Harmful algae survey in Vancouver Harbour

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Introduction

As part of the work conducted during the Practical Workshop sponsored by the Marine Environmental Quality Committee of the North Pacific Marine Science Organization (PICES), a harmful algae survey was carried out, including shellfish PSP distribution, ARTOX test and cyst distribution. Samples were collected during May 23 to June 8, 1999, at 9 stations in Vancouver Harbour (Fig. 1).

Material and methods

Shellfish sample collection

Shellfish samples collected for algal toxin analysis at each station are shown in Table 1. About 500g of whole mussels *Mytilus trossuous* were collected at each intertidal sampling site. Clam samples were also obtained from some interdital beaches (*Ruditapes philippinarium*, *Venerupis staninea*) and from benthic trawling (*Clinocardium nuttallii*, *Yoldia sp*.). Samples were weighed and processed immediately after collection, and then frozen for later lyophilizing. After lyophilization, samples were weighed and then stored in the laboratory before analysis.

Mouse bioassay

The AOAC mouse bioassay method (AOAC 1990) for PSP was used in the investigation. Mice (strain ICR) were purchased from the Medical Inspection Institute of Qingdao. A dry sample (0.5g) was extracted with 3 ml 0.1N HCl, ultra-sonicated for 8 × 10 seconds, then centrifuged at 10,000 rpm for 10 minutes. 1 ml of supernant was used for mouse injection, and 1 ml 0.1N HCl was used as control. Purified STX at concentrations of 0.147 μg/ml and 0.294 μg/ml of STX (purchased from the National Research Council, Canada) were also tested. Symptoms exhibited by mice after injection were observed, and lethal time was recorded.

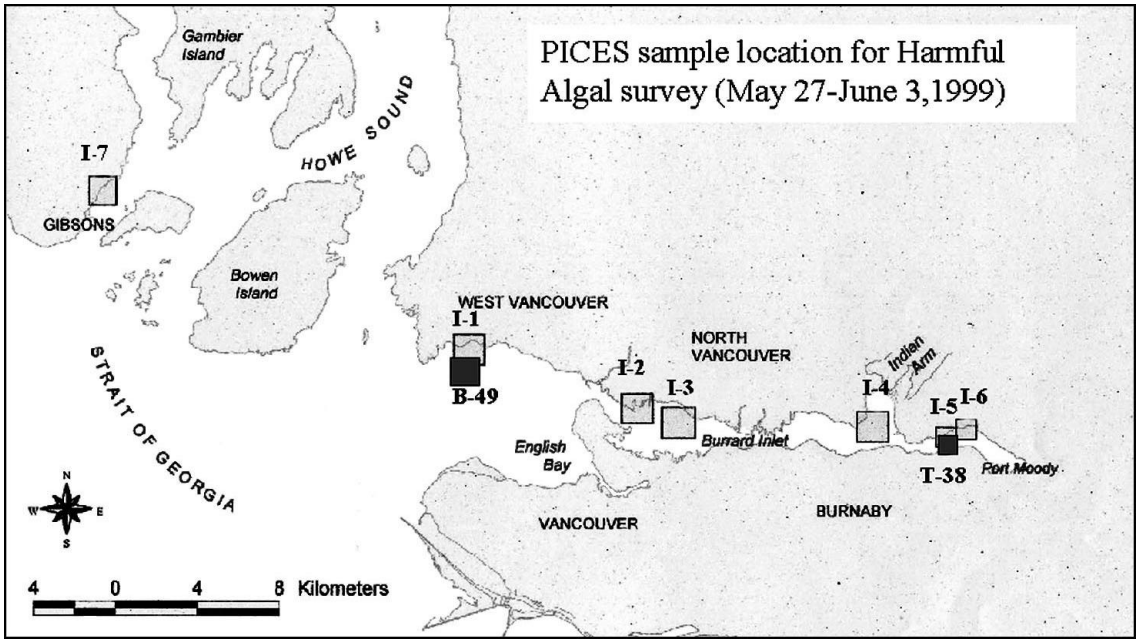

Fig. 1 Sampling sites in Vancouver Harbour.

