Gunpowder/Bird River Fish Kill
December 18, 2016

Maryland Department of the Environment

In Partnership With

Maryland Department of Natural Resources

University of Maryland Center for Environmental Science

February 14, 2017

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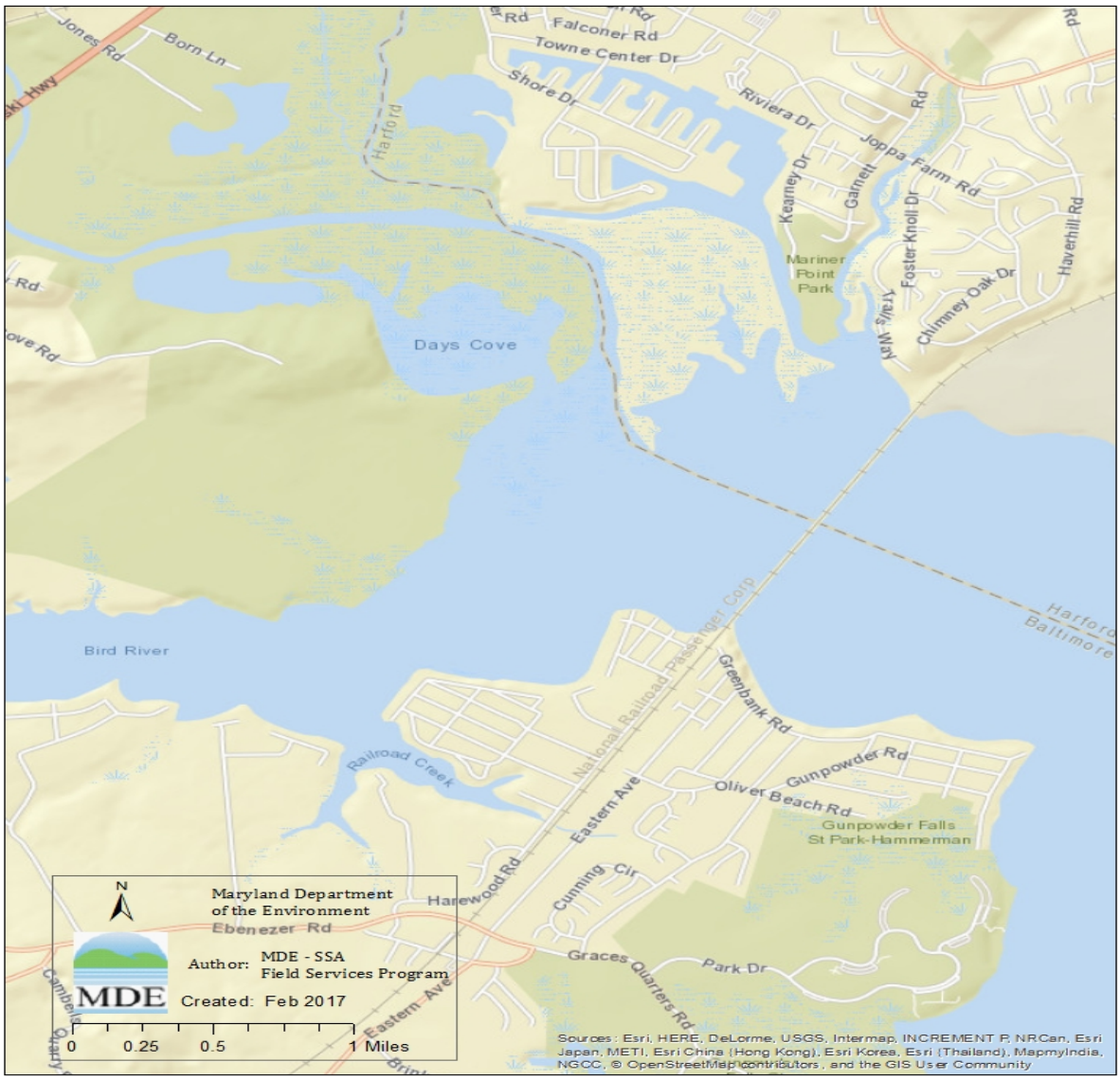
Executive Summary

The Maryland Department of the Environment (MDE) investigated a large fish kill in the upper Gunpowder and Bird rivers near Joppatowne (Harford/Baltimore Counties) on December 19th (Figure 1). A follow-up investigation was performed on December 26, followed by periodic field observations during the month of January. MDE continued to receive inquiries from the public and various state agencies through the end of January, 2017.

Figure 1: Upper Gunpowder/Bird River



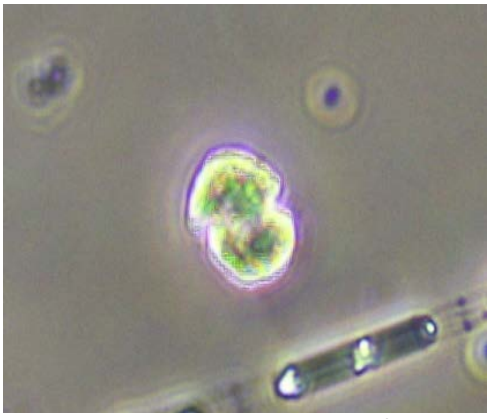
An estimated 20,553 fish died during this fish kill event. Ten fish species were affected: chain pickerel, yellow perch, largemouth bass, bluegill sunfish, pumpkinseed sunfish, carp, black crappie, gizzard shad, channel catfish and spottail shiner. All affected fish were freshwater species, except for gizzard shad, which occasionally migrate to more saline waters in the bay. Many of the gizzard shad displayed lesions typical of chronic cold stress, which is common for this species. All other fish species were virtually pristine looking with no signs of disease or chronic stress, except their gills were bright red and bleeding.

