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Phycotoxin analyses in St. Lawrence Estuary beluga

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ABSTRACT

The St. Lawrence Estuary is frequently subjected to harmful blooms (colloquially termed “red tides”) of the toxic dinoflagellate *Alexandrium tamarense* which can cause outbreaks of Paralytic Shellfish Poisoning (PSP), with consequent health risks for marine organisms and humans. PSP toxins can be transferred throughout the food web, and have been observed in a wide variety of organisms other than shellfish, both in the St. Lawrence Estuary and elsewhere. PSP and other algal toxins have been implicated in the mortalities of various marine mammal species around the world, and their potential effects on the endangered population of St. Lawrence Estuary beluga is a matter of concern, particularly in light of an intense bloom of *A. tamarense* in 2008 which coincided with an unusually high number of beluga mortalities in a short period. Between 1995 and 2012, 66 beluga carcasses stranded in the Estuary were tested for the presence of PSP toxins using High Performance Liquid Chromatography (HPLC) and Enzyme-Linked ImmunoSorbent Assay (ELISA) techniques. Overall, 18 of the 66 animals tested positive for the presence of saxitoxins in one or more tissues, 6 of these during the 2008 red tide. A total of 7 animals showed positive results in more than one tissue. All positive results occurred in years when *A. tamarense* was relatively abundant, and there is a positive relationship between the normalized anomalies in the number of mortalities and cumulative *A. tamarense* abundance. The simultaneous occurrence of PSP toxins in prey species and beluga digestive tracts indicates that beluga were ingesting toxic prey, while the absence of other evident illnesses or lesions (determined by necropsy) in at least one of these animals suggests that intoxication may have been the ultimate cause of death. We conclude that algal toxins should be considered as potential factors in any unexplained marine mammal mortalities. The potential effects of both major toxic algal blooms and chronic low-level exposure to toxins on vulnerable marine mammal populations should be of concern, especially in the context of climate change and the apparently increasing frequency of toxic algal blooms worldwide.

Analyse des phycotoxines dans les bélugas de l'estuaire du Saint-Laurent

RÉSUMÉ

L'estuaire du Saint-Laurent est souvent sujet à des proliférations nuisibles (appelées familièrement « marées rouges ») du dinoflagellé toxique *Alexandrium tamarense*, qui peut causer des épidémies d'intoxication par phycotoxine paralysante, ce qui entraîne d'importants risques pour la santé des organismes marins et des êtres humains. Les toxines responsables de l'intoxication par phycotoxine paralysante peuvent être transmises par le réseau trophique, et il a été observé chez une panoplie d'organismes autres que des mollusques et des crustacés, dans l'estuaire du Saint-Laurent et ailleurs. Les toxines responsables de l'intoxication par phycotoxine paralysante et d'autres toxines provenant d'algues sont en partie responsables de la mort de mammifères marins de diverses espèces dans le monde entier, et leurs effets potentiels sur la population en voie de disparition de béluga de l'estuaire du Saint-Laurent constituent un sujet préoccupant, surtout à la suite de la forte prolifération de *A. tamarense* survenue en 2008, qui a coïncidé avec un nombre inhabituellement élevé de mortalités de bélugas sur une brève période. Entre 1995 et 2012, on a soumis à des tests 66 carcasses de bélugas s'étant échouées dans l'estuaire afin de déceler la présence des toxines responsables de l'intoxication par phycotoxine paralysante au moyen de techniques de chromatographie liquide à haute performance (CLHP) et de dosage immunoenzymatique. Au total, 18 des 66 animaux ont affiché des résultats positifs à la présence de saxitoxines dans un ou plusieurs échantillons de tissus, dont six durant la marée rouge de 2008. Un total de sept animaux ont affiché des résultats positifs dans plus d'un échantillon de tissus. Tous les résultats positifs ont été obtenus au cours des années où *A. tamarense* était relativement abondant, et il existe un lien positif entre les anomalies normalisées dans le nombre de mortalités et l'abondance cumulative de *A. tamarense*. L'occurrence simultanée des toxines responsables de l'intoxication par phycotoxine paralysante chez les espèces de proies et dans l'intestin de bélugas indique que les bélugas consommaient des proies intoxiquées, et l'absence d'autres maladies ou lésions évidentes (déterminées par une nécropsie) chez au moins un de ces animaux révèle que l'intoxication était probablement la cause ultime de la mort. Nous en avons conclu que les toxines des algues doivent être considérées comme des facteurs potentiels des mortalités inexplicables de mammifères marins. Les effets potentiels des proliférations d'algues toxiques et de l'exposition chronique de faible intensité aux toxines sur les populations vulnérables de mammifères marins devraient être considérés comme préoccupants, surtout dans le contexte du changement climatique et de la fréquence visiblement croissante des proliférations d'algues toxiques à l'échelle mondiale.

1.0 INTRODUCTION

Like many other temperate coastal areas, the St. Lawrence Estuary is subject to recurrent blooms of the harmful dinoflagellate *Alexandrium tamarense* (formerly *Gonyaulax tamarensis*), a producer of paralytic shellfish toxins which include saxitoxin (STX) and its derivatives (Fig. 1). The cell concentration (abundance) of the dinoflagellate varies greatly, from a few cells per litre to millions under favourable environmental conditions. Dense blooms are colloquially known as red tides, due to the rusty colour imparted to the surface waters by these strongly pigmented cells. Red tides and their link with paralytic shellfish poisoning (PSP) in humans have been known for millennia, and the role of *A. tamarense* and other related species as causative agents has been recognized since at least the 1930s (Sommer et al. 1937), and documented in Canadian waters since the 1940s (Needler 1949). Early research also linked red tides to mass mortalities of fish (Connell & Coss 1950), suggesting that PSP toxins could bioaccumulate elsewhere in the food web. Further research has confirmed that PSP toxins can cause intoxication and/or mortality in a wide range of species including fish (White 1980), birds (Coulson et al. 1968), pinnipeds (Hernández et al. 1998; Reyero et al. 1999) and cetaceans (Geraci et al. 1989; Doucette et al. 2006). In addition to PSP toxins, other phycotoxins produced by various species of algae have been implicated in mortalities of birds, fish and marine mammals around the world (Trainer and Baden 1999; Scholin et al. 2000; Flewelling et al. 2005; Fire et al. 2007).