Table 1. Shellfish sample number, collection station and collection time.

Sample No.	Site Time	I1 0527	B49 0527	I3A 0528	I5B 0529	I6 0529	T38 0529	I4 0530	I2A 0601	I7 0602
	Mytilus trossulos	1, 2		4, 5	6, 7	8, 9		14, 15	18, 19	20, 21
Intertital	*Ruditapes philippinarium*					10				
	Venerupis staninea							16, 17		
Benthic	*Clinocardium nuttallii*		3				11,12			
	Yoldia sp.						13			

Artemia Toxicity Test (ARTOX)

Several species of macroalgae were collected at each intertidal sampling site. Attached microalgal cells were scraped from macroalgae and concentrated for testing.

Artemia cysts were obtained from the *Artemia* Center in Belgium and kept at a low temperature (about 4°C) during transportation and storage. Hatching was initiated 2 days before experiments in Petri-dishes. 10 *Artemia* larvae at the second or third instar stage were transferred under a dissection microscope to 4 wells of the 6 × 4-well plates. Each well contained 1 ml of test algal culture. Each group consisted of three replicate wells and one rinsing well which was used to minimize dilution of the test solution during shrimp transfer. The *Artemia* were observed during the exposure at several-hour intervals and surviving *Artemia* were counted after 24 hours of incubation in the darkness. Seawater was used as a control in the *Artemia* test. Quality control tests were carried out using potassium dichromate $K_2Cr_2O_7$ as the positive control toxin according to the standardized protocol of the method.

Replicate sediment core samples were collected near sites I-7, I-3 and I-6 (Fig. 1). The surficial sediment of each core was incubated using phytoplankton growth medium and optimal light conditions for approximately 3 weeks. Sub-samples were collected every few days and preserved in Lugol's Solution. These samples will be analyzed for phytoplankton abundance and composition. The germination of potentially harmful phytoplankton will be documented.

Results

PSP analysis in shellfish samples

Wet and dry weight of shellfish samples collected from each station are shown in Table 2. Table 3 includes only intertidal samples. Only mussel samples were found to contain PSP. PSP was not determined in other shellfish samples, not even in shellfish collected from the sites which were very close to the site where PSP has been detected in mussel samples such as *Venerupis staninea* from site I-4 and *Clinocardium nuttallii* from site I-1. Table 4 shows that the PSP concentrations in mussels were all lower than eqv. STX 20 µg/100g ww, which is below the common limit of eqv. STX 80µg / 100g ww.

The results indicated that PSP was found only in mussels, and only in English Bay and Burrard Inlet, but not in Port Moody and Gibsons. The concentrations showed a decreasing trend from the West Vancouver to the east of Vancouver Harbour (Fig. 2).

Table 2. Wet and dry weight of shellfish collected.

Sample No.	Wet W.(g)	Dry W. (g)	W:D	Sample No.	Wet W. (g)	Dry W. (g)	W:D
1*	94.1	16.0	5.9	12	29.6	4.2	7.0
2*	83.4	14.5	5.8	13	5.3	0.9	5.9
3	20.5	3.0	6.8	14*	117.1	8.9	6.2
4*	103.6	17.5	5.9	15*	97.3	17.2	5.7
5*	89.5	14.8	6.0	16	98.3	16.1	6.1
6*	101.4	22.2	4.5	17	93.7	16	5.9
7*	106.5	26.0	4.0	18*	97.3	14.9	6.5
8*	78.3	16.5	4.7	19*	98.0	15.2	6.4
9*	76.5	15.7	4.9	20*	81.9	16.9	4.8
10	90.1	17.2	5.2	21*	56.7	11.3	5.0
11	117.7	18.9	8.1				