Ten moribund fish were collected and preserved in 10 percent formalin on December 19 for histological examination. No additional animals-- including insects, worms, crayfish, birds, or mammals – appeared to be affected. Latent effects on fish occurred well into January, affecting mainly carp and channel catfish.

No discharges of pollutants were identified during the investigation, thus acute pollution was not suspected to be a factor. All water quality parameters were within normal ranges during the investigation, except salinity, which was about 7ppt (parts per thousand) and unusually high for this typically fresh region of the bay. In addition, a strong cold front swept through the area just prior to the fish kill. Extreme low tide and skim ice was evident on the 19th, with numerous dead fish stranded on mud flats.

Multiple water analyses were performed at several locations during the course of the investigation. Water sample results for dissolved metals, nitrate/nitrite, chlorine, and cyanide were unremarkable. Algae analysis results from December 19 demonstrated that the ichthyo-toxic algae *Karlodinium veneficum* was present at cell counts as high as 4,300 cells/ml (Figure 2). A follow-up investigation on Monday December 26 indicated that the algae appeared to be dying off. Karlodinium cells were 40/ml or less during this period. A number of catfish and carp were observed sluggishly milling around in shallow water, from Gunpowder State Park-Hammerman Area to the Lower Bird River.

Figure 2: Karlodinium veneficum



C. Luckett 2016

Liquid Chromatograph/Mass Spectrophotometer results performed by Dr. Allen Place of the University of Maryland's Institute of Marine and Estuarine Technology (IMET) on January 10, 2017 identified a karlotoxin strain as the probable cause of the fish kill. The toxin was present at levels as high as168ng/ml (nanograms/milliliter), based on the laboratory results of water samples obtained during the December 19 investigation. In addition, the toxin was demonstrated to be hemolytic (i.e. destroys red blood cells) in the laboratory. This algae produces several forms of karlotoxin. Dr. Place will be doing further laboratory analysis to identify the precise strain of karlotoxin involved with this event. The algae will be analyzed to see if it is a genetic strain new to the region.

Fish histology was performed by Mark Matsche of the MD-DNR/NOAA Oxford Cooperative Laboratory. His results indicate that the fish "died as a result of respiratory distress and failure from marked changes in the gill architecture (host reaction) and hemorrhage following exposure to an irritant and adverse water quality conditions." This diagnosis is consistent with what has been seen in the past with karlotoxin fish kills.

The results of the investigation indicate that *Karlodinium veneficum* pushed remarkably far into the fresh end of the Chesapeake Bay estuary in 2016. As a consequence, a localized population of freshwater fish was adversely affected by a toxic algae species that is more commonly observed in higher saline water. These results indicate that the fish were subjected to a lethal one-two punch to the respiratory system (i.e. gill function) induced by a combination of karlotoxin and salinity stress.

Investigation

The initial report of dead and dying fish in Joppatowne was relayed via text to MDE by Joseph Love of Maryland Department of Natural Resources Fisheries at 5:06 p.m. December 18, 2016. MDE personnel immediately contacted the complainant for details and arranged to meet at Mariner Point Park in Joppatowne at 9:30 a.m. December 19. The original complainant reported that several fishermen reported seeing "lots" of dead and dying fish in the vicinity of Mariner Point Park in Joppatowne. Initial reports of affected fish included gizzard shad, crappie and yellow perch.

The preliminary investigation at 9:30 a.m. confirmed the presence of numerous freshly dead and dying fish near the Mariner Point Park boat ramp with hundreds of gulls actively feeding on struggling fish. An extreme low tide was observed with extensive mud flats exposed throughout the area. Hundreds of dead fish were stranded on the mud flats. The wind was northwest at 10 mile per hour and the air temperature was 32 degrees Fahrenheit. Skim ice covered much of the Joppatowne canal network.

To clarify geography in this report, the canal that borders Mariner Point Park to the west, connecting the public boat ramps to the Gunpowder River, often referred to as "Joppatowne Canal" is herein referred to as Thomas Creek. The tidal creek to the east of the park is herein referred to as Foster Branch. Mariner Point is at the confluence of these two creeks.

Dead fish Calculations

The complainant accompanied an MDE biologist as he explored several locations along the Mariner Point Park property on foot, made observations, and performed fish counts. They included:

1) A 120-yard section of beach on Thomas Creek above Mariner Point Boat Ramp contained 68 dead fish of six species, including gizzard shad (40), pumpkinseed sunfish (6), bluegill sunfish (9), black crappie (4), yellow perch (8) and spottail shiner (1). Many of the gizzard shad displayed lesions typical of chronic cold stress, which is also typical for this species. All other fish species were virtually pristine looking with no signs of disease or chronic stress. Their gills however were observed to be bright red and bleeding.
2) A 100-yard section of beach north of a small fishing pier on Thomas Creek, approximately 200 yards south of the boat ramp contained 23 dead fish of four species, including gizzard shad (15), yellow perch (3), bluegill sunfish (3) and black crappie (2).
3) A 100-yard section of beach west of Mariner Point Park on Thomas Creek contained approximately 50 dead fish.
4) A 50-yard section of beach on Foster Branch contained approximately 50 dead fish.
5) Foster Branch at Joppa Farm Road. Approximately 138 dead fish were observed within the 30 yard stretch directly below the bridge. They included gizzard shad (100), yellow perch (50), spottail shiner (5), small sunfish (25) and (3) largemouth bass. Many were stranded on the exposed mud bottom. Hundreds of live fish were observed in a pool under the bridge, extending upstream into the flowing freshwater stretch. No dead or dying fish were observed above this point. At the Trimble road crossing a small discharge of sediment was observed, which was believed to be related to the previous cold front/rain event that occurred over the weekend, but was not believed to be related to the fish kill.
6) Another 75 dead fish were observed within a 50-yard stretch of Foster Branch approximately 100 yards below Joppa Farm Bridge (Figure 3).
7) A mud flat southeast of Mariner Point (observed from distance at low tide) contained approximately 500 dead fish, including gizzard shad (250), unknown species (250).
8) Nine dead fish were observed within a 25 foot section of shallow cove at a community access point on the Gunpowder River near Greenbank Road and Patuxent Road intersection (Spottail shiner 6, sunfish species 3).

