Characterization of cyanobacteria in White Pond, Concord, MA: An evaluation of cyanobacterial populations, biomass, cyanotoxin concentrations and trophic interactions

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Background

The Town of Concord, MA adopted the cyanoCasting™ Program for implementation at White Pond beginning in May 2022 to assist in routine public health monitoring and long-term management planning for this beloved "Jewel in the Woods". The program has two elements that make it unique, 1) gathering information that supports a proactive versus reactive monitoring program and 2) a "train the trainer" component. Within the monitoring program, a tiered sampling design allows for the novel assessment of the plankton populations (e.g. bloom-forming cyanobacteria) and the broader trophic influences. This is important because what we collect and analyze is simply a "snapshot" representing the influence of many variables which may include traditional water quality parameters (e.g. abiotic variables such as nutrients, light, temperature, turbidity) but can benefit from considerations of biological factors (e.g. biotic variables including presence/absence of fish, structure of zooplankton population).

Sample Collection and Results

Beginning on May 4, 2022, we started collecting plankton samples that would provide information on the trophic influence of fish (as potential predators) on the zooplankton (as their food source and prey) which could then result in changes in the cyanobacteria (the food source of the zooplankton). This phenomenon, known as a trophic cascade, has been documented in many Cape Cod freshwater ponds[1,2] acting as a primary variable dictating the dynamics of their cyanobacterial populations. The early season zooplankton data suggested that a trophic cascade was not occurring in White Pond. This helped us interpret whether and to what extent cyanobacteria might be present in the water samples, where this was close to non-detectable until June 1. On June 1, the "proactive" bloom-forming cyanobacteria (BFC) sample indicated that particular cyanobacterial genus (e.g. *Dolichospermum* and *Microcystis*) were responding to changes in light levels (an abiotic variable) and beginning to appear in the water column of White Pond at a rapid rate. On June 15, when sampling at the Town Beach was initiated prior to its official opening date, an accumulation of cyanobacteria along the shoreline was observed. Throughout the remainder of the season weekly sampling at White Pond was conducted, using the results from the BFC sample to help the Health Division make decisions regarding beach access.

Recommendations for 2023

1) The information from the 2022 sampling season demonstrated that the bloom-forming cyanobacteria (BFC) sample played an important role in the early warning system that allowed for a proactive cyanobacteria monitoring program for the Town of Concord. The tiered monitoring program should continue in the future until data suggests otherwise.
2) The "train-the-trainer" program successfully familiarized Town of Concord staff with the collection of cyanobacteria and zooplankton samples. Objectives for the 2023 season should focus on making arrangements for staff to take on the responsibility of sample processing, fluorometric analysis and interpretation. Any cyanotoxin analysis should continue to be conducted at an outside laboratory.
3) The collection of plankton samples from White Pond offers the Town an opportunity to create a robust educational program focusing on freshwater ecology.

Project Monitoring Plan

For the purposes of the initial assessment of White Pond we used a suite of sample types including surface grab samples (when available), bloom-forming cyanobacteria (BFC), whole lake water (WLW), less than 50 micron (<50 µm) and zooplankton. The samples were collected from early May until mid-October at a series of sites across the pond, as shown in Figure 1, including the Town beach, Deep Site #1, Deep Site #2 and rotating cove sampling to confirm the degree of homogeneity and suggest where exposure to cyanotoxins might occur at permitted and unpermitted swimming sites.

Description of methods

The samples collected in White Pond followed the U.S. EPA QAPP[2], including the bloom forming cyanobacteria (BFC), whole lake water (WLW) less than 50 micron (<50µm) and zooplankton samples. The BFC and zooplankton samples were collected as a 3meter vertical net tow using a 50µm plankton net, placed in a darkened container for 2 hours, placed into the Pocket ZAPPR™ for a total of 30 minutes to allow for separation, followed by collection of the BFC and zooplankton samples. The BFC sample was collected from the "top" of the Pocket ZAPPR™ using a 5 mL pipette, placed in a 5 mL microvial and frozen for future fluorometric and cyanotoxin analysis. The zooplankton sample was collected from the "bottom" of the Pocket ZAPPR with a 5 mL and preserved with 4% formalin-sucrose for future analysis. The WLW sample was collected using an 3m integrated tube sampler and placed in a darkened container. The WLW sample was thoroughly mixed, and a 100 mL subsample was passed through a 50µm nylon mesh ring net to collect the <50µm sample. Subsamples (5 mL) of the WLW and <50µm were placed in a microvial and frozen for future fluorometric and cyanotoxin analysis. When surface scum or shoreline accumulations were present, a surface grab sample was collected by skimming a sample from the water surface or shoreline sediment with a darkened container. A 5 mL subsample was removed with a pipette, placed in a microvial and frozen for future fluorometric and cyanotoxin analysis.

Sample processing and analysis: Samples were prepared for fluorometric analysis using the extraction method[4,5] (single freeze-thaw), placed in a calibrated (µg L^{-1}) Amiscience hand-held multi-channel fluorometer and readings taken for phycocyanin (PC) and phycoerythrin (PE). Following fluorometry, the samples were refrozen for ELISA (enzyme-linked immunosorbent assay) cyanotoxin analysis using a triple freeze-thaw extraction method.

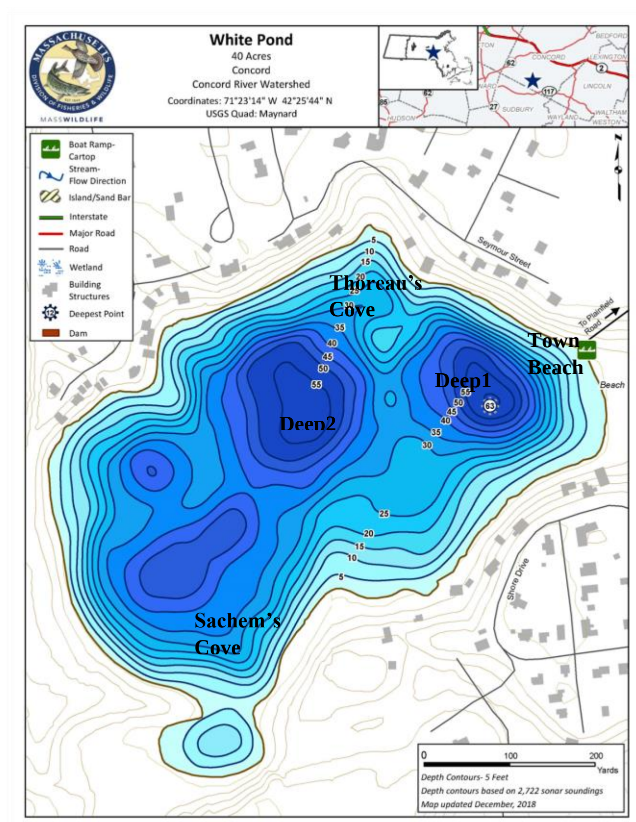

Figure 1. Map showing cyanobacteria sampling stations

Early Season Monitoring May 4 – July 19:

