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Office of Research and Development

(Lead ORD Laboratory/Center/Office)

(Division/Branch)

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QA Category:

5

6

(Category)

ORD National Program:

(Acronym)

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(Number)

Task ID:

(Number)

Approvals

Prepared by:

Name	Date	Organization
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QA Manager:

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A3. Distribution List

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Betty Kreakie, ACESD
Stephen Shivers, ACESD

A4. Problem Definition/Background

The National Research Programs of the Environmental Protection Agency (EPA) provide a framework for research within the Office of Research and Development (ORD). One of these programs, Safe and Sustainable Water Resources (SSWR), is tasked with ensuring the integrity of the nation's waters to maintain human and environmental health. Harmful Algal Blooms (HABs) are a particular area of interest (e.g. Project 4.0.1). Understanding HABs is important because HABs have the potential to produce toxins, such as microcystin, and to reduce O₂ levels upon bloom collapse. These blooms negatively affect both human health (through direct contact and drinking water) and the ecosystem when present. Much research has been conducted on the spatial or temporal dynamics of HABs, but few have addressed the spatial and temporal dynamics at high frequency (biweekly sampling).

A5. Project/Task Description

Project Description

The overarching goal of this project is to investigate the spatial and temporal dynamics of cyanobacterial blooms. Specifically, this project will address the following questions:

1. How does the total algal and cyanobacterial population change over space and time?
 - a. Does landuse/landcover affect total algae and cyanobacteria?
2. Are there toxins produced in the bloom?
 - a. How does toxin concentration change over space and time?
3. How do physical and/or chemical drivers affect cyanobacterial blooms?
 - a. Are cyanobacterial population changes preceded by changes in N or P?
4. How does the zooplankton community change over space and time?
 - a. Are cyanobacteria populations affected by changes in zooplankton?

The effects of land use/land cover on HAB dynamics will be evaluated by initially selecting two lakes in southern New England with contrasting land uses (urban vs. forested). Warwick Pond (medium density urban) and Yawgoo Pond (forested) were selected as the study lakes (Figure 1). After the first study season, a third pond (Mashapaug Pond) was added as a high density urban pond with recurring cyanobacterial blooms.

Site Description

Warwick Pond is a small (34.4 hectare) lake located near the city of Warwick, Rhode Island. The watershed drains 4.23 km² of land dominated by urban use (residential, commercial, and industrial) (Figure 2). T.F. Green International Airport is adjacent to the lake and contributes runoff during storm events (Figure 3). The lake is shallow (max depth = 7.9 m) and has a residence time of 517 days (Figure 4). The inflows into Warwick Pond are: an unnamed tributary, surface and storm water runoff, groundwater, and precipitation. The outflow is

Buckeye Brook which empties into Narragansett Bay. HAB advisories have been issued for Warwick Pond during the summer/fall of 2015 and 2016.

Yawgoo Pond is larger (57.9 hectares), deeper (max depth = 11 m), and has a shorter residence time (215-246 days) than Warwick Pond (Figure 4). The watershed is 3.65 km² and is primarily forested (Figures 2 and 3). The inflows into Yawgoo Pond are the Chickasheen Brook, surface water runoff, groundwater, and precipitation, and the outflow is the Chickasheen Brook. Historically, Yawgoo Pond has had cyanobacterial blooms, but these were caused by point source inputs of P from two shellfish processing plants and from beavers damming the inflow. After remediating these sources, water quality improved and blooms have not been observed recently.

Mashapaug Pond is intermediate in size (46.1 hectares), shallow (max depth = 5.2 m), and is the largest freshwater pond in the city of Providence, RI. The watershed drains 4.7 km² of urban landscape. Mashapaug Pond receives flow from an unnamed tributary connected to Spectacle Pond, ground water, and storm water; the outflow provides flow to the Roger Williams Park pond complex. Mashapaug Pond has a long history of anthropogenic influences extending back to the 1600s. More recently, the Gorham Manufacturing Company operated between 1890 and 1986 adjacent to the pond and contributed to contamination within the pond. HABs occur frequently and persist from summer into the fall.

Task Description

To address the four project questions, a field sampling campaign will begin in May 2017 and will continue through the end of the growing season each year (~November-December). Sampling trips to the lakes will occur twice per week during the sampling campaign when the bloom is active and weekly otherwise. A deployable YSI sonde will be used to collect physical data (temperature, conductivity, dissolved oxygen (DO), and pH), and a bbe AlgaeTorch sonde will collect biological data (chlorophyll *a* and % cyanobacteria). Water samples will be collected and returned to the lab for chemical analyses (turbidity, TN, TP, NO₃, PO₄, NH₄, cyanotoxin, phycocyanin, and chlorophyll *a*). A Secchi disk will be used to determine Secchi transparency.