The strain of *A. tamarense* native to the St. Lawrence Estuary is noted for being extremely toxic. As few as 1000 cells L⁻¹ are sufficient to cause toxicity in mussels above the legal harvest limit (80 µg of toxin 100 g⁻¹ of tissue) (Blasco et al. 1998). The Canadian Food Inspection Agency (CFIA) regularly monitors toxicity levels in shellfish during the summer months and harvesting areas in the Estuary are routinely closed to protect public health. Mortalities of fish and birds are occasionally reported by the public and specimens have been brought to the Maurice Lamontagne Institute (MLI) for testing. Beginning in 1995, we have tested stranded marine mammal carcasses, with particular emphasis on beluga (*Delphinapterus leucas*) for the presence of PSP toxins. Three major red tides have occurred in the past two decades, in 1996, 1998 and 2008. During the exceptional red tide of August 2008, 7 beluga were found dead in one week. To date a total of 66 beluga carcasses from the St. Lawrence Estuary have been tested for PSP toxins.

1.1 PARALYTIC SHELLFISH TOXINS (SAXITOXIN AND RELATED MOLECULES)

Paralytic toxins were the first class of so-called « shellfish » toxins to be chemically characterized and remain the best known and most prevalent worldwide. Although produced by phytoplankton cells, they were originally isolated from the Alaskan butter clam *Saxidomus giganteus* (Schantz et al. 1957), and are often collectively referred to as *saxitoxins*. The class is now known to comprise over twenty related molecular structures of substituted purines (Fig. 1). The potency of these neurotoxins ranges two orders of magnitude from the most toxic “carbamate” group that includes saxitoxin (STX), neosaxitoxin (NEO) and gonyautoxins (GTX) 1 through 4, to the least toxic N-sulfocarbamoyl analogues, the decarbamoyl group being of intermediate toxicity (Doucette et al. 2006). All share the same mode of action as *sodium channel blockers* which interrupt the transmission of nerve impulses, especially in vertebrates. Saxitoxin is extremely toxic, with an oral lethal dose (LD50) in humans of 5.7 µg kg⁻¹. Intoxication causes acute paralysis which leads to death from respiratory failure.

2.0 TOXICITY TESTING METHODS

Detection of PSP toxins is challenging by virtue of their wide spectrum of different chemical analogues. A number of analytical methods are currently available, the most prevalent of which are liquid chromatography (formerly High Performance Liquid Chromatography - HPLC) and Enzyme Linked ImmunoSorbent Assay (ELISA) each offering different advantages and disadvantages (see below). The data in this report have been generated using both of these methods and caution must be used when comparing results from different years, since the specificity, sensitivity and detection limits of the two methods are different.

2.1 HPLC

In the late 1990's and early 2000's, the measurements done at MLI were performed using HPLC with post-column oxidation and fluorescence detection, according to the method of Sullivan and Wekell (1987). Tissue samples were extracted in dilute acetic acid (0.1 M) and analyzed on a Varian HPLC 5000. In general, HPLC detection of PSP toxins offers excellent discrimination between the different toxin structures but has the disadvantages of relatively slow processing times, high infrastructure and operating costs, and the need for specialized operator training. The HPLC configuration used at MLI during this period provided a detection limit of approximately 10 µg STX 100 g⁻¹ of tissue.

2.2 ELISA

ELISA is commonly employed for the detection of many types of biomolecules. It offers the advantages of relatively low cost and rapid processing times using common spectrophotometric plate readers. The method gives an overall picture of the toxicity of the sample but is not able to reveal which of the numerous PSP toxins is present. Moreover its sensitivity varies by up to two orders of magnitude between those derivatives, being most sensitive to STX and least sensitive to NEO, GTX 1 and GTX 4. Thus it is only semi-quantitative in samples where a mixture of toxins is present and may underestimate the actual toxin concentration. It is, however, quite sensitive overall, with detection limits in the range of 1 – 3 µg STX per 100 g⁻¹ of tissue, at typical sample dilution levels. Owing to its simplicity and relatively low cost, ELISA has been the method of choice at MLI since 2008, replacing the Varian HPLC system which had become obsolete.

To complement and validate the ELISA assays performed during the 2008 red tide, selected samples were analysed using advanced, more sensitive HPLC techniques (LCMS-LCFLD) at the Institute for Marine Biosciences, Halifax. (Starr et al., unpublished data¹).

2.3 COMPARISON BETWEEN THE TWO METHODS

Since the analytical protocols were originally developed for testing shellfish destined for human consumption, the results are expressed in the customary units for this purpose: µg STX equivalent per 100 g of tissue. As discussed above, the nature of the ELISA test means that the toxin concentration expressed in "STX equivalents" may be underestimated in samples where a

¹ Maurice Lamontagne Institute, Mont-Joli, Qc, G5H 3Z4

mixture of toxins is present. The HPLC method can measure all the toxin analogues individually, but since their toxicity varies, a simple sum of their concentrations does not necessarily provide a good index of the actual toxicity of the sample. HPLC results are therefore weighted using the relative toxicities of the analogues (originally determined by mouse bioassay) (Oshima and Yasukatsu 1995) and the results thus expressed in STX equivalents give a more accurate representation of the actual toxicity of the mixture. Notwithstanding these differences, we have chosen to report the toxin concentrations from both methods in units of $\mu\text{g STX eq. } 100 \text{ g}^{-1}$ to facilitate comparison of samples in different years and to provide a measure of the total concentration of toxins present, but the reader must bear in mind that the comparison is not absolutely direct.