9) Eleven dead fish were observed within a 25 foot section of shallow cove at a community beach access point at the end of Crooks Road on the Bird River (Spottail shiner 8, sunfish spp 3).

Figure 3: Dead fish along Foster Branch

A boat was launched at 11:30 to more extensively investigate the area. Dead fish that were floating (not deposited on the shoreline) were counted and identified.

10) 223 dead, floating fish were observed in Thomas Creek from the boat ramps to Mariner Point, including gizzard shad (141), largemouth bass (3), pumpkinseed sunfish (6), sunfish spp. (72), common carp (1).
11) 68 dead floating fish were observed north and west of the boat ramp in Thomas Creek/canals, including gizzard shad (55), bluegill sunfish (4), pumpkinseed sunfish (3), black crappie (1), yellow perch (1), common carp (1), and sunfish sp. (3). The area north and west was only partially assessed due to ice.
12) Approximately 100 dead floating fish were estimated from a distance, along a shallow shoreline with limited access east of railroad bridge near Piney Point, which included gizzard shad (50), unidentified fish (50).
13) 165 dead floating fish were observed in a 770 yard stretch of Foster Branch, including gizzard shad (113), sunfish (47), and yellow perch (5).

December 19 dead fish sub-totals for Mariner Point Park area:
Staff biologists employed two methods to calculate the number of dead fish. The total number was the sum of dead fish based on discrete counts of floating fish combined with the sum of dead fish exposed on the shoreline. Fish on shorelines were extrapolated from random sub-counts of measured shoreline distances.

Based on Observations 1-6 above:
Shoreline totals/yard: 68/120, 23/100, 50/100, 50/50, 183/30, 75/50- yields 449/450 yards= 0.998 dead fish/yard.

Subtotal calculated using Google Earth-
Mariner Point Peninsula (2,385 yards) Shoreline, plus opposite Peninsula (2,408 yards) Total Shoreline= 4,793 yards.

Shoreline length 4,793 yards x fish density on shoreline 0.998 fish/yard=**4,783** fish

December 19 dead fish sub-totals for Lower Bird River and SW Lower Gunpowder to Oliver Point:
Two observations were made at two 25 feet sections of the south shoreline on the lower Bird River and Gunpowder River between the railroad bridge and Oliver Point. The total affected shoreline of the south shore of the Bird River (from Google Earth) was 14,160 feet. If we assume that the dead fish counted and identified were representative of the area:

Based on Observations 8-9 above:
Shoreline totals/yard: 9/25ft, 11/25ft- yields 19/50 feet= 0.40 dead fish/foot.

Subtotal calculated using Google Earth-
South shoreline of Bird River= 14,160 feet.

Shoreline length 14,160 feet x 0.38 fish/foot= **5,664** fish

North Shore of Bird River and Days Cove area:
No extrapolation was performed for the northern shoreline in the lower Bird River, including Days Cove, due to extreme low tide during the investigation and the remoteness of the area. Up to an additional 5,000 dead fish may have been in this area based on one local fisherman's personal account. This is plausible, given that the size of the area and its shoreline are comparable to the two other areas assessed on Dec. 19.
Total shoreline extrapolations yields- 4,783 + 5,664 = **10,447**
Dead fish estimate of north shore of Bird River and Days Cove = **5,000**
Total direct boat counts plus estimates of dead fish on the Mariner Point mud flat (Observation #7) = **1,056**

Total December 19 dead fish count is **16,503**.

Water Sample Analysis

Water samples were collected at the following locations on December 19 (Figure 4 and Table 1):

Table 1: December 19, 2016 Water Samples

Date	Location	Station	Depth	Lat	Long
12/19/2016	Thomas Creek and canals, 250 yards NW of Boat Ramps	1	Surface	39.40318	76.35403
12/19/2016	Thomas Creek, End of Dock at Mariner Pt. Park	2	Surface	39.40219	76.35171
12/19/2016	Thomas Creek, 200 yards S of Boat Ramps	3	Surface	39.40043	76.35164
12/19/2016	Mouth of Thomas Ck, entering Gunpowder R.	4	Surface	39.39444	76.34816
12/19/2016	Gunpowder River, midchannel, 50 yards D/S of RR Bridge	5	Surface	39.38887	76.34238
12/19/2016	Gunpowder River, midchannel, 50 yards D/S of RR Bridge	5	Bottom	39.38887	76.34238
12/19/2016	Lower Foster Branch	6	Surface	39.39793	76.34796
12/19/2016	Bird River at Crooks Road	7	Surface	39.37627	76.38841
12/19/2016	Gunpowder River at Patuxent Road	8	Surface	39.37532	76.35077

Figure 4: December 19th Water Samples

Ten moribund fish were collected from Station 6 (Lower Foster Branch) and preserved in 10 percent formalin on December 19 for histological examination (Appendix 2). Fish histology was performed by Mark Matsche of the MD-DNR/NOAA Oxford Cooperative Laboratory. His results indicate that the fish "died as a result of respiratory distress and failure from marked changes in the gill architecture (host reaction) and hemorrhage following exposure to an irritant and adverse water quality conditions." This diagnosis is consistent with what has been seen in the past with karlotoxin fish kills.

Water quality was tested in situ at four of these locations using either an YSI 6600 v2 or Eureka Manta 2 multi-parameter instrument (Table 2). The results indicate that all water quality

parameters were well within acceptable state standards. *Note that the observed salinity was indicative of what would be expected in a mesohaline environment.

Water sample results for dissolved metals, nitrate/nitrite, chlorine, and cyanide were unremarkable (Appendix 1). There was no evidence of pollution observed throughout the area.