Monitoring for cyanobacteria was initiated on May 4, 2022 at Deep Site #1, Deep Site #2, Sachem's Cove and Thoreau's Cove with undetectable biomass in the bloom forming cyanobacterial sample and proceeded biweekly. On June 1, there was a rapid increase in bloom-forming cyanobacterial biomass noted at Deep Site #1, Deep Site #2 and Thoreau's Cove , with Sachems's Cove not being sampled that day and sampling was increased to a weekly basis. On June 15, sampling at the Town Beach was initiated with a shoreline accumulation being observed that day composed of *Dolichospermum lemmermanii,* The bloom-forming cyanobacterial (BFC) biomass sample was composed of *Dolichospermum lemmermanii* reaching its maximum on this sampling date, consistent with an "early season recruitment event". Of particular note is the extent to which this phenomenon was observed at all five sampling sites, suggesting that the water column of White Pond was being "seeded" by this genus. Two weeks later, on June 28, a similar "early season recruitment event" consisting of *Microcystis aeruginosa* was observed at all five sampling sites across the pond and a shoreline accumulation being observed at the Town Beach. The results confirmed a relatively homogeneous distribution of buoyant cyanobacteria in White Pond and suggested that three sampling sites would be sufficient for continued assessment activities. By July 19, the pond was "seeded" with *Microcystis* as well as *Dolichospermum*. "Seeding" is part of the life history and ecology of both of these cyanobacterial genus and can be expected to recur annually following a profile similar to that shown in Figure 2, although the timing may vary somewhat. Of particular interest during this very active period are the differences in the pigment and microcystin concentrations in the *Dolichospermum* versus the *Microcystis* samples, best exemplified in the "Grab" samples. (Table 3). While the phycocyanin concentrations are very similar (1788 versus 1820 µg L^{-1}), the microcystin concentrations are quite different (non-detect versus 69.2 µg L^{-1}), respectively. During the spring recruitment, shoreline accumulations at the Town Beach collected on July 5 had measured microcystin concentrations of 232.4 µg L^{-1}. During this time we noted low-levels of cyanobacterial biomass (as phycocyanin) measured in the whole lake water samples (5.2 µg L^{-1}) and microcystin concentrations less than 0.40 µg L^{-1}.

Monitoring July 26 – October 4

Monitoring continued at Deep Site #2, Thoreau's Cove and the Town Beach from July 26 – October 4 on a bi-weekly basis as shown in Figure 3. The patterns of cyanobacterial population remained similar to that described for May 4- July 19, where all three sites had similar composition and dominance (*Dolichospermum* spp. versus *Microcystis* spp.) and increases/decreases in the phycocyanin concentrations. The values from samples collected at the Town Beach remained elevated over those for either the Deep Site #2 or Thoreau's Cove, suggesting that wind action and water movement play a role in the concentration of the sample along this westward facing shoreline. These results appear to confirm the concurrent investigation conducted by Higgins Environmental Associates ("Higgins") associated with the passive A-pod algal harvesting system. Notable events after July 19 include the shoreline accumulation of *Dolichospermum* on August 9, when a grab sample was collected from the water, as well as a vertical profile series of samples from the sandy shoreline to a depth of approximately 0.5 inches. These samples were tested for in the laboratory for anatoxin-a, a cyanotoxin associated with animal exposure and potentially small children playing along the shoreline. The anatoxin-a concentrations rapidly decreased through the sand profile, as seen in Table 3 from a high of 0.5 µg L^{-1} to 0.1 µg L^{-1}.