3.0 SAMPLE COLLECTION

3.1 TISSUE SAMPLING AND EXTRACTION

A total of 66 beluga carcasses of all ages (newborn calf to adult) were sampled between 1995 and 2012, with the majority being sampled since the start of systematic testing in 2008. Most animals (46) were female, only 19 were male. One was of undetermined sex. Testing initially focussed on liver tissue (58/66 animals), however during the exceptional red tide of 2008 (and thereafter) we have systematically tested additional tissues, including brain (18 animals), stomach (including stomach contents, if any: 21 animals), feces (2 animals), intestine (5 animals), kidney (28 animals), blood from the heart (20 animals) and urine (2 animals). In the winter of 2013 we conducted a retrospective analysis of frozen samples from 2004 onwards to test for the presence of toxins in mammary gland tissue (22 animals) and milk (5 animals).

Tissue samples were collected either in the field at the stranding site, or during necropsy at the Faculté de médecine vétérinaire, Université de Montréal, in Ste-Hyacinthe, QC. Tissues were transported, usually frozen, to MLI for sub-sampling and analysis. Depending on the condition of the carcass, a maximum 50 g of each tissue were collected and frozen (-80°C) in plastic centrifuge tubes or Whirl-pak bags to await extraction and testing.

Thawed sub-samples (5 – 15 g) were homogenized in an equal volume of cold acetic acid (0.1 M) using a blender or tissue grinder and centrifuged to remove the solids. The supernatants were transferred to microcentrifuge tubes for serial dilution prior to toxin testing, either by HPLC or ELISA methods. Acetic acid extraction was used, even for the ELISA method (in spite of the recommended HCl extraction for commercial shellfish testing), to permit further testing of the same samples for individual toxins via HPLC if necessary. The alternative (HCl) method converts N-sulfocarbamoyl toxins into their hydrolysed analogues, and potential information on the toxin composition is lost.

3.2 ELISA TESTING PROTOCOL

Acetic acid extracts were assayed using ELISA kits for saxitoxin (Abraxis LLC, Warminster, PA, USA). Samples, standards and controls were diluted according to the ELISA kit instructions, loaded in duplicate onto the microtiter plate pre-coated with secondary antibody and incubated with peroxidase enzyme conjugate and saxitoxin antibodies for 30 min at room temperature. The plate was then washed with a buffer solution and incubated for another 30 min with enzyme substrate (colour) solution. After stopping the reaction with H_2SO_4 , colour development

was determined spectrophotometrically at 490 nm on a microplate reader and concentrations were calculated against the standard curve response.

3.3 ABUNDANCE OF TOXIC ALGAE

The MLI has maintained a harmful algae monitoring program at several sites around the St. Lawrence Estuary and elsewhere in the Gulf of St. Lawrence from 1994 until 2011. Specimens were collected weekly at coastal locations (typically public wharves and aquaculture sites) via a surface bucket sample and a vertical plankton net tow. Phytoplankton samples were preserved using Lugol's iodine and counted with an inverted microscope according to the protocol of Lund et al. (1958). This report uses algal abundances from two monitoring stations in the Estuary: Tadoussac (located within the region of critical beluga habitat) and Ste-Flavie (MLI pier), approximately 125 km to the east.

4.0 RESULTS

The toxin concentrations in tissues from all 66 beluga carcasses tested are shown in Table 1. The detection limit for each assay was determined based upon standard criteria (3x chromatogram baseline noise amplitude in the case of HPLC, and the threshold level of the lowest-concentration standard in the case of ELISA). Results lower than detection limits are considered as negative for the presence of PSP toxins and are indicated as 0.0 in the table.

Positive test results were not evenly distributed amongst the different tissues, and some tissues (mammary gland, milk and urine) showed no positive results, even when other tissues from the same animal tested positive in some cases. The results can be summarized as follows: Liver (1/58 positive), brain (13/18 positive), stomach (9/21 positive), feces (1/2 positive), intestine (2/5 positive), kidney (3/28 positive), heart blood (1/20 positive), mammary gland (0/22 positive), milk (0/5 positive) and urine (0/2 positive).

In liver, only one positive result of $2.7 \mu\text{g STX eq } 100 \text{ g}^{-1}$ tissue was observed during the red tide of 2008. Brain results remained generally low and near the detection limit (maximum $1.5 \mu\text{g STX eq } 100 \text{ g}^{-1}$), and are thus only weak positives, all being observed in recent years. The only two brain samples tested in 2008 were both negative by ELISA, while one was positive by HPLC. The highest toxin concentrations were observed during the 2008 red tide in stomach (maximum $63.2 \mu\text{g STX eq } 100 \text{ g}^{-1}$) and feces samples (maximum $56.5 \mu\text{g STX eq } 100 \text{ g}^{-1}$), with lower results for intestine (maximum $4.3 \mu\text{g STX eq } 100 \text{ g}^{-1}$), kidney (maximum $7.3 \mu\text{g STX eq } 100 \text{ g}^{-1}$) and heart blood (maximum $5.1 \mu\text{g STX eq } 100 \text{ g}^{-1}$). Overall, 18 of the 66 animals tested positive for the presence of saxitoxins in one or more tissues, 6 of these during the 2008 red tide. A total of 7 animals showed positive results in more than one tissue, 3 of these being highly-intoxicated specimens from the 2008 event.

Necropsies were performed on 61 of 66 stranded beluga using standard laboratory techniques (Lair et al. 2014) to determine the cause of death (COD), which is also listed in Table 1. No apparent cause (COD=7) was determined in 11 of the beluga examined at necropsy. None of these animals with undetermined COD tested positive for saxitoxins. However, the necropsy of one beluga carcass from the 2008 red tide (DL-05-2008) failed to find any other significant pathologies or injuries and the diagnosed COD is listed as "probable STX intoxication" based upon the circumstances of the event (Lair et al. 2014). This animal tested negative for saxitoxins via ELISA, but positive via HPLC.

Figure 2 shows the weekly abundance (cell concentration) of *A. tamarens* at Tadoussac from 1995 to 2011. Annual peaks in cell concentration are observed each summer, but are highly variable from year to year. The data also suggest a long-term trend of increasing maximum bloom concentrations. The highest cell concentrations occurred in 2008 with a maximum value of 74000 cells L⁻¹.