A6. Project/Task Organization

Project oversight will be provided by Jeff Hollister and Betty Kreakie. Stephen Shivers will contribute to overall project design as well as day to day operational procedures, including field data collection and laboratory analysis.

A7. Quality Objectives and Criteria for Measurement Data

The overall quality objective for this project is to generate field data to evaluate the spatial and temporal dynamics of cyanobacterial blooms. Because this study is not inference based but is exploratory in nature, exact values for completeness are unknown at present. Furthermore, the use of non-parametric and/or machine learning techniques alleviate many of the statistical considerations required of traditional parametric inference. As such, data completeness is of minimal concern with this research project. The quality objectives will be maintained by utilizing appropriate quality control measures in both the lab and the field. Regular calibration of field instruments (See Section B7 for schedule) should minimize error produced by the sondes (see Tables 1 and 2 for measurement range and resolution). Rigorous application of QA/QC policies in EPA LOPs and SOPs (see Section B4 for analytical methods) will be applied during laboratory procedures to ensure data quality and minimize instrumentation

or procedural error.

A8. Special Training Requirements/Certifications

None of the field tasks require special training or certification. Standard training using analyzers at ACESD will be provided, as needed, by ACESD technicians or others who have expertise with the method. Training will be documented via the ORD competency forms.

A9. Environmental concerns and mitigation

At this time, there are no projected environmental impacts that require mitigation action. Future potential impacts will be reevaluated as needed. Waste generated from laboratory analysis will be disposed of in accordance with local ACESD policy. A “leave no trace” practice will be utilized during field operations to limit the impact of field sampling on the environment. In order to minimize transference of nuisance species, the boat and all field gear will be washed at the end of each sampling trip. If two ponds are sampled on the same day, the boat and all field gear will be washed with high pressure spray (car wash) between ponds.

A10. Documentation and Records

Jeff Hollister will be responsible for maintaining and updating this QAPP. All weekly field equipment calibrations will be logged in the field notebook upon completion. Any deviance that requires recalibration will also be noted. Dates and times of calibration standard creation for lab procedures will be noted in the lab notebook. Results from standard curves will also be added to the lab notebook.

Field Tasks

Data acquisition in the field using non-data logging sondes and site observations will be recorded in Rite in the Rain notebooks (see Section A.5 for description of data to be collected). Data will be entered and transferred to EPA server storage, which is backed up regularly. This study falls into QA Category B for basic environmental research and will follow records schedule 1035b, records will be held for twenty years after project is completed.

Laboratory Tasks

The Astoria Pacific autoanalyzer (analytical chemistry) and microplate reader (ELISA) will output data in spreadsheet form. (See Section A.5 for description of data to be collected). These spreadsheets will be inspected for potential problems before being stored on EPA server storage. Data output from the Turner Designs Trilogy fluorometer will be transferred to a local laptop, converted to spreadsheet format using R, and stored on EPA server storage. All laboratory notes will be handwritten in an EPA approved laboratory notebook (#226). This study falls into QA Category B for basic environmental research and will follow records schedule 1035b, records will be held for twenty years after project is completed.

B. Measurement / Data Acquisition

B1. Sampling Process Design (Experimental Design)

Samples will be collected from three lakes (Mashapaug Pond, Warwick Pond and Yawgoo Pond) weekly or twice weekly over a six-month period (June-November). Seven sampling locations (six littoral and one index site) were established for each lake and coordinates for the sites are in Table 3. At the six littoral sites (minimum depth of 2 m), physical parameters (Secchi transparency, temperature, conductivity, DO, and pH) and biological parameters (chlorophyll *a* and cyanobacteria) will be measured using sondes and a Secchi disk. Integrated photic zone water samples will be collected in duplicate in amber bottles for turbidity, chlorophyll *a* and phycocyanin determination. At the index sites, the littoral site activities will be repeated and triplicate integrated water samples will be collected for chemical analysis (TN, TP, NO₃, PO₄, NH₄). This sampling framework is an expansion of the field design used for the National Lakes Assessment (2007 and 2012).