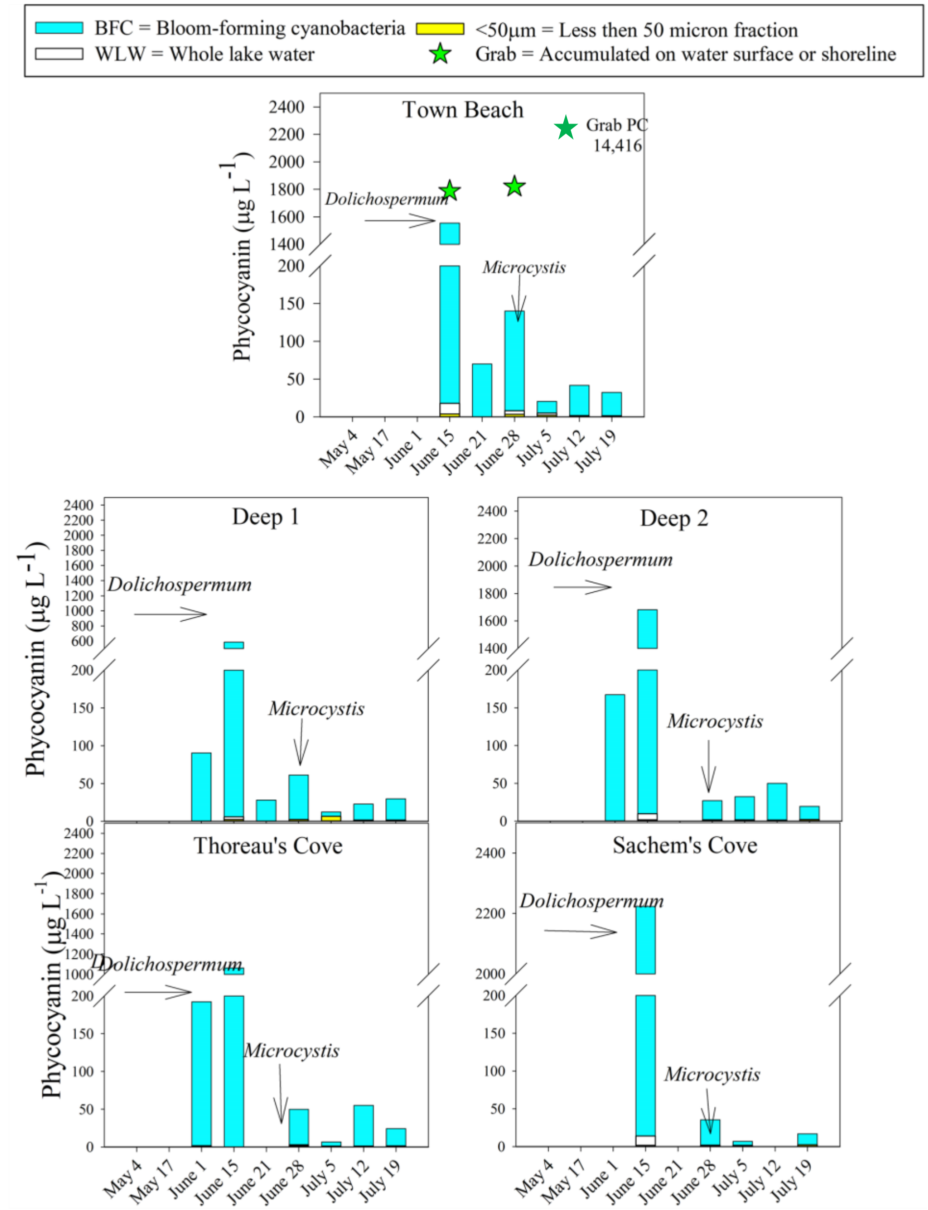

Figure 2. Phycocyanin concentrations at 5 sites in White Pond, May 4 – July 19

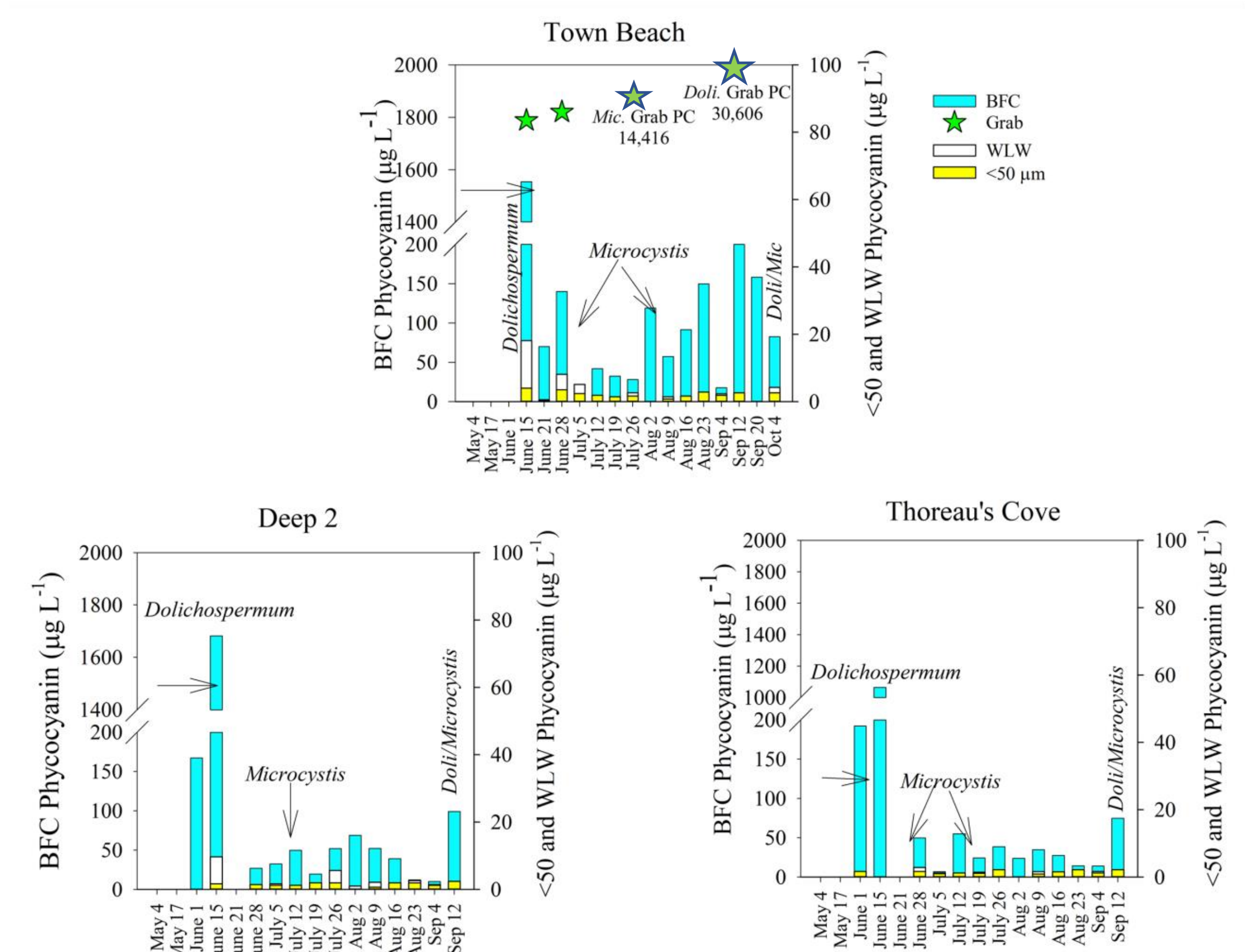

Figure 3. Phycocyanin concentrations at 3 sites in White Pond May 4 – October 4

Evaluation of trophic interactions in White Pond

The question arose as to whether the presence of vertebrate planktivores were exerting a strong trophic influence on the cyanobacterial populations in White Pond. Zooplankton play a central role in the processing algal (and cyanobacterial) biomass and can serve as a trophic indicator providing information on the health and balance within aquatic systems. The CyanoCasting ™ Program utilizes the Pocket ZAPPR™ to isolate and collect positively phototactic microcrustaceans (zooplankton) for qualitative analysis. This approach offers a simple and effective method to assess trophic influences. Zooplankton samples were collected from May 4 – October 4 from Deep Site #2 between the hours of 7AM-9AM and identified to the species level when present for a description of community composition. As shown in Table 12, the zooplankton displayed a degree of diversity from early May through June 1, after which only calanoid copepods were observed. Rotifers were notably absent from the zooplankton samples, suggesting that *Daphnia* spp. were present in White Pond[6]. A single night time sample (8 PM) collection was conducted on September 14, revealing a diverse microcrustacean population with an average body length of 0.942 mm and predator:panfish ratio of 0.474[7]. Evidence of invertebrate planktivory in the zooplankton sample consisted primarily of the pointed helmet shield on *Daphnia ambigua*, which forms in the presence of *Chaoborus punctipennis* [8]. We were able to collect and identify *C. punctipennis* during our night time monitoring effort. The presence of the diverse microcrustacean population at this time (after sun-down) suggested that the zooplankton were actively using visual refuge to avoid vertebrate planktivory during daylight hours. The predator:panfish ratio suggests an aquatic system which supports a fishery that has a balanced trophic structure[7]. Examples of the microcrustaceans in White Pond are shown in Appendix A along with a key to their distinguishing characteristics.