Table 2 shows the normalized anomalies (in standard deviations) of total annual beluga mortalities and the cumulative weekly abundance of the toxic dinoflagellate *A. tamarens* at Tadoussac and Ste-Flavie for each year of the study. Data from 2010 were excluded because sampling began on 26 July, which is very late in the season and could have allowed significant *A. tamarens* abundances to be missed. Over the period of 1995 – 2011, the average cumulative abundance of *A. tamarens* observed before 26 July was 51% of the annual cumulative abundance. In 5 of 16 years, the figure exceeded 90%. Note also that at the time of publication there were no *A. tamarens* data available for 2012.

Figure 3 shows the occurrence of positive and negative toxin tests for each animal compared with the cumulative abundance of *A. tamarens* at Tadoussac during the same year. The year 2008 was clearly exceptional with the highest cumulative abundance of *A. tamarens* for the entire time series. Positive tests occurred only in years where *A. tamarens* was present. These data suggest that beluga are being intoxicated on a punctual basis as a result of high *A. tamarens* abundance but there is no significant long-term accumulation of phycotoxins in their tissues.

5.0 DISCUSSION

The data show that PSP toxins accumulate in various body tissues of the St. Lawrence Estuary beluga, although the levels are highly variable from tissue to tissue and from year to year. The presence of high toxin levels in the stomach and feces, as well as other tissues during the red tide event of 2008 strongly indicates that the beluga were ingesting toxic prey, although the uptake of toxin into body tissues was highly variable. For example, ELISA analyses of liver produced only one positive test, even though many of these animals tested positive in other tissues. The degree to which PSP toxins are absorbed and retained by mammalian liver is unknown, although it is known to accumulate in the livers of fish such as mackerel (*Scomber scombrus*) (Castonguay et al. 1997). The results are nevertheless consistent with the hypothesis that the beluga experienced acute poisoning. Low-level STX concentrations in virtually all the brain tissue samples tested, including many individuals with positive stomach tests and in years with relatively lower *A. tamarens* abundance, suggest that chronic low-level exposure may be present. Unfortunately the time series for brain tissue is short and covers only recent years, so we cannot fully evaluate this possibility, but we have confidence that these observations are reliable, since similar samples collected from 8 hunted beluga at Sanikiluaq (Hudson Bay) in 2008 showed no positive results for any tissues, including brain (data not shown). There is no record of toxic phytoplankton blooms in Hudson Bay and hence those animals should not have been exposed to any toxins.

There is very little information in the literature regarding the prevalence of saxitoxins in other species of cetaceans. Geraci et al. (1989) observed toxins in the kidney, liver and stomach contents of humpback whales (*Megaptera novaeangliae*) near Cape Cod, which were apparently derived from ingesting intoxicated mackerel. Doucette et al. (2006) observed toxins in the feces of North Atlantic right whales (*Eubalaena glacialis*) in the Bay of Fundy which were derived from intoxicated copepods. To our knowledge, the present observations in beluga

constitute the only other clearly established case of PSP toxins in a population of cetaceans. However, the transmission of PSP toxins to cetaceans is probably common, though its prevalence is difficult to estimate due to the paucity of observations. Note that we have also observed saxitoxins in single specimens of minke whale (*Balaenoptera acutorostrata*), northern bottlenosed whale (*Hyperoodon ampullatus*) and harbour porpoise (*Phocoena phocoena*) (S. Michaud, unpublished data²).

The red tide of 2008 was very intense and persistent, with high maximum cell counts (74000 cells L⁻¹ at Tadoussac in early August) and a total observed duration of at least 10 days. There had been numerous shellfish harvesting closures in the St. Lawrence Estuary throughout the season, indicating a high general abundance of *A. tamarensis* in the region. This situation provided ample time for beluga to be exposed to phycotoxins via their prey. A comprehensive testing program during August and September 2008 revealed very high levels of saxitoxins in a wide variety of other marine organisms, including invertebrates, fish, birds and seals. Indeed, the toxin levels in some fish and invertebrates were an order of magnitude higher than those considered safe for human consumption (Starr et al., unpublished data²). The wide distribution of the phycotoxins in the food chain coupled with the unusual mortality of a large number of beluga in a short period, several of which were shown to have high levels of toxins in their digestive tracts strongly suggests that poisoning was a contributing factor in these deaths. Not all the beluga carcasses were examined at necropsy, but in one case (DL-05-2008) pathologists were unable to find any other significant pathologies or injuries and the COD is listed as “saxitoxin intoxication” (Lair et al. 2014). Moreover, necropsy and toxicity data from other marine organisms including birds and seals was unequivocal in supporting death due to intoxication with no other significant pathological processes diagnosed in the majority of cases (Starr et al. unpublished data).

Evaluating the overall effect of phycotoxins on beluga mortality is difficult given a relatively sparse data set which contains many gaps, but 2008 was clearly an exceptional year, as the normalized anomalies show, and there is a slight positive correlation between the anomalies in mortality and cumulative *A. tamarensis* abundance at Tadoussac ($r^2 = 0.36$). It is also clear from the temporal distribution of positive toxin tests that when *A. tamarensis* is abundant there is a potential for PSP toxins to be transmitted through the food chain to beluga, and fatal intoxication could occur if highly contaminated prey are ingested.

One significant difficulty experienced in this investigation has been the effects of carcass deterioration on the preservation of the toxins. After analysing hundreds of samples from a wide variety of species, we have noticed that toxins are less likely to be detected in specimens which have begun to decompose, a particular problem when dealing with stranded marine mammal carcasses. Freshly-killed specimens are more likely to give positive results, and care must be taken to freeze samples quickly after collection, if toxin extraction cannot be immediately performed. It therefore seems plausible that the true number of intoxicated animals could be higher than we observed.

It is unclear what may be the role of sublethal PSP toxin exposure in terms of beluga survival. The prevalence in recent years of unusual mortalities of adult females (dystocia), deaths of dependent calves and other deaths of undetermined cause could be related to sub-lethal toxin exposure which renders the animals vulnerable to other stressors. For example, dependent

² Maurice Lamontagne Institute, Mont-Joli, Qc, G5H 3Z4

calves often show no significant pathological lesions and may have died due to abandonment, lack of maternal care, inability to nurse or to follow the mother, or the death of the mother (Lair et al. 2014). The persistent presence of low-level toxicity in beluga brain samples in 2011 and 2012 is interesting in this context. Further investigation to determine the potential role of intoxication in these phenomena is necessary.