Table 2: Water Quality Stations Dec 19, 2016

Location	Depth Ft	Temperature	Conductivity	*Salinity	pH	D.O.	%Sat
Station 1	2.5	0.78	11,450	6.39	7.59	13.16	96.2
Station 5	2	0.81	12,870	7.20	7.59	14.22	104.6
Station 5	4.6	0.78	12,860	7.19	7.93	12.56	92.2
Station 6	2.83	1.23	12,500	6.93	7.64	13.74	102.2
Station 7	Surface	3.08	11,500	6.40	7.30	10.72	93.2

The results of algae toxin analysis by the IMET laboratory (Table 3) indicate that Karlotoxin concentrations ranged from 18.25 ng/ml at station 3, to 168 ng/ml at station 5. *Karlodinium veneficum* cell counts ranged from 4,282 cells/ml at station 2, to 81 cells/ml at station 7. A second species of dinoflagellate *Heterocapsa rotundata* was present with cell counts as high as 3,313cells/ml at station 8. This alga is a typical winter bloom species that is not known to be toxic.

Table 3: December 19, 2017, Predominant Algae Cell Counts/ml and Karlotoxin Levels

Location	Station	Depth	K. veneficum/ ml	H. rotundata/ ml	Karlotoxin_KmTx ng/ml
Thomas Creek, 250 yards NW of Boat Ramps	1	S	4040	404	37.09
Thomas Creek, End of Dock at Mariner Pt. Park	2	S	4282	1374	46.33
Thomas Creek, 200 yards S of Boat Ramps	3	S	3151	404	18.25
Mouth of Thomas Ck, entering Gunpowder R.	4	S	2424	646	32.56
Gunpowder River, 50 yards D/S of RR Bridge	5	S	3394	485	168.08
Gunpowder River, 50 yards D/S of RR Bridge	5	B	3232	162	64.77
Lower Foster Branch	6	S	1454	485	24.73
Bird River at Crooks Road	7	S	81	81	Not analyzed
Gunpowder River at Patuxent Road	8	S	566	3313	Not analyzed

Follow-up investigation of December 26

On December 26 a follow-up investigation occurred in response to citizen information indicating that fish were still actively dying in the Gunpowder and lower Bird Rivers (Figure 5). Eight locations were visited and six additional water samples were taken (Table 4). Algae sample analysis indicated that the Karlodinium concentration had fallen to 40 cells/ml or less at all locations (Table 4). Several hundred struggling fish (primarily channel catfish and common carp) were observed near the shoreline in the mouth of Bird River and Gunpowder River near the State Park. This count was based on observations made in a 500 yard section of beach at Gunpowder Falls State Park (Hammerman Area), which yielded 142 dead carp and 28 dead catfish. This equated to a density of .284 dead carp/yard and .056 dead channel catfish/yard. A 11,900 yard section of shoreline was identified from the lower Bird River to Gunpowder State Park which contained recently dead and moribund fish. Based on this information and extrapolation, an additional 4,049 freshly dead fish were estimated over this range, which included 3,382 carp and 667 channel catfish.