B2. Sampling Methods Requirements

Physical parameters will be measured with sondes and devices using EPA SOPs, LOPs, and FOPs. Secchi transparency will be measured using a Secchi disk. The Secchi disk will be lowered on the shaded side of the boat until it disappears and the depth will be recorded in the field notebook (EPA 841-B-11-003). The disk will be lowered 0.5 m, raised slowly until it reappears, and the reappearance depth will be recorded. To minimize error, the same person will take Secchi disk depth readings at each site. Temperature, conductivity, DO, pH, and salinity will be measured using a YSI multiprobe sonde (Table 1). Measurements will be made from the surface to just above the sediment at each sampling site creating a depth profile for each parameter (EPA 841-B-11-003). All data will be recorded in the field notebook. Total chlorophyll ($\mu\text{g chl-a/L}$) and cyanobacteria concentration ($\mu\text{g chl-a/L}$) will be measured at the surface at each site using a bbe AlgaeTorch (Table 2).

Water sample collection will follow a modified procedure (direct transference into collection bottles instead of a 4 L cubitainer) of the National Lake Assessment (EPA 841-B-11-003). A Minnesota Pollution Control Agency (MPCA) integrated water sampler will be used for collecting integrated water samples within the photic zone (upper 2 m of water column). At the index site and the littoral sites, the sampler will be triple rinsed with lake water before collecting water. After rinsing, water will be collected and dispensed into acid-washed 1 L amber bottles. The bottle shall be triple rinsed with lake water before collecting the final sample. Bottles will be placed in a cooler on ice until returning to the lab.

Zooplankton collection will follow the procedure of the National Lake Assessment (EPA 841-B-11-003). Two plankton nets (150 μm and 50 μm mesh size) will be towed vertically through the water column at a steady rate (0.3 m/s or 16.7 sec for each tow) at the index site within each lake. Using two different net sizes will allow for collection of different size fractions of zooplankton. A single 5 m tow will be used for each net size (3 m at Mashapaug). After towing, zooplankton will be rinsed into a bucket using a squirt bottle filled with DI water, narcotized using CO₂ tablets and transferred to labeled storage containers containing 70% ethanol.

B3. Sample Handling and Custody Requirements

Field collection bottles will be clearly labeled at the time of collection. Bottles will be stored in a cooler on ice until returning to the lab to prevent sample degradation caused by heat

and/or light.

All samples will be filtered and/or stored within 24 hours of collection.

Chlorophyll *a*, phycocyanin, NO₃, PO₄, and NH₄ samples will be filtered using pre-ashed GF/F (0.7 µm) filters.

Chlorophyll *a* will be filtered under reduced light conditions. 400 mL of water will be filtered onto filters for chlorophyll *a* analysis. If chlorophyll *a* concentrations are high causing reduced filtration rates, smaller volumes of water may be filtered. The volume of water that was filtered will be written in the lab notebook. Filters will be wrapped in foil and stored in the freezer until extraction. To start extraction, filters will be placed in 15 mL polystyrene tubes (prefilled with 90% acetone). The tubes will be stored in the freezer for a minimum of 12 hours before analysis.

Phycocyanin An additional 400 mL of water will be filtered onto filters for phycocyanin analysis. Filters will be wrapped in foil and stored in the freezer until extraction.

Nutrient analysis The water that was filtered will be used for nutrient analysis (NO₃, PO₄, and NH₄) and will be distributed to 20 mL scintillation vials for storage (below 0 °C) until analysis. Unfiltered water for nutrient analysis (TN and TP) will also be distributed to 20 mL scintillation vials for storage (below 0 °C) until analysis, which will occur within 48 hours of digestion.

Cyanotoxin Unfiltered water for cyanotoxin analysis will be distributed into 20 mL glass scintillation vials, frozen, and held until processing.

Zooplankton samples will be stored in pre-labeled containers and stored in 70% ethanol until analysis.

B4. Analytical Methods

Chlorophyll *a* Chlorophyll *a* determination will use fluorometric analysis. A known quantity of water will be filtered through 47 mm GF/F filters and that volume will be recorded in the lab notebook. Frozen filters will be placed in 15 mL polystyrene tubes (containing 10 mL of 90% acetone) and sonicated in a sonicating water bath for 20 minutes. Determination will proceed following the ACESD LOP for non-acid determination of chlorophyll *a* using a Turner Designs Trilogy fluorometer (J-ACESD-MAB-SOP-1425-0, [Non-Acid Determination of Chlorophyll a Using a Turner Designs Trilogy Fluorometer](#)).