Results and Discussion:

Train-the Trainer:

The “Train-the Trainer” program with Town Staff should continue during 2023, and be expanded to include the processing of samples for fluorometric analysis and interpretation of data. Samples should continue to be analyzed for cyanotoxins (microcystins and anatoxin-a) at an outside laboratory as deemed necessary.

Tiered monitoring from 2022:

The use of the tiered monitoring approach was able to confirm that an aquatic trophic cascade that could negatively affect the water quality in White Pond was not occurring. The structure of the zooplankton population that was collected in early June and again during the mid-September night-time collection confirmed the presence of large-bodied cladocerans (a type of zooplankton). The absence of cladocerans in the routine daytime sampling from the Deep site suggests that they are making use of visual refuge to avoid fish predation, thus making night-time sampling the preferred protocol. Additionally, aquatic trophic cascades tend to result in observable shifts in the composition in the cyanobacterial populations towards an increase in the picocyanobacterial biomass, which would have been measured using the $<50\mu m$ water sample. The non-detect cyanobacteria biomass in these $<50\mu m$ samples (data not shown) supports this conclusion.

The bloom-forming cyanobacteria (BFC) samples were able to support the proactive approach that was desired by the Town, and the data was key to anticipating cyanobacterial accumulations at the shoreline of the Town Beach. In this manner, the Health Division was able to improve the decision making process for beach closures. The composition of the BFC samples (*Dolichospermum*s spp. and *Microcystis* spp.) suggests that visible scums will continue to be observed in White Pond for the foreseeable future. The cyanoCasting™ monitoring program should be continued during 2023 at three sites in White Pond, including the Town Beach, Deep Site #2 and Thoreau’s Cove. Sample collection should occur at least every 2 weeks and weekly as needed. The ecology of these two common genus suggests that the presence of a cyanobacterial “footprint” in White Pond should be confirmed during 2023 through the collection of sediment samples to confirm presence via visual inspection, pigment and toxin analysis. Sample collection could take place during “ice-on” conditions as well as early “ice-off” conditions using a gravity core device. This will confirm the source of cyanobacteria for future seeding of the water column.

Tables 1-3. Cyanobacterial pigment and toxin analysis in White Pond Town Beach

Table 1. Town Beach Whole Lake Water (WLW)

		Estimate	Measured
Dates	PC (µg L^{-1})	MC (µg L^{-1})	**MC (µg L^{-1})**
5/4/2022			
5/17/2022			
6/1/2022			
6/15/2022	18.1	0.01	
6/21/2022	**0.6**	0.00	
6/28/2022	8.2	0.24	0.32
7/5/2022	5.2	0.14	0.30
7/12/2022	**1.5**	0.03	
7/19/2022	**1.2**		
7/26/2022	**2.6**		
8/2/2022	**0.1**		
8/9/2022	**1.5**		
8/16/2022	**1.5**		
8/23/2022	**2.6**		
9/4/2022	**2.4**		
9/12/2022	**1.9**		
9/20/2022	**0.1**		
10/4/2022	4.2		

Bold RED text denotes values less than 3 µg L^{-1} (Limit of Detection)

Table 2. Town Beach Bloom-forming Cyanobacteria (BFC)

				Estimate	Measured
Dates	Composition & Dominance	PC (µg L^{-1})	Growth rate (µ day^{-1})	MC (µg L^{-1})	**MC (µg L^{-1})**
5/4/2022					
5/17/2022					
6/1/2022					
6/15/2022	Dolichospermum (100%)	1554.0		0.13	
6/21/2022	Dolichospermum (100%)	70.0		0.03	
6/28/2022	Microcystis (95%)	140.2		6.39	1.57
7/5/2022	Doli%/Mic% (50%/50%)	20.7		0.71	0.84
7/12/2022	Microcystis (95%)	41.9	0.10	1.60	
7/19/2022	Microcystis (95%)	32.6		1.20	
7/26/2022	Microcystis (99%)	28.1		1.01	
8/2/2022	Microcystis (99%)	119.1	0.21	5.30	0.99
8/9/2022	Dolichospermum (95%)	57.4		0.02	
8/16/2022	Dolichospermum (90%)	91.6		0.03	
8/23/2022	Dolichospermum (100%)	149.9		0.04	
9/4/2022	Dolichospermum (100%)	17.7		0.01	
9/12/2022	Doli/Mic (70%/30%)	287.1		0.05	0.21
9/20/2022	Dolichospermum (99%)	158.3		0.04	N/D
10/4/2022	Dolichospermum (100%)	82.8		0.03	

Town Beach Grab

			Estimate	Measured
	Dates	PC (µg L^{-1})	MC (µg L^{-1})	MC (µg L^{-1}) or ATX (µg L^{-1})
	5/4/2022			
	5/17/2022			
	6/1/2022			
	6/15/2022	1788.0	0.14	N/D
	6/21/2022			
	6/28/2022	1820.0	121.22	69.19
	7/5/2022	14416.0	1304.23	232.38
Sediment profile				
Surface	8/9/2022	30640.0		ATX 0.50 µg L-1
0-0.1"	8/9/2022	16120.0		ATX 0.50 µg L-1
0.1-0.2	8/9/2022	181.0		ATX 0.20 µg L-1
0.2-0.3	8/9/2022	67.0		ATX 0.10 µg L-1

Tables 4-5. Cyanobacterial pigment and toxin analysis in White Pond Deep Site #1

Table 4. Deep 1 Whole Lake Water (WLW)

		Estimate	Measured
Dates	PC (µg L^{-1})	MC (µg L^{-1})	**MC (µg L^{-1})**
5/4/2022	**0.1**		
5/17/2022	**0.1**		
6/1/2022			
6/15/2022	5.8	0.01	
6/21/2022			
6/28/2022	**1.7**	0.04	**0.28**
7/5/2022	**3.5**	0.09	
7/12/2022	**1.0**	0.02	
7/19/2022	**1.7**		

Bold RED text denotes values less than 3 µg L^{-1} (Limit of Detection)

Table 5. Deep 1 Bloom-forming Cyanobacteria (BFC)

				Estimate	Measured
Dates	Composition & Dominance	PC (µg L^{-1})	Growth rate (µ day^{-1})	MC (µg L^{-1})	**MC (µg L^{-1})**
5/4/2022		**0.1**			
5/17/2022		**0.1**			
6/1/2022	Dolichospermum (100%)	90.5	0.49		
6/15/2022	Dolichospermum (100%)	585.7	0.13	0.08	
6/21/2022	Dolichospermum (100%)	28.1			
6/28/2022	Microcystis (95%)	61.2		2.47	**0.99**
7/5/2022	Doli%/Mic% (50%/50%)	12.3		0.39	
7/12/2022	Microcystis (95%)	22.8	0.09	0.80	
7/19/2022	Microcystis (95%)	29.8	0.04	1.08	