Clearly the potential role of phycotoxins in beluga mortality should not be dismissed. The 2008 event demonstrates that this species is potentially vulnerable to the effects of toxic algal blooms, and observations of intoxication in other cetaceans underscore this vulnerability. The risk may be particularly acute in endangered species such as the St. Lawrence Estuary beluga or the North Atlantic right whale in the Bay of Fundy whose small, isolated populations could be significantly affected by a single intoxication event. During unusual mortality events, especially those involving multiple species of marine animals, phycotoxins should be considered as possible causes and appropriate analyses performed. Given climatic variability, oceanographic changes and the increasing frequency and severity of toxic algal blooms (Anderson et al. 2012) such monitoring is greatly needed, especially when other causes of death cannot be determined during standard necropsies.

6.0 CONCLUSIONS

- PSP toxins accumulate in various body tissues of the St. Lawrence Estuary beluga.
- The observed occurrence of beluga intoxication coincides with the presence of toxic algal blooms and strongly suggests that poisoning was a contributing factor in mortality.
- High toxin concentrations throughout the food web and in the beluga digestive tract during the 2008 red tide indicate that the beluga ingested toxic prey.
- There is a positive relationship between the anomalies in beluga mortalities and cumulative abundance of *A. tamarensis* at Tadoussac over the study period.
- Carcass deterioration causes significant challenges for the accurate measurement of toxin concentrations and may result in underestimation of the effects of toxic algal blooms on beluga.
- Low-level toxicity in brain tissue may indicate chronic sublethal exposure with unknown implications for beluga survival. Further investigation of this is recommended.
- Algal toxins should be considered as potential factors in any unexplained marine mammal mortalities.
- The potential effects of a major toxic algal bloom on an isolated, vulnerable marine mammal population should be of concern, especially in the context of climate change and the apparently increasing frequency of toxic algal blooms worldwide.

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8.0 TABLES

Table 1: Beluga carcasses tested for the presence of paralytic toxins since 1995. Values under tissue types indicate concentration of toxin in $\mu\text{g STX eq } 100 \text{ g}^{-1}$ of tissue; “-” indicates no data available (tissue not tested). Positive values are highlighted in red. A superscript symbol next to the toxin concentration value indicates a positive (+) or negative (-) result by HPLC (2008 only). Cause of death (determined by necropsy, Lair et al. 2014): 1) bacterial/viral infection; 2) parasitic infection; 3) malignant neoplasia (cancer); 4) neonatal (death of dependent calf); 5) dystocia (difficult birth, post-partum complications); 6) trauma (hit by boat, trapped in net); 7) not determined; 8) other; NA) not available (no laboratory necropsy performed)

Animal Number	Location	Date observed	Sex	Age	Liver	Brain	Stomach contents	Feces	Intestine	Kidney	Blood	Urine	Mammary	Milk	Cause of Death
DL-8-1995	Bic	1995-08-10	F	0	0.0	-	-	-	-	-	-	-	-	-	4
DL-9-1995	Pointe-au-Pic	1995-08-18	M	3	0.0	-	-	-	-	-	-	-	-	-	2
DL-1-1996	Escoumins	1996-04-10	M	42	0.0	-	-	-	-	-	-	-	-	-	1
DL-4-1996	Bic	1996-07-22	n.d.	34	0.0	-	-	-	-	-	-	-	-	-	1
DL-6-1996	Ste-Luce	1996-08-11	F	0	0.0	-	-	-	-	-	-	-	-	-	4
DL-8-1996	Petite Matane	1996-10-18	F	2	0.0	-	-	-	-	-	-	-	-	-	2
DL-2-1997	Ile aux Lièvres	1997-05-23	F	57	0.0	-	-	-	-	-	-	-	-	-	7
DL-3-1997	Riviere Ouelle	1997-06-08	F	43	0.0	-	-	-	-	-	-	-	-	-	1
DL-4-1997	Bic	1997-07-01	M	0	0.0	-	-	-	-	-	-	-	-	-	4
DL-5-1997	Ile St-Barnabé	1997-07-15	M	12	0.0	-	-	-	-	-	-	-	-	-	1
DL-2-1998	Ste-Flavie	1998-05-23	M	26	0.0	-	-	-	-	-	-	-	-	-	1
DL-3-1998	Anse-au-Persil	1998-05-24	F	59	0.0	-	-	-	-	-	-	-	-	-	3
DL-4-1998	Ste-Flavie	1998-05-24	F	42	0.0	-	-	-	-	-	-	-	-	-	3
DL-7-1998	Petite Matane	1998-08-07	M	36	0.0	-	-	-	-	-	-	-	-	-	2
DL-01-2004	Sainte-Luce	2004-05-10	F	12	-	-	-	-	-	-	-	-	0.0	-	2
DL-02-2004	Port-au-Persil	2004-06-04	F	60	-	-	-	-	-	-	-	-	0.0	-	3
DL-06-2005	Baie des Ha!Ha!, Saguenay	2005-09-11	F	48	-	-	-	-	-	-	-	-	0.0	-	7