*mussel sample

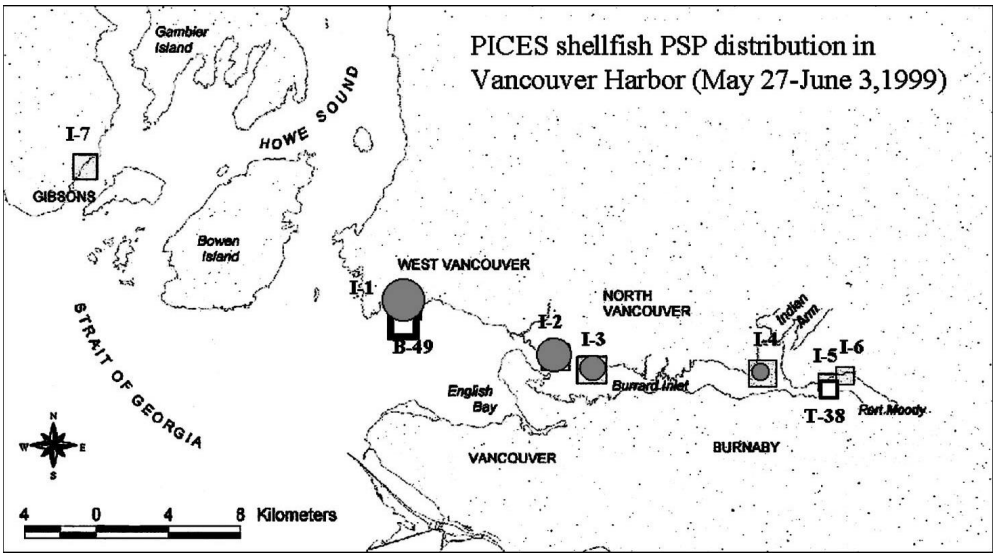

Fig. 2 Distribution of PSP in shellfish collected May 27-June 3, 1999, in Vancouver Harbour. The size of the dots indicates the concentration of PSP.

Table 3. Average lethal time of mouse injected with shellfish sample extraction.

Sample No.	Average lethal time (h)	Sample No.	Average lethal time (h)
1*	12	12	–
2*	1.5	13	–
3	–	14*	+(>24)
4*	12	15*	+(>24)
5*	24	16	–
6*	–	17	–
7*	–	18*	+(>24)
8*	–	19*	1.1
9*	–	20*	–
10	–	21*	–
11	–	Control	–

*mussel sample

Table 4. Determination of PSP concentration in shellfish samples using purified STX.

Sample	Average lethal time	PSP in extraction (eqv. STX µg/ml)	PSP in mussel (eqv. STX µg/100g ww)
STX 0.294µg /ml	9.5 min		
I 1 mussel	l 6.5h	0.15-0.2	15-20
I 2A mussel	12h	0.15-0.2	15-20
STX 0.147µg /ml	15h		
I 3A mussel	18h	<0.15	<15
I 4 mussel	+ >24h	<0.15	<15

+(>24h): showed classical PSP symptoms, such as paralyzed legs, slow but deep respiratory, twitching, trembling head, but survived after 24h.

Table 5. *Artemia* test results of samples from each station.

	Site Date	I 1 5.27	I 3A 5.28	I 5B 5.29	I 6 5.29	I 4 0530	I 2A 0601	I 7 0602
Artemia		–	+	–	–	–	–	–

+: swimming behavior of *Artemia* was inhibited and 24h LC50 of *Artemia* was about 50%.

Artemia test

Positive results of the sample from Longsdale Quay I-3A (Table 5) indicated that toxic algae such as *Heterosigma* or DSP producer *Prorocentrum lima* might be present in the water (Demaret *et al*. 1995). However, no toxicity was found from re-samples collected 4 days later.

Discussion

PSP contents and toxin profile will be further studied using HPLC. Cyst distribution work undertaken by T. Sutherland will be submitted once finished.

References

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