Bold RED text denotes values less than 3 µg L^{-1} (Limit of Detection)

Tables 6-7. Cyanobacterial pigment and toxin analysis in White Pond Deep Site #2

Table 6. Deep 2 Whole Lake Water (WLW)

		Estimate
Dates	PC (µg L^{-1})	MC (µg L^{-1})
5/4/2022	**0.1**	
5/17/2022	**0.1**	
6/1/2022		
6/15/2022	9.7	0.01
6/21/2022		
6/28/2022	**1.2**	0.03
7/5/2022	**1.7**	0.04
7/12/2022	**1.2**	0.03
7/19/2022	**1.5**	
7/26/2022	5.6	
8/2/2022	**1.0**	
8/9/2022	**2.1**	
8/16/2022	**1.5**	
8/23/2022	**2.6**	
9/4/2022	**1.5**	
9/12/2022	**1.9**	

Bold RED text denotes values less than 3 µg L^{-1} (Limit of Detection)

Table 7. Deep 2 Bloom-forming Cyanobacteria (BFC)

				Estimate
Dates	Composition & Dominance	PC (µg L^{-1})	Growth rate (µ day^{-1})	MC (µg L^{-1})
5/4/2022		**0.1**		
5/17/2022		**0.1**		
6/1/2022	Dolichospermum (100%)	167.2	0.53	
6/15/2022	Dolichospermum (100%)	1682.0	0.16	0.14
6/21/2022				
6/28/2022	Microcystis (95%)	27.0		0.96
7/5/2022	Doli%/Mic% (50%/50%)	32.3		1.19
7/12/2022	Microcystis (95%)	49.8	0.06	1.94
7/19/2022	Microcystis (95%)	19.5		
7/26/2022	Microcystis (99%)	51.9	0.14	2.04
8/2/2022	Microcystis (99%)	68.8	0.04	2.82
8/9/2022	Dolichospermum (100%)	52.2		0.02
8/16/2022	Dolichospermum (100%)	39.1		0.02
8/23/2022	Dolichospermum (100%)	12.1		0.01
9/4/2022	Dolichospermum (100%)	10.0		0.01
9/12/2022	Doli%/Mic% (70%/30%)	99.1		0.03

Bold RED text denotes values less than 3 µg L^{-1} (Limit of Detection)

Tables 8-9. Cyanobacterial pigment and toxin analysis in White Pond Thoreau's Cove

Table 8. Thoreau's Cove Whole Lake Water (WLW)

		Estimate
Dates	PC (µg L^{-1})	MC (µg L^{-1})
5/4/2022	**0.1**	
5/17/2022		
6/1/2022	**1.2**	
6/15/2022		
6/21/2022		
6/28/2022	**2.8**	0.07
7/5/2022	**1.2**	0.03
7/12/2022	**1.2**	0.03
7/19/2022	**1.5**	
7/26/2022	**2.2**	
8/2/2022	**0.1**	
8/9/2022	**1.6**	
8/16/2022	**1.5**	
8/23/2022	**2.2**	
9/4/2022	**1.7**	
9/12/2022	**1.9**	

Bold RED text denotes values less than 3 µg L-1 (Limit of Detection)

Table 9. Thoreau's Cove Bloom-forming Cyanobacteria (BFC)

				Estimate
Dates	Composition & Dominance	PC (µg L^{-1})	Growth rate (µ day^{-1})	MC (µg L^{-1})
5/4/2022		**0.1**		
5/17/2022				
6/1/2022	Dolichospermum (100%)	192.2		
6/15/2022	Dolichospermum (100%)	1064.0	0.12	0.11
6/21/2022				
6/28/2022	Microcystis (95%)	49.8		1.95
7/5/2022	Doli%/Mic% (50%/50%)	6.8		0.20
7/12/2022	Microcystis (95%)	55.1	0.30	2.19
7/19/2022	Microcystis (95%)	24.2		
7.26.2022	Microcystis (99%)	38.6	0.07	1.45
8/2/2022	Microcystis (99%)	23.7		
8/9/2022	Dolichospermum (100%)	34.7		0.02
8/16/2022	Dolichospermum (100%)	27.4		0.02
8/23/2022	Dolichospermum (100%)	14.4		0.01
9/4/2022	Dolichospermum (100%)	14.0		0.01
9/12/2022	Doli%/Mic% (70%/30%)	74.8		0.03

Bold RED text denotes values less than 3 µg L^{-1} (Limit of Detection)

Tables 10-11. Cyanobacterial pigment and toxin analysis in White Pond Sachems' Cove

Table 10. Sachems Cove Whole Lake Water (WLW)

		Estimate
Dates	PC (µg L^{-1})	MC (µg L^{-1})
5/4/2022		
5/17/2022	**0.1**	
6/1/2022		
6/15/2022	14.1	0.01
6/21/2022		
6/28/2022	**2.0**	
7/5/2022	**1.7**	
7/12/2022		
7/19/2022	**1.5**	

Bold RED text denotes values less than 3 µg L-1 (Limit of Detection)

Table 11. Sachems Cove Bloom-forming Cyanobacteria (BFC)

				Estimate
Dates	Composition & Dominance	PC (µg L^{-1})	Growth rate (µ day^{-1})	MC (µg L^{-1})
5/4/2022				
5/17/2022		**0.1**		
6/1/2022				
6/15/2022	Dolichospermum (100%)	2223.0		0.16
6/21/2022				
6/28/2022	Microcystis (95%)	35.3		
7/5/2022	Doli%/Mic% (50%/50%)	7.0		
7/12/2022				
7/19/2022	Microcystis (95%)	17.0		

Bold RED text denotes values less than 3 µg L^{-1} (Limit of Detection)

Table 12. List of microcrustaceans in White Pond

Sample Date	Microcrustacean
4-May	Calanoid copepods
	Microcyclops rubellus
	Holopedium gibberum
17-May	*Eubosmina longispina*
1-Jun	*Daphnia ambigua*
	Daphnia catawba
	Daphnia mendotae
15-Jun	Zooplankton absent
21-Jun	Calanoid copepods
28-Jun	Calanoid copepods
5-Jul	Calanoid copepods
	Epischura nordenskioldi
12-Jul	Calanoid copepods
	Epischura nordenskioldi
19-Jul	Calanoid copepods
26-Jul	Calanoid copepods
2-Aug	Calanoid copepods
9-Aug	Calanoid copepods
16-Aug	Calanoid copepods
23-Aug	Calanoid copepods
4-Sep	Calanoid copepods
12-Sep	Calanoid copepods
14-Sep	Calanoid copepods
	Eubosmina longispina
	Holopedium gibberum
	Diaphanosoma brachyurum
	Daphnia ambigua
	Daphnia catawba
	Daphnia mendotae