Animal Number	Location	Date observed	Sex	Age	Liver	Brain	Stomach contents	Feces	Intestine	Kidney	Blood	Urine	Mammary	Milk	Cause of Death
DL-03-2006	Tadoussac	2006-09-21	F	35	-	-	-	-	-	-	-	-	0.0	-	5
DL-02-2007	Sainte-Luce	2007-05-27	F	44	-	-	-	-	-	-	-	-	0.0	-	7
DL-05-2007	Trois Pistoies	2007-06-08	M	0	0.0	-	-	-	-	0.0	-	-	-	-	4
DL-06-2007	Saint-Simon-sur-mer	2007-08-17	F	55	-	-	-	-	-	-	-	-	0.0	-	3
DL-08-2007	Rimouski	2007-09-11	F	38	-	-	-	-	-	-	-	-	0.0	-	7
DL-01-2008	Rivière Ouelle	2008-05-03	M	21	0.0	-	-	-	-	0.0	0.0	-	-	-	8
DL-02-2008	Portneuf-sur-Mer	2008-06-07	F	37	0.0	-	0.0	-	4.3 ⁻	0.0	0.0	-	-	0.0	3
DL-03-2008	Escoumins	2008-06-16	F	52	0.0	-	-	-	-	0.0	0.0	-	-	0.0	8
DL-05-2008	Saguenay	2008-08-11	F	50	0.0 ⁻	-	0.0 ⁺	-	0.0 ⁺	0.0	0.0	-	0.0	-	8 tox.
DL-06-2008	Ile Rouge	2008-08-13	F	0	0.0	-	-	-	-	-	-	-	-	-	4
DL-07-2008	Ste-Flavie	2008-09-18	F	57	0.0	-	-	-	0.0	0.0	5.1 ⁺	-	-	-	8
DL-110-2008	St-Simon	2008-08-11	F	22	0.0 ⁺	-	63.2 ⁺	-	-	7.3 ⁺	-	-	-	-	NA
DL-111-2008	Ile aux Basques	2008-08-11	F	36	0.0	0.0	-	-	3.5	0.0	-	-	-	-	NA
DL-112-2008	Ile Verte	2008-08-11	M	13	2.7 ⁺	-	16.1 ⁺	-	-	6.6 ⁺	-	-	-	-	NA
DL-113-2008	Ste-Flavie	2008-08-15	F	39	-	0.0	-	0.0 ⁺	-	0.0 ⁺	-	-	-	-	NA
DL-116-2008	Ste-Luce	2008-09-10	F	11	-	-	0.0 ⁺	56.5 ⁺	-	2.7 ⁺	-	-	-	-	NA
DL-01-2009	Ste-Luce	2009-06-16	F	12	0.0	-	-	-	-	-	-	-	-	-	8
DL-02-2009	Tadoussac	2009-07-29	M	2	0.0	-	-	-	-	-	-	-	-	-	7
DL-03-2009	Ste-Flavie	2009-08-01	M	49	0.0	-	-	-	-	-	-	-	-	-	2
DL-04-2009	Matane	2009-09-03	M	0	0.0	-	-	-	-	-	-	-	-	-	6
DL-05-2009	Pointe-au-Père	2009-09-15	F	48	0.0	-	-	-	0.0	-	-	-	0.0	-	7
DL-01-2010	St-Ulric	2010-03-09	F	2	0.0	-	-	-	-	-	-	-	-	-	2
DL-02-2010	Ste-Félicité	2010-03-21	F	1	0.0	-	-	-	-	-	-	-	-	-	2
DL-03-2010	Sainte-Luce-sur-Mer	2010-04-30	F	7	0.0	-	-	-	-	-	-	-	0.0	-	8
DL-04-2010	Sainte-Luce-sur-Mer	2010-06-09	F	42	0.0	-	-	-	-	-	-	-	0.0	0.0	2

Animal Number	Location	Date observed	Sex	Age	Liver	Brain	Stomach contents	Feces	Intestine	Kidney	Blood	Urine	Mammary	Milk	Cause of Death
DL-05-2010	Rocher-Blanc	2010-06-10	F	51	0.0	-	-	-	-	-	-	-	-	-	2
DL-06-2010	Rimouski	2010-07-16	F	18	0.0	-	-	-	-	-	-	-	0.0	-	5
DL-07-2010	Ste-Flavie	2010-07-31	F	40	0.0	-	-	-	-	-	-	-	0.0	-	5
DL-08-2010	Ste-Flavie	2010-08-29	F	31	0.0	-	-	-	-	-	-	-	0.0	-	5
DL-09-2010	Rimouski (Sacré-Coeur)	2010-11-04	M	1	0.0	-	-	-	-	-	-	-	-	-	2
DL-10-2010	St-Fabien	2010-12-31	M	2	0.0	-	-	-	-	-	-	-	-	-	1
DL-11-2010	Cloridorme	2010-12-31	F	35	0.0	-	-	-	-	-	-	-	-	-	8
DL-01-2011	Ste-Flavie	2011-04-07	M	2	0.0	1.5	-	-	-	0.0	0.0	-	-	-	6
DL-02-2011	Rimouski	2011-05-19	F	44	0.0	0.0	0.0	-	-	0.0	0.0	-	0.0	-	5
DL-03-2011	Matane	2011-06-08	F	30	0.0	-	0.0	-	-	0.0	0.0	-	0.0	-	5
DL-04-2011	Percé	2011-07-12	F	56	0.0	1.1	1.2	-	-	0.0	0.0	-	0.0	0.0	3
DL-05-2011	Métis-sur-Mer	2011-08-09	F	14	0.0	1.2	1.2	-	-	0.0	0.0	0.0	0.0	-	5
DL-06-2011	Matane-sur-Mer 48 29' 39"N, 68 29' 35"W	2011-09-10	F	68	0.0	1.2	1.3	-	-	0.0	0.0	-	-	-	8
DL-07-2011	La Martre	2011-10-09	M	55	0.0	1.1	0.0	-	-	0.0	0.0	-	-	-	7
DL-08-2011	Saint-Fabien	2011-10-28	F	22	0.0	1.2	0.0	-	-	0.0	0.0	-	0.0	-	2
DL-09-2011	Pointe-aux-Outardes	2011-11-09	M	43	0.0	1.2	1.2	-	-	0.0	0.0	-	-	-	7
DL-01-2012	Baie-des-Sables	2012-05-19	F	26	0.0	1.1	0.0	-	-	0.0	0.0	-	0.0	-	5
DL-02-2012	Grosse-Roche	2012-05-27	M	23	0.0	0.0	1.0	-	-	0.0	0.0	-	-	-	2
DL-03-2012	Île-aux-Fraises	2012-07-16	F	21	0.0	1.3	0.0	-	-	0.0	0.0	-	0.0	-	7
DL-04-2012	Rivière Ouelle	2012-08-12	F	0	0.0	1.3	1.0	-	-	0.0	0.0	-	-	-	4
DL-06-2012	Tadoussac	2012-08-23	F	0	0.0	1.1	0.0	-	-	0.0	-	-	-	-	4
DL-07-2012	Saint-Fabien-sur-Mer	2012-10-03	F	3	0.0	1.3	1.0	-	-	0.0	-	0.0	-	-	2
DL-08-2012	Métis-sur-Mer	2012-11-05	M	53	0.0	1.1	0.0	-	-	0.0	0.0	-	-	-	7
DL-09-2012	Rivière-du-Loup	2012-11-07	F	30	0.0	0.0	0.0	-	-	0.0	0.0	-	0.0	0.0	7

Table 2. Normalized anomalies (in standard deviations) of total beluga strandings in the St. Lawrence Estuary and the cumulative weekly abundance of the toxic dinoflagellate *A. tamarensis* at Tadoussac and Ste-Flavie. In 2010 the algal abundance data time series was too short for adequate statistics. There are no abundance data for 2012.

