			100	6500			

<b>e) <i>Prorocentrum minimum</i></b>							
day/month	Sept-Îles	Mont-Louis	Gaspé	Gascons	TSI	TASO	TIDM
15, 24/05	20			60			
1, 2, 3/06	20	40		180	40	60	960
6, 7/06	460	320	20	140			
12/06	280	200	880	300			
16, 19, 20/06	100		4480	580			260
26, 29, 30/06	420		540	560			
4/07			20	2240			

<b>f) <i>Pseudo-nitzschia seriata</i></b>							
day/month	Sept-Îles	Mont-Louis	Gaspé	Gascons	TSI	TASO	TIDM
15, 24/05							
1, 2, 3/06							600
6, 7/06							
12/06				80			
16, 19, 20/06	160	80					1680
26, 29, 30/06	1400	80					
4/07							

### **Zooplankton samples:**

The zooplankton samples collected during the seasonal St. Lawrence survey were analysed using the Abraxis ELISA assay for the presence of PSP toxins (saxitoxin and its derivatives). Results show non-detectable to negligible concentrations of PSP in the zooplankton. In fact, under the conditions of dilution recommended by the ELISA kit manufacturer (1/1000), our results suggest that the zooplankton samples would have all tested negative for PSP. When tested at 1/250 dilution, only eight stations of a total of 32 were weakly positive at the detection limit or just above, the highest being only 2.8 µg per 100 g. At 1/250 dilution, the detection limit of the assay was 0.92 µg/100 g, and the regulatory limit for human consumption of seafood is 80 µg per 100 g, so these are very low levels. Some of those samples were also tested at an intermediate dilution of 1/500 and appeared to be negative, as expected.

The oral lethal dose in humans (LD50) for saxitoxin is around 5.7 µg STX per kg of body weight (Meyer 1953). Assuming a 40 000 kg whale consuming up to 4% of its body weight in zooplankton daily (Durbin et al., 2002), and that this zooplankton contained the maximum value observed here of 2.8 µg STX per 100 g, we estimate that the oral dose of this individual would be roughly equivalent to 1.1 µg STX per kg body weight. This represents less than one fifth of the lethal dose for humans. Assuming a lethal dose in whales similar to that observed in humans, this simple calculation shows that phycotoxin concentrations observed in zooplankton are unlikely to have been fatal for whales.

In addition, comparing similar results in the literature from the Bay of Fundy (Turner et al. 2000; Doucette et al., 2005, 2006) we note that the concentrations observed in the current case are more than an order of magnitude below levels previously observed in zooplankton and in the feces of living right whales, suggesting that the animals can tolerate levels far higher than those observed here.

Another point worth noting is that this mortality event appears to have been confined to right whales, whereas if a major toxic bloom were involved, we would expect to find carcasses of other species in the marine food web, including other mammals, as has been observed elsewhere (Starr et al., 2017). It is unlikely that a phycotoxin event would affect only one species of predator.

### **Conclusions:**

We tested samples of phytoplankton and zooplankton that may have been potential food for the right whales that were found dead after migrating into the Gulf of St. Lawrence. The phytoplankton cells of *Alexandrium tamarense* and *A. ostenfeldii* (PSP toxins), of *Dinophysis acuminata*, *D. norvegica*, and *Prorocentrum minimum* (DSP toxins), and *Pseudo-nitzschia seriata* (ASP toxins), were all present, but in relatively low abundances and at some stations only. The zooplankton samples tested negative for PSP toxins, with the exception of trace concentrations found in 8 samples out of 32. Those results suggest that intoxication from the plankton was not implicated in this mass mortality. This is consistent with the results obtained from the Canadian Food Inspection Agency who found no phycotoxins in tissue samples obtained by necropsy from several of the right whale carcasses (Daoust et al., 2017).

While the evidence we present here does not support a conclusion of acute intoxication, we recognise the possibility that the whales may have become intoxicated outside the Gulf of St. Lawrence during their migration. In this context it is worth noting that the east coast of the United States has experienced large blooms of *Pseudo-nitzschia* spp. in late 2016 when the

Division of Marine Fisheries in the Commonwealth of Massachusetts posted [Information regarding the current shellfish area closures in Massachusetts associated with Amnesic Shellfish Poisoning](#) and the Providence Journal reported that [Mysterious toxic algae that shut down RI shell fishing last fall is back](#). Large blooms of *Alexandrium* spp. were also present in the Gulf of Maine during the spring and summer of 2017 when the Portland Press Herald reported [Red tide prompts state to close many clam flats](#).

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