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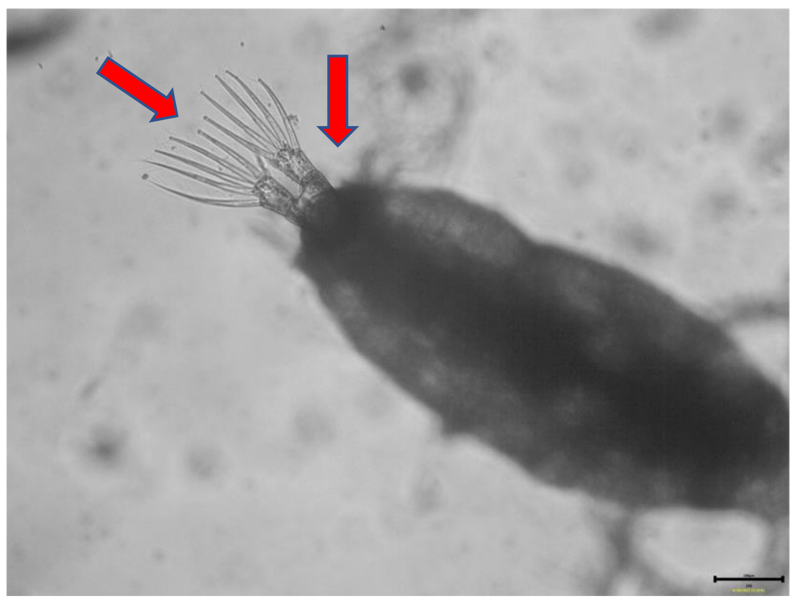
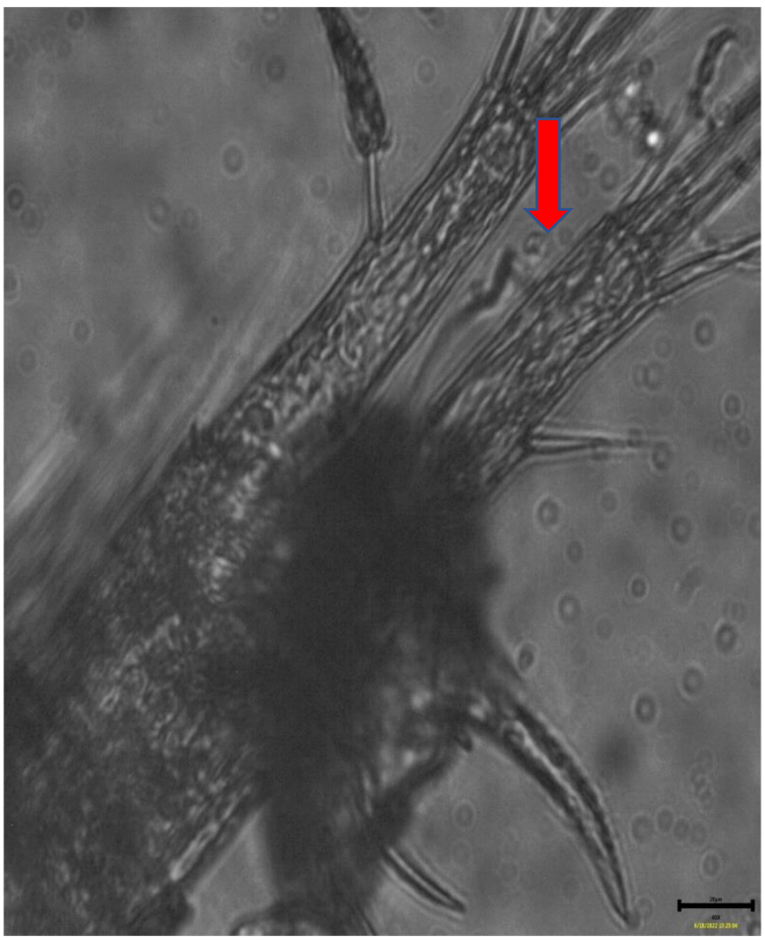
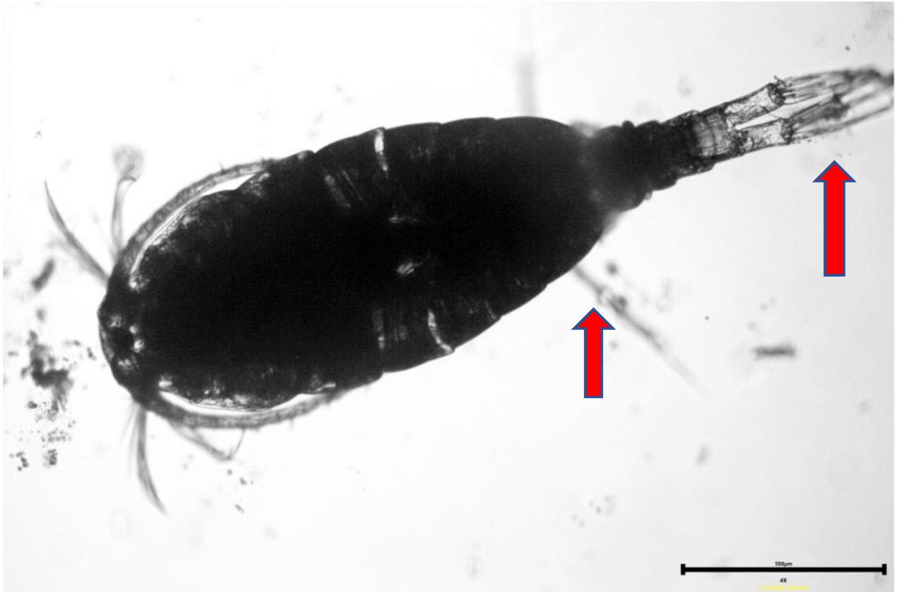
Appendix A: Microcrustaceans in White Pond

<u>Calanoid copepod:</u>
Antennules long
5 thin caudal setae
Caudal length <3x caudal width
Setae of similar length
Male has right geniculate antennule