Year	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Number of strandings	14	14	13	14	17	14	12	17	15	19	9	11	17	24	13	22	15	27
Strandings – anomalies (stdev)	-0.34	-0.34	-0.61	-0.34	0.45	-0.34	-0.87	0.45	-0.08	0.97	-1.66	-1.13	0.45	2.29	-0.61	1.76	-0.08	3.08
<i>A. tamarensis</i> at Tadoussac - Anomalies (stdev)	-0.50	-0.35	-0.13	0.28	-0.52	-0.48	0.08	-0.12	1.69	-0.37	-0.39	-0.45	-0.51	3.42	-0.34		-0.27	
<i>A. tamarensis</i> at St.Flavie Anomalies (stdev)	-0.75	1.22	-0.72	3.15	-0.73	-0.73	-0.07	-0.59	0.55	-0.73	0.01	0.03	-0.49	0.83	0.29		-0.61	

9.0 FIGURES

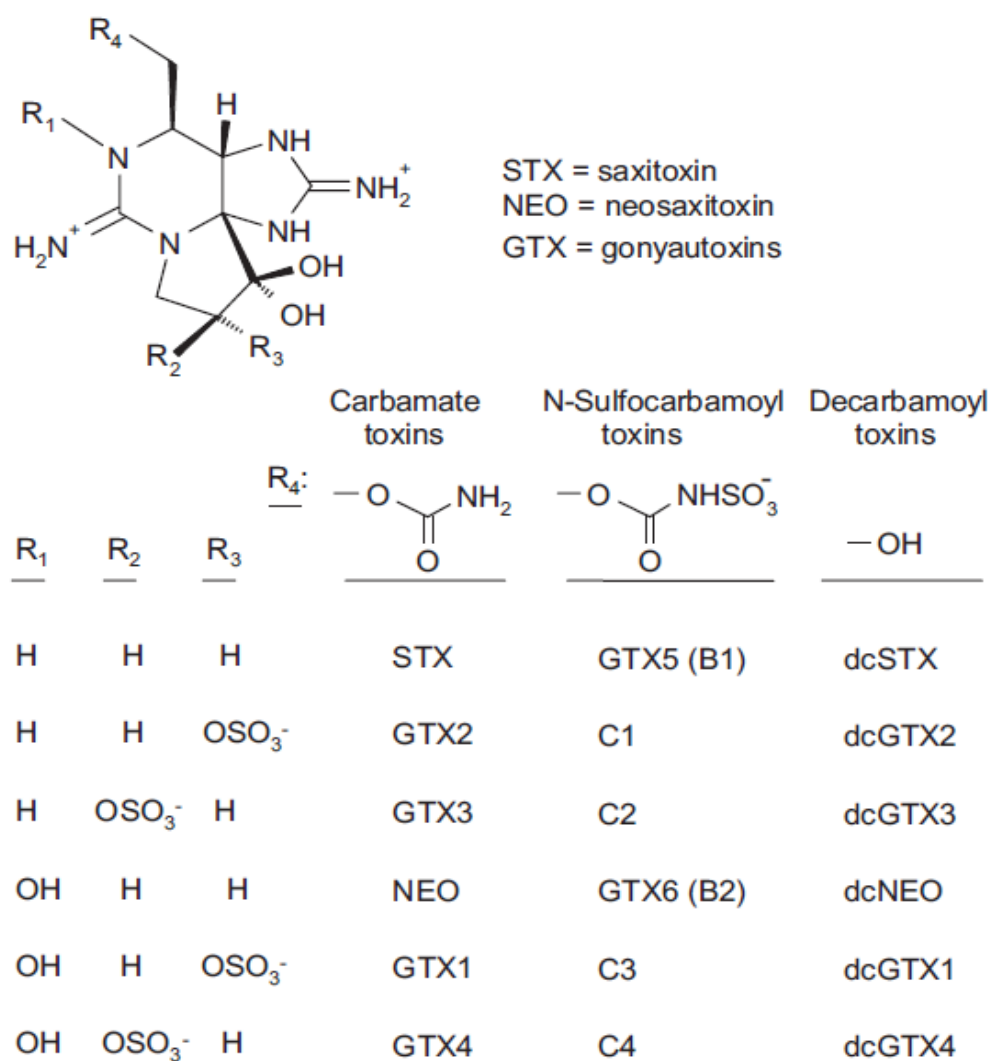


Figure 1. Saxitoxin and its derivatives (from Donovan et al. 2008)