Figure 5: December 26 Water Samples

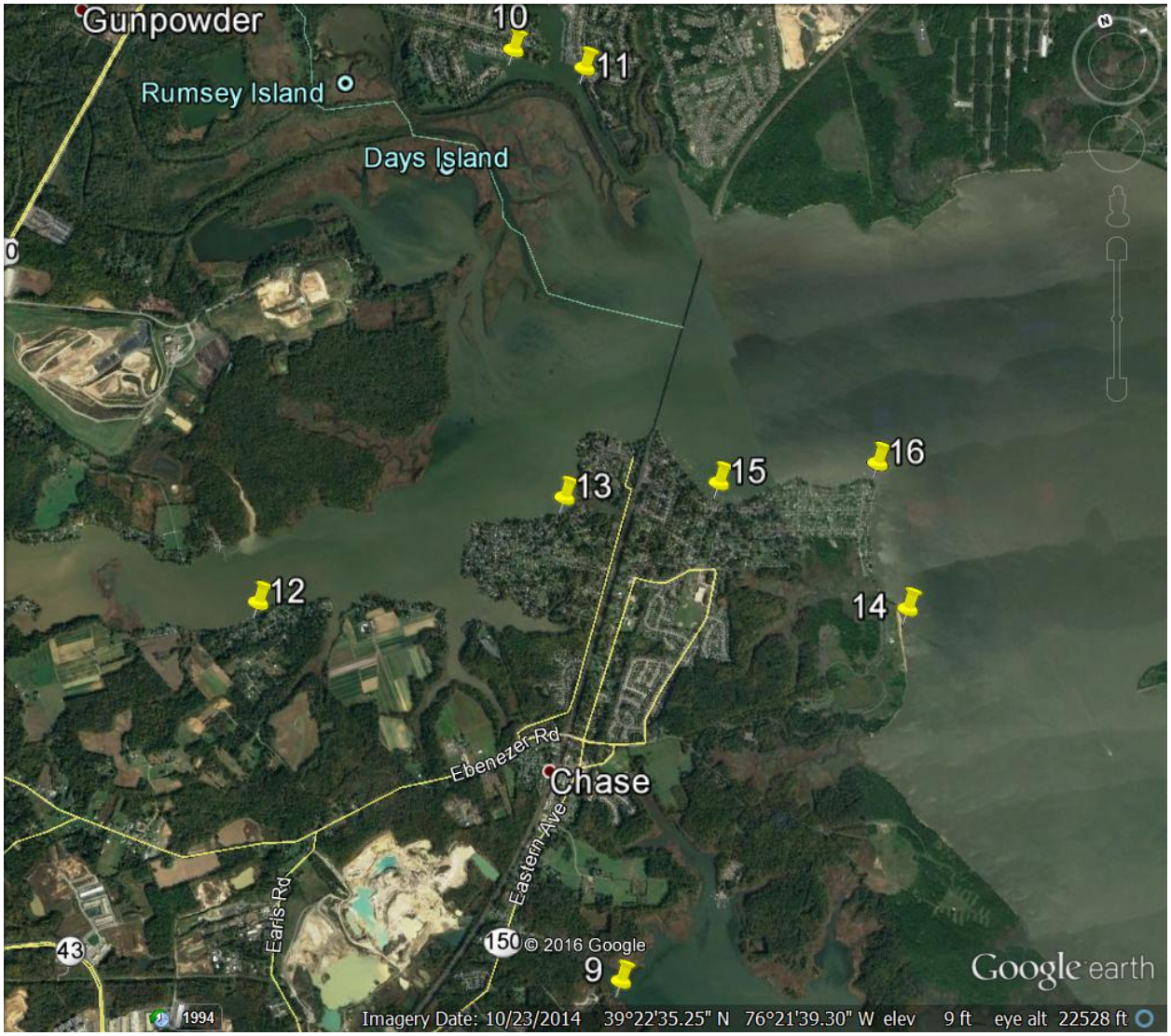


Table 4: December 26, 2016 Predominant Algae cell counts/ml

Station	Location	Karlodinium veneficum	Heterocapsa rotundata	Observations
16	Gunpowder River at Oliver's Point	0	404	30 sluggish catfish
14	Gunpowder River at Gunpowder State Park	40	1414	142 dead carp, 28 dead channel catfish/500 yards, numerous sluggish fish observed
10	Canal at Neptune Dr.	40	5656	no dead fish
11	Thomas Creek at Mariner Pt. Park	0	1980	1 dead, 1 sluggish catfish
12	Bird River at Crooks Road	0	3070	several sluggish catfish
16	Gunpowder River at N. Shore Dr.	0	525	22 dead fish
9	Dundee Creek at Marshy Point Nature Center	Not Sampled, no dead fish observed		
15	Gunpowder River at Greenbank Rd. x Patuxent Rd	Not Sampled, No dead fish observed		

Numerous large (10-20 pound) decomposing carp continued to float ashore in this area during the month of January (Figure 6) that were either initially killed or ultimately succumbed to algae toxin released during the December 18 incident. Cold water temperature significantly slowed down the decomposition rate of the larger fish; hence they remained in this area longer than the smaller fish species.

Figure 6: Dead carp continuing to float ashore in January.

Fish Species Composition

During the initial investigation, about 1,500 fish were identified to species during multiple shoreline counts and boat transects. All of those identifications and counts were added together to provide the species distribution (in percent of total) for the event. These percentages were extrapolated into un-assessed but affected areas to arrive at the total fish killed by species for the entire event.

In some cases, bluegill and pumpkinseed sunfish were counted separately yielding a percentage for each species. Due to time constraints there were a large number of sunfish (bluegill and pumpkinseed sunfish) counted but not separated to species. The percentage of bluegill (47 percent) and pumpkinseed (53 percent) from the earlier counts was applied to the unidentified sunfish to estimate the final total for each of these two species.

The two discrete areas that were observed from a distance (e.g. mud flat off Mariner Point and the shallow shoreline east of the railroad bridge near Piney Point) were composed of half gizzard shad and half "unidentified". The species distribution (percentages of the whole) of non-gizzard shad fish from other areas was extrapolated into the unidentified fish in these two cases.

The results of the follow-up investigation on December 26, where dead Common Carp and Channel Catfish were the predominant species were kept separate from the initial results, extrapolated separately, and then added into the total mortalities for the event.

Table 5: Total mortalities and species distribution of the Gunpowder/Bird River fish kill					
Species	**Counted 12/19**	**Percent of TOTAL**	**Total from 12/19**	**Result from follow-ups**	**GRAND TOTAL**
gizzard shad	880	59.66	9,846	0	9,846
pumpkinseed sunfish	199	13.49	2,226	0	2,226
bluegill sunfish	174	11.80	1,947	0	1,947
yellow perch	136	9.22	1,522	0	1,522
spottail shiner	40	2.71	447	0	447
largemouth bass	30	2.03	335	0	335
black crappie	14	0.95	157	0	157
common carp	2	0.14	23	3,382	3,405
channel catfish	0	0.00	0	667	667
chain pickerel*	0	0.00	0	1	1
TOTAL	**1475**	**100.00**	**16,503**	**4,050**	**20,553**

*Based on picture from citizen.

The estimated total number of fish killed during this event is 20,553, which is the culmination of dead fish observed on December 19 and follow-up investigations (16,503 and 4,050 respectively).

Conclusion

This fish kill was caused by a late bloom of the fish killing algae *Karlodinium veneficum*. The identified karlotoxin is believed to be a newly discovered strain of KmTX (Appendix 3). All 7 sample sites analyzed from December 19, 2016 contained the toxin and all produced a strong hemolytic reaction in the laboratory. Although this species bloomed elsewhere in Maryland in November (Northeast River, Middle River and St Martin River -personal communication Maryland Department of Natural Resources) the toxin was only found in the Gunpowder samples. Observations made during the Gunpowder event indicate that the relatively low numbers of Karlodinium cells (<4,300 cells/ml) were in a heterotrophic state, which is the life phase of highest toxicity and lowest photosynthetic activity. Insitu water quality readings support this observation with near 100 percent dissolved oxygen saturation and neutral pH readings of 7.3 to 7.93.

Research thus far has shown *Karlodinium* typically blooms in the Chesapeake Bay between 7-10 ppt salinity and at temperatures about 77^{o} F (Allen Place, personal communication). Fish kills generally occur when cell counts are above 10,000 cells/ml., and when the cells are actively preying on other algae (heterotrophic). New data from Jen Wolny, DNR, indicates that Karlodinium is capable of heterotrophy at winter temperatures (St Martin River sample 1/25/17 at 46^{o} F).