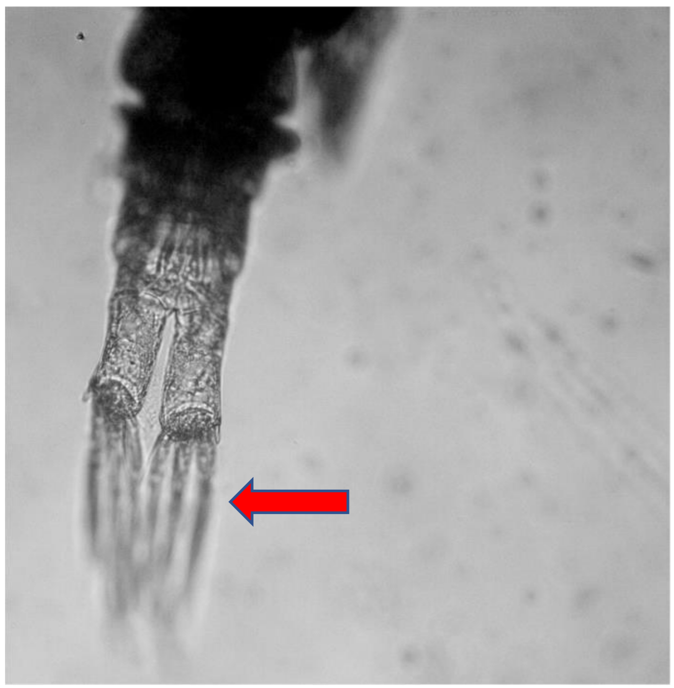


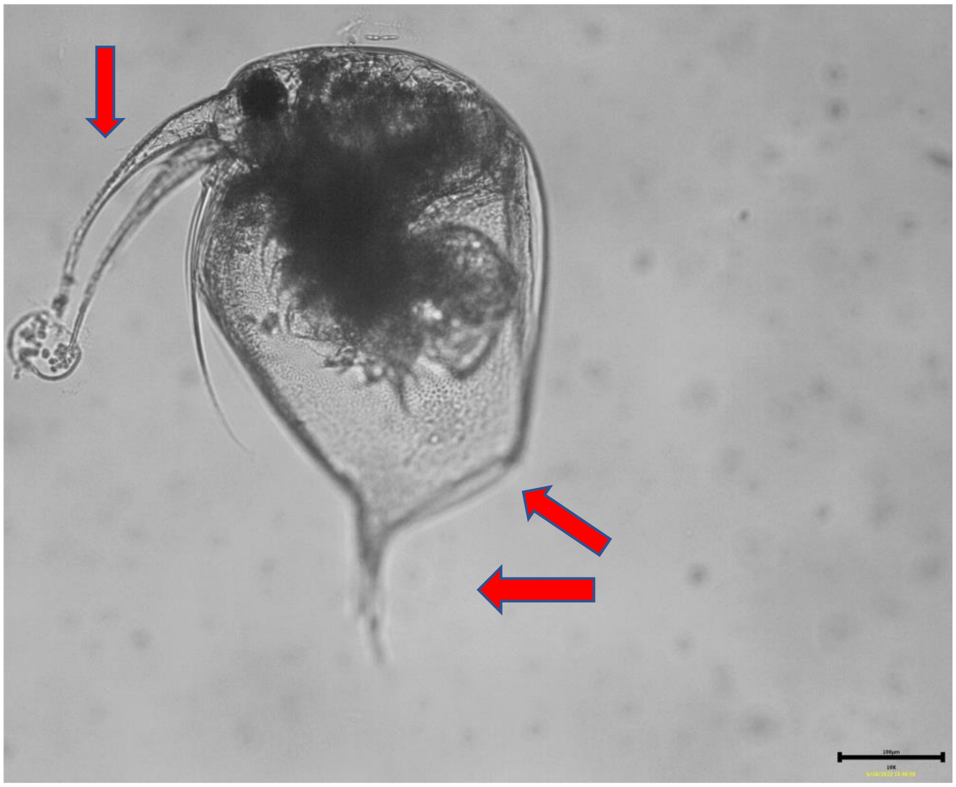


<u>*Microcyclops rubellus*</u>
Antennules short (<18 segment)
No apical claw
Prosome tapers towards urosome
First antennae 11 segments or less
Hairs absent from inner margin of caudal ramus

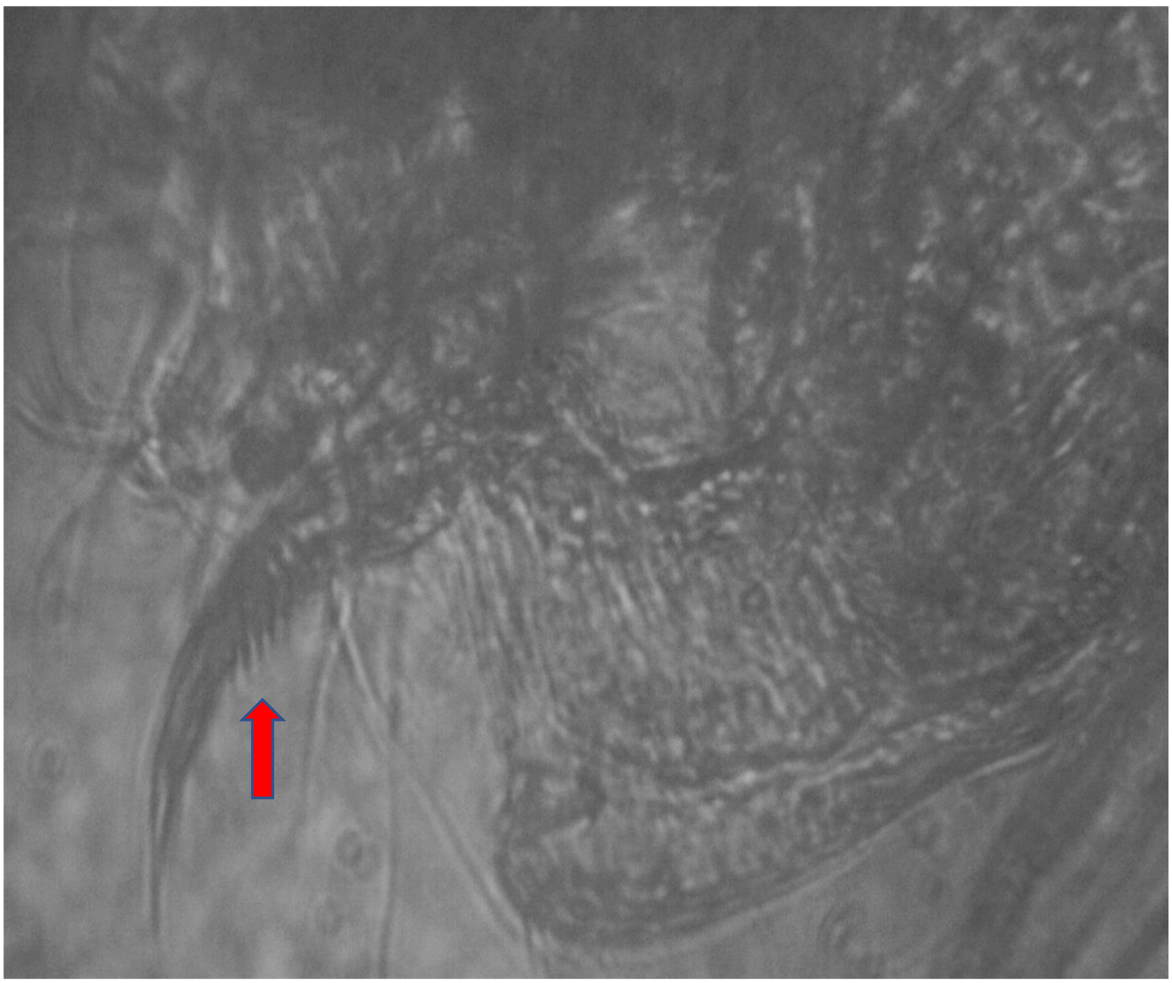


Epischura nordenskioldi
Antennules long
3 stout caudal setae
Outer setae shorter than ramus