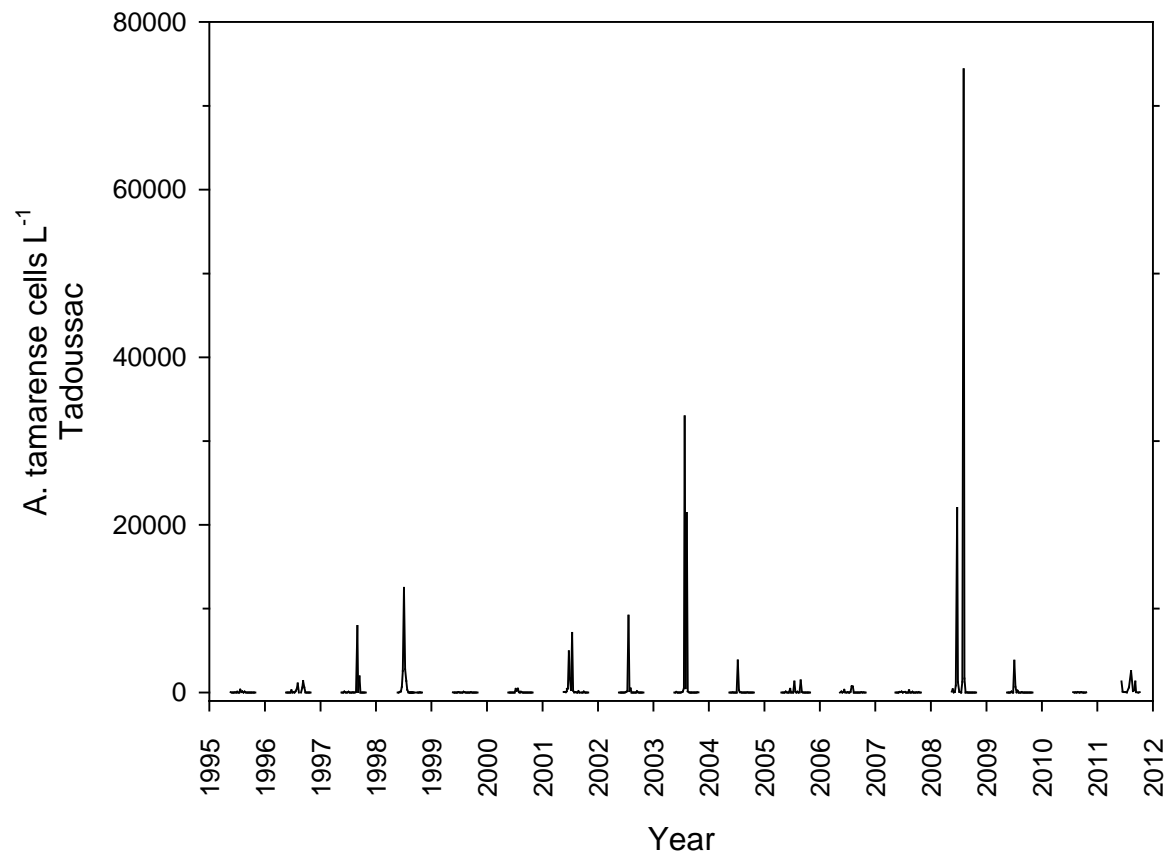


Figure 2. Time series of weekly *A. tamarensis* abundance at Tadoussac, 1995 – 2011.

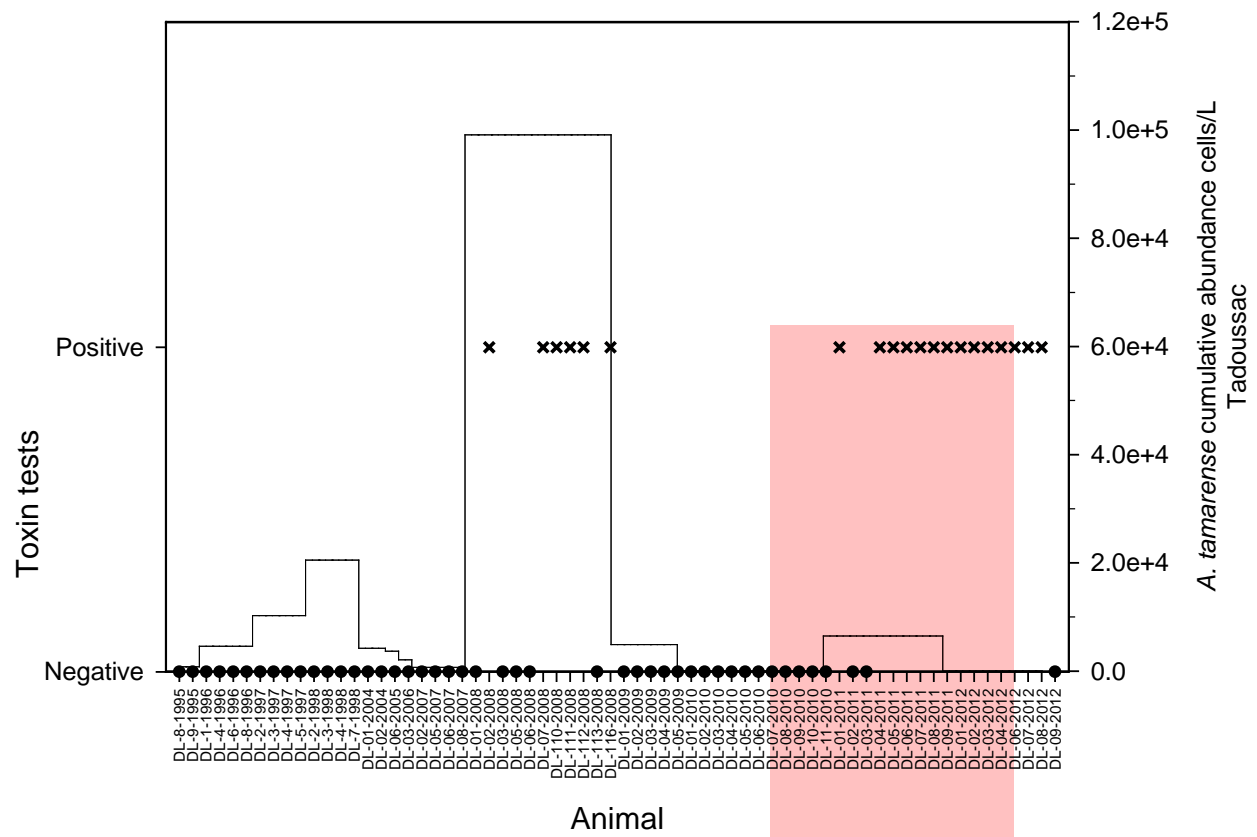


Figure 3. Occurrence of positive (x) and negative (●) toxin tests in all stranded beluga tested compared with cumulative weekly *A. tamarensis* abundance in the same year at Tadoussac. There are no cumulative abundance data for 2010 or 2012.