EPA Bay Program data suggests that the bay water temperature remained high (>55 ^{o}F) well into November 2016. In addition, the salinity wedge apparently moved further into predominately fresh areas, presumably due to low rainfall. DNR bay program data (J. Wolny DNR, personal communication) indicates that a Karlodinium bloom of 11,000/ml was documented in the Northeast River on November 17, 2016, which is late in the season and extremely far north into the freshwater portion of Chesapeake Bay. On December 14, 2016, just prior to the fish kill, they documented a bloom near the mouth of the Gunpowder River (Station WT 2.1) at 4,900 cells/ml with a salinity of 7 ppt.

This data indicates that Karlodinium may have pushed further into the typically fresh end of the estuary with the result of adversely effecting a historically native freshwater population. This data also suggests that the affected freshwater fish were subjected to a one-two punch of karlotoxin and salinity stress.

Appendix 1

Station 2 Water Chemistry Results From December 18, 2016

Send Report To:
Charles Poukish, MDE
416 Chinquapin Round Rd
Annapolis MD 21401

State of Maryland
DHMH-Laboratories Administration
Division of Environmental Chemistry
INORGANICS ANALYTICAL LABORATORY
1770 Ashland Ave
Baltimore, Maryland 21205
WATER ANALYSIS

Lab No. | Date Received

Do not write above this line.

SAMPLE ID

Bottle Number 113 Name Gunpowder Falls County Harford County Code

Location Mariner Pt. Park, Joppatowne, MD Data Category Code

Collected: Date 12-19-2016 Time 10 am Collector & Phone Charles Poukish 443482-2732 Submitter Code

CHECK (one per box)
Drinking Water / Landfill / Stream / Other tidal creek
Community / Non community / Private / Other state/fed waters
Source (raw water) / Distribution (treated) / MCL
Emergency / Routine / Recheck / Special
Federal Project

FIELD

Plant No. Sampling Station Preservation (Iced) Acid Type of Acid

pH Chlorine: Free Total Specific Conductance

Notes to Lab/Remarks: pH 6.5 - ALIQUOT PRESERVED WITH NaOH to pH >12 ca

CHECK TESTS	TESTS	Error Code	RESULTS
	Alkalinity (Total)		
	Ammonia - N		
	Chloride		
	Conductance*, Spec.		
	Dissolved Solids (Total)		
	Hardness		
	Fluoride		
	Nitrite, N		(—)
	Nitrate - Nitrite, N		(—)
	Sulfate		
	Total Solids		
	Turbidity*		
	Other: Cyanide		(—)
	SULFIDE		(—)
	TOTAL CHLORINE		(—)
	FREE CHLORINE		(—)

* Results reported in Units, all others in milligrams per liter (ppm)

Number of Tests Requested

DHMH 30 A 1/15

Supervisor Section Chief [signature] Date Reported 12/21/16

ORIGINAL LABORATORY

State of Maryland
DHMH-Laboratories Administration
Division of Environmental Chemistry
TRACE METALS LABORATORY
1770 Ashland Avenue, Baltimore, Maryland 21205
Robert Myers, Ph.D., Director

ACCREDITED
Certificate # 3525.02

Certificate of Analysis

HARFORD CO HD ENVIRO HLTH
PO BOX 797 / 120 S HAYS ST
BEL AIR, MD 21014

Lab Project No: E17002541 Date Coll.: 12/19/2016 Date Received: 12/20/2016 Submitted By: Poukish

Field ID: 112
Lab No.: E17002541001

Method	Element	Result	Units	Date Analyzed
EPA 200.7	Aluminum	0.798	ppm	12/27/2016
EPA 200.7	Manganese	0.11	ppm	12/27/2016
EPA 200.7	Sodium	1733.00	ppm	12/27/2016
Method	**Element**	**Result**	**Units**	**Date Analyzed**
EPA 200.8	Arsenic	0.010	ppm	12/23/2016
EPA 200.8	Chromium	<0.010	ppm	12/27/2016
EPA 200.8	Lead	<0.005	ppm	12/27/2016
EPA 200.8	Nickel	<0.050	ppm	12/27/2016
EPA 200.8	Selenium	<0.025	ppm	12/27/2016
EPA 200.8	Silver	<0.050	ppm	12/27/2016
EPA 200.8	Zinc	<0.050	ppm	12/27/2016
Method	**Element**	**Result**	**Units**	**Date Analyzed**
EPA 245.1	Mercury	<0.00050	ppm	12/23/2016

Comments:

Approved by: Yongzhao Chen Approval date: 12/29/2016

**The following methods are included in our A2LA Scope of Accreditation: EPA 200.7, EPA 200.8, EPA 245.1.

This document contains confidential health information that is privileged, confidential and exempt from disclosure under law. If you have received this information in error, please call (410) 767-6944 and arrange for return or destruction.

Appendix 2

Histology Report

Accession #:	16-GP-XX-##	Study:	Fish Kill
Species:	Various		
Date Collected:	12/21/2016	Notes:	
Collection Site:	Gunpowder River: Joppatowne		

History: The fish submitted here were collected in response to a fish kill event in the Gunpowder River, near Joppatowne on December 19th. Nine species of fish were documented in the fish kill. The gills of the fish were bright red and bleeding. No other animals seemed to be effected, including insects, worms, crayfish, birds, or mammals. Latent effects occurred well into the 1st of the year, affecting mainly carp and channel catfish. No saltwater fish appeared to be involved.

Samples were analyzed by MDE for dissolved metals, cyanide, nitrite, chlorine. All results were below levels of concern. No pollution sources were identified. All water quality parameters were acceptable. However the salinity was 7ppt., which was totally unexpected for this normally fresh area. Karlodinium veneficum was present at 4,300 cells/ml on December 19th. Water samples were submitted to Dr. Place for Karlotoxin analysis (results pending).

Comments: Samples were delivered to the COL intact in 10%NBF or were trimmed on site and preserved in formalin. External lesions were noted only from the gills (hemorrhage).