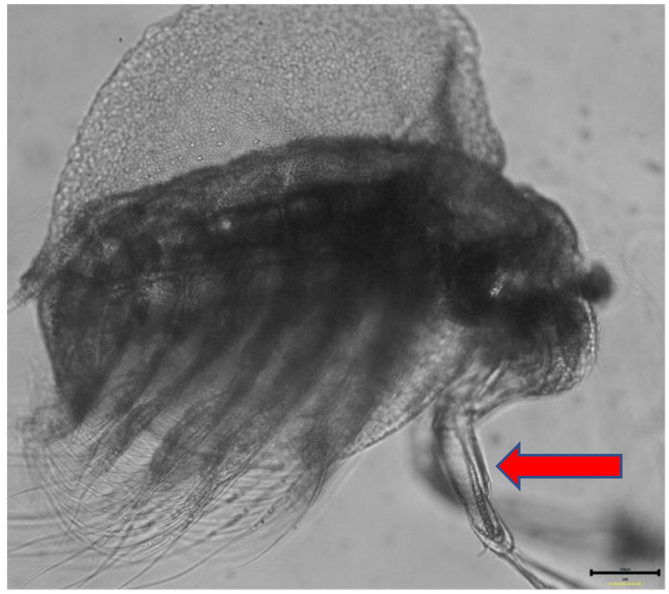




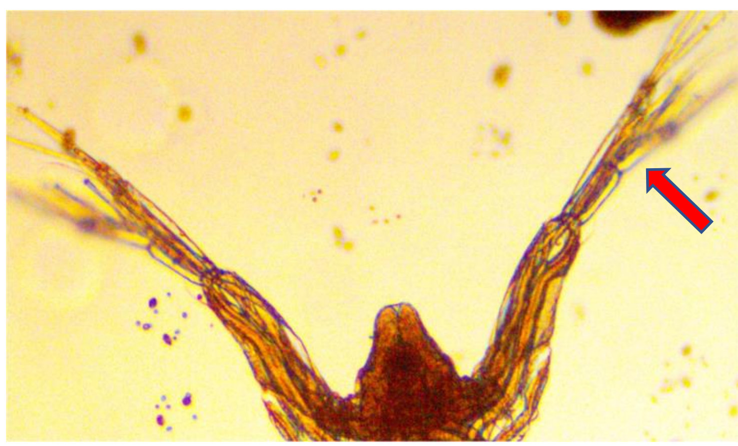
Eubosmina longispina
Bivalve carapace
Antennules separate
Post abdominal claw with proximal pecten only
Mucro present

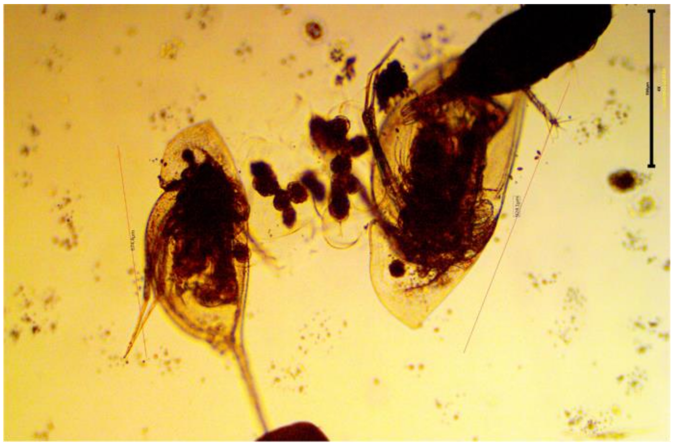


Holopedium gibberum
Unbranched antennule



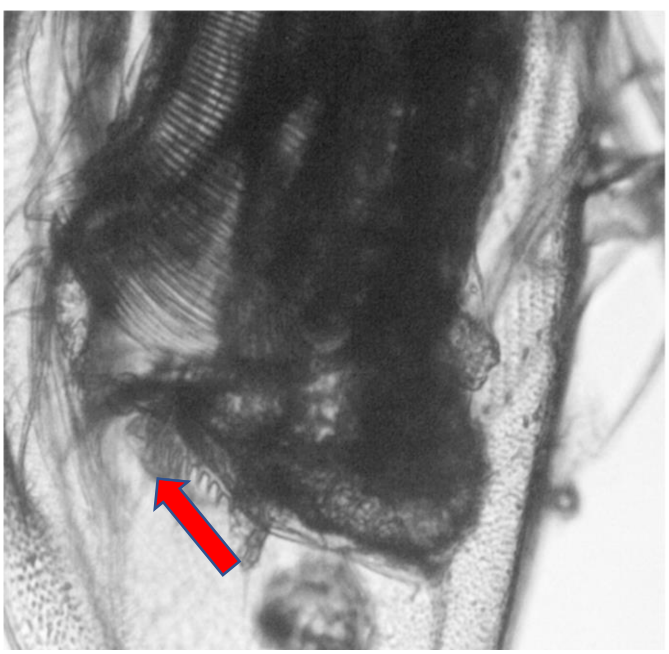
Diaphanosoma brachyurum
Bivalve carapace
Branched antennae
Post abdominal claw with three spines
Dorsal ramus with two segments





Daphnia. ambigua
Adult body length less than 1.24 mm
Pecten of post abdominal claw same size
2^{nd} segment antennule hair extends beyond tip of ramus
Helmet shield when C. punctipennis present

Daphnia mendotae:
Adult body length greater than 1.25 mm
Pecten of post abdominal claw same size
2nd segment antennule hair extends beyond tip of ramus
Rostrum long and acute
Head not twice as deep as long



Daphnia catawba
Teeth (4-6) of middle pecten stout
Optic vesicle not touching head margin
Dorsal spinules far apart (distance 2-3x spinule length)
D. schloderi similar but spinules almost overlapping