Fish 1-5

Organ ▼ / Fish ▸	16-GP-BG-01	16-GP-BG-02	16-GP-BG-03	16-GP-BG-04	16-GP-BG-05
	Bluegill	Bluegill	Bluegill	Bluegill	Bluegill
	12/21/2016	12/21/2016	12/21/2016	12/21/2016	12/21/2016
	Gunpowder River	Gunpowder River	Gunpowder River	Gunpowder River	Gunpowder River
Skin	NE	NE	NE	NE	NE
Eye	NE	NE	NE	NE	NE
Nares	NE	NE	NE	NE	NE
Muscle	X	F-parasitic cyst possible cestode	X	X	X
Gills	M-lamellar swelling and epithelial lifting/separation, M-moderate lamellar fusion and epithelial hyperplasia, D-mild inflammatory infiltrates, M-moderate hemorrhage	M-lamellar swelling and epithelial lifting/separation, M-moderate lamellar fusion and epithelial hyperplasia, D-mild inflammatory infiltrates, M-moderate sloughing or epithelial erosion	M-lamellar swelling and epithelial lifting/separation, M-severe lamellar fusion and epithelial hyperplasia, D-moderate inflammatory infiltrates, M-moderate sloughing and hemorrhage	M-moderate telangiectasia, M-severe lamellar fusion and epithelial hyperplasia, D-mild inflammatory infiltrates, M-moderate sloughing and hemorrhage	M-moderate telangiectasia, M-severe lamellar fusion and epithelial hyperplasia, D-mild inflammatory infiltrates, M-moderate sloughing and severe hemorrhage
Pseudobranch	NE	NE	NE	NE	NE

Organ ▼ / Fish ▸	16-GP-BG-01	16-GP-BG-02	16-GP-BG-03	16-GP-BG-04	16-GP-BG-05
Heart	X	F-ventricular metacercaria	F-moderate ventricular valve enlargement with parasitic cyst	X	X
Liver	X, No lipid or glycogen	X, little lipid or glycogen	X	F-ascitic cyst	X
Esophagus	NE	X	X	X	X
Stomach	X	X, food	X, food	X, food	X, food
Intestine	X	X	X	X, food	X, food
Peritoneum	NE	NE	NE	NE	NE
Swim bladder	NE	NE	NE	NE	NE
Pancreas	NE	NE	NE	NE	NE
Spleen	X	F-large digene cyst	X	X	X
Head Kidney	NE	X	NE	X	X
Trunk Kidney	X	X	X	X	X
Repro	female	NE	NE	female	male
Brain	NE	NE	NE	NE	NE
Nervous	NE	NE	NE	NE	NE
Misc.					

Fish 6-10

Organ ▼ / Fish ▸	16-GP-PM-01	16-GP-PM-02	16-GP-PT-01	16-GP-PT-02	16-GP-PT-03
	Pumpkinseed	Pumpkinseed	Spottail Shiner	Spottail Shiner	Spottail Shiner
	12/21/2016	12/21/2016	12/21/2016	12/21/2016	12/21/2016
	Gunpowder River	Gunpowder River	Gunpowder River	Gunpowder River	Gunpowder River
Skin	NE	NE	NE	NE	NE
Eye	NE	NE	NE	NE	NE
Nares	NE	NE	NE	NE	NE
Muscle	X	X	X	X	X
Gills	M-lamellar swelling and epithelial lifting/separation, M-moderate telangiectasia, M-moderate lamellar fusion and epithelial hyperplasia, M-moderate to severe hemorrhage	M-moderate telangiectasia, M-severe lamellar fusion and epithelial hyperplasia, D-mild inflammatory infiltrates, M-severe sloughing and hemorrhage	M-moderate telangiectasia, M-moderate lamellar fusion and epithelial hyperplasia, D-none to mild inflammatory infiltrates, M-mild to moderate sloughing and hemorrhage	M-moderate telangiectasia, M-moderate lamellar fusion and epithelial hyperplasia, D-none to mild inflammatory infiltrates, M-moderate sloughing and hemorrhage	M-moderate telangiectasia, M-severe lamellar fusion and epithelial hyperplasia, D-mild inflammatory infiltrates, M-severe sloughing and hemorrhage
Pseudobranch	NE	NE	NE	NE	NE

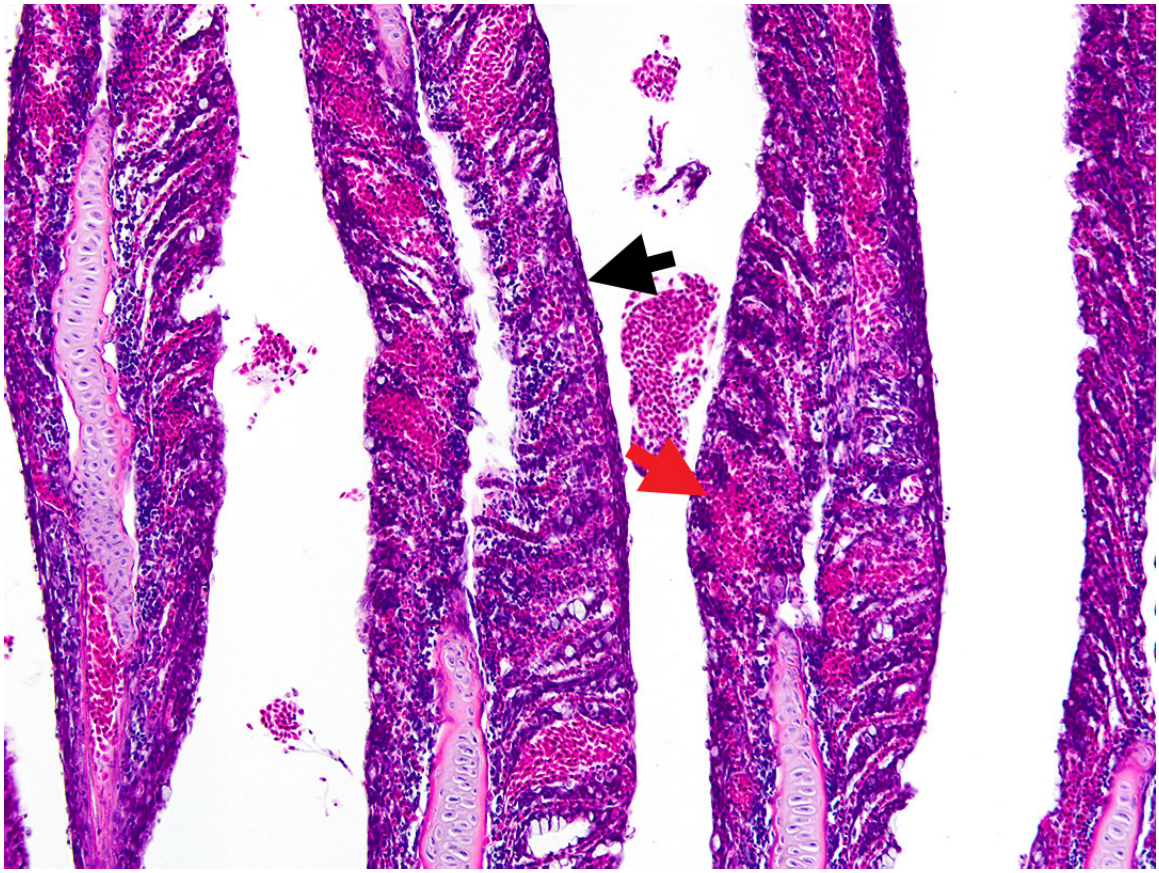
Organ ▼ / Fish ▸	16-GP-PM-01	16-GP-PM-02	16-GP-PT-01	16-GP-PT-02	16-GP-PT-02
Heart	X	X	X	X	X
Liver	M-Large digene cysts, no lipid or glycogen	X, no lipid or glycogen	X, small amount of lipid	X, small amount of lipid	X,some lipids
Esophagus	X	X	X	X	X
Stomach	X, fluid	X, food	X, food	X	X, lots of food
Intestine	X, digenes between loops	X, food, M-submucosal cysts probably parasitic	X, food	X	X, lots of food
Peritoneum	NE	NE	NE	NE	NE
Swim bladder	NE	NE	NE	NE	NE
Pancreas	NE	NE	NE	NE	NE
Spleen	X	X	X	X	↑ macrophage aggregates
Head Kidney	X	NE	NE	X	NE
Trunk Kidney	NE	X	X	X	X
Repro	male	female	Female, multiple oocyte stages	Female, multiple oocyte stages	Female, multiple oocyte stages
Brain	NE	NE	NE	NE	NE
Nervous	NE	NE	NE	NE	NE
Misc.					

Comments: The primary lesions evident in fish submitted to COL were associated with the gills. Two different types of response were evident in branchial tissue. First, several areas of gill tissue from all fish showed signs of osmotic-related changes, including swelling, fluid accumulation and separation of epithelial layer from lamellae. These types of changes can indicate a general stress response and can be seen in a fish exposed to wide variety of stressors, particularly abrupt changes in water quality conditions. Secondly, and of greater importance, a moderate to severe reaction to an irritant was evident in the gill tissue of all fish. Less severe changes were indicated by slight folding of lamellae and hyperplasia of epithelial tissue. More severe changes involved lamellar fusion and "walling off" of distal lamellar layer with epithelial tissue, inflammatory cells and some goblet cells. Changes in several fish appeared to be chronic in nature as indicated by sloughing or erosion of epithelial tissue, breakdown in lamellar structure and hemorrhage.

Minor lesions noted related to low intensities of digenean metacercariae and few cestodes, and were unrelated to cause of death.

Conclusion: Fish died as a result of respiratory distress and failure from marked changes in the gill architecture (host reaction) and hemorrhage following exposure to an irritant and adverse water quality conditions. Reports from field investigations indicate presence of *Karlodinium venificum*, a potentially toxic algal species, and unusually high salinity.

Image



Gill tissue from juvenile spottail shiner collected during a fish kill in the Gunpowder River, December, 2016. Tips of lamellae are fused with a thickened layer (black arrow) of epithelial cells and some inflammatory cells. There is also a general breakdown of lamellar architecture and hemorrhage (red arrow). Branchial lesions were evident in all fish submitted to COL, and ranged in severity from moderate to severe.

Appendix 3- IMET LC/MS Karlotoxin Results

Table 1

Bottle Name	Cell Count (cells/ml)	Volume	UV Area	LC_MS Area	m/z (+2)	m/z(+2) II	Mass	Total KmTx (ng)	Concentration (ng/ml)
Bottle 1	4282	300	15880.5	765867	690.3977		1380.7954	13897.7537922987	46.33
Bottle 6475	4040	300	12715.8	545107	690.3962	689.3874	1380.7924	11128.1796966161	37.09
Bottle 007	3394	300	57618.8	2051719	690.3970	689.3877 682.3988	1380.7940	50424.8541423571	168.08
Bottle 3118	3232	300	22201.8	861004	690.3965	689.3893	1380.793	19429.813302217	64.77
Bottle 4771	3151	300	6255	253033	690.3943	689.3911	1380.7886	5474.03733955659	18.25
Bottle 91	2424	300	11160.3	394222	690.3980	689.3911	1380.7960	9766.89031505251	32.56
Bottle 5912	1454	300	8476	396306	690.3965	689.3911	1380.793	7417.73628938156	24.73
Stds	Injected Mass (5 ul of 1/100 dilution) ng			UV Area per ng					
4,5-dihydro-KmTx 2	50		104245	2084.9					
,5-dihydro-dechloro-KmTx 2	60		20569	342.82					

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