Phycocyanin determination will use fluorometric analysis. A known quantity of water will be filtered through 47 mm GF/F filters and that volume will be recorded in the lab notebook. Frozen filters will be placed in 30 mL centrifuge tubes containing 20 mL of 50 mM phosphate buffer and sonicated in a sonicating water bath for 15 minutes under reduced light. The samples will be refrigerated for 2 hours then placed in a dark storage cabinet to warm to room temperature (total extract time of 3 hours). The samples will be analyzed for phycocyanin using a fluorometer fitted with a phycocyanin module (Orange) based on Kasinak et al 2015 and will follow J-ACESD-MAB-SOP-3949-0, [Determination of Phycocyanin Using a Turner Designs](#)

Trilogy Fluorometer.

Samples will be digested before TN/TP analysis. This digestion will follow ACESD LOP, which is currently in development.

Nutrient (TN, TP, NO₃, PO₄, and NH₄) determination will use segmented flow analysis performed on an Astoria-Pacific Micro-Segmented Flow Autoanalyzer. The following EPA standard methods will be used as guidance:

- Method 350.1 Determination of Ammonia Nitrogen
- Method 353.2 Determination of Nitrate-Nitrite
- Method 365.1 Determination of Phosphorus

These methods have been modified for use by the ACESD laboratory and the ACESD LOP describes a modified procedure (J-ACESD-EMRB-SOP-3076-1, [Nutrient Analysis by the Astoria-Pacific Astoria2 Micro-Segmented Flow Autoanalyzer](#)).

Cyanotoxin determination will use enzyme-linked immunosorbent assays (ELISA). Unfiltered water samples in 20 mL glass scintillation vials will undergo a freeze thaw cycle three times. After the third cycle, water will be filtered using a 25mm glass fiber syringe filter (1.2 µm) and transferred to a new glass scintillation vial. The assays will proceed according to kit manufacturer instructions and EPA guidelines (EPA Method 546 and EPA 841-B-11-004).

Zooplankton identification and analysis will follow the EPA National Lake Assessment methodology (EPA 841-B-11-003) as well as methods described in Mack et al. 2012.

Zooplankton samples will be rinsed with DI water in an appropriately sized sieve for the sample and then rinsed into a graduated cylinder. The cylinder will be rinsed and the total volume of water for the rinses will be recorded as the dilution volume. Aliquots will be counted until 200 individuals of a taxa are counted or until 5 aliquots are counted. Cladocerans will be identified to genus and copepods to order.

B5. Quality Control

QC checks, such as spikes and duplicates, are integral to ensuring data integrity and will be used whenever possible. QC checks are method dependent and are discussed in detail in the methods listed in the appendix.

B6. Instrument/Equipment Testing, Inspection, and Maintenance Requirements

All analytical equipment (Astoria-Pacific segmented flow autoanalyzer, fluorometer, and microplate reader), sondes, pipettes, and balances are maintained in accordance with manufacturer standards by ACESD. The Secchi disk will be inspected for proper rope attachment before each use.

B7. Instrument Calibration and Frequency

Instrument calibration is critical for ensuring data quality and will be performed frequently. The DO, turbidity, conductivity, and pH sensors will be calibrated weekly with known standards and checked before each sampling trip. The AlgaeTorch is factory calibrated

every two years per manufacturer recommendation and is maintained by Anne Kuhn. Manufacturer calibration is verified by using a calibration test cylinder. Accuracy of the AlgaeTorch will also be verified by correlating sonde output vs. chlorophyll *a* measured by fluorometry. Fluorometer accuracy will be assessed before each sample run using a secondary solid calibration standard. Other analytical equipment will be calibrated before each sample run by the designated operator at ACESD.

B8. Inspection/Acceptance Requirements for Supplies and Consumables

All research team members are responsible for ensuring all necessary supplies and consumables (i.e. pH buffers and conductivity standards) are available when needed.

B9. Data Acquisition Requirements (Non-direct Measurements)

Both Warwick Pond and Yawgoo Pond have been continuously monitored for over 20 years. Historical data collected by the University of Rhode Island's WaterWatch monitoring program will be used as historical context to aid in interpretation of data collected during this project.

B10. Data Management

All field data will be recorded on Rite-In-The-Rain paper to prevent reduced legibility from contact with water. All handwritten data will be transferred weekly to the database. Data from analytical equipment will be transferred to the database immediately upon procedure completion. Data for this task is to be under version control (e.g. via git) and will be stored both locally and remotely on GitHub. Access to the database will be available for all project collaborators via GitHub.

As this project will combine data from multiple field sensors, lab instruments, and hand written notes, great care will need to be taken in merging the data into an analytical dataset. The dataset itself can be fairly simply constructed as a flat .csv file. Raw data from laboratory instruments (immediately following procedure completion) and files from field sensors with data loggers (weekly) will be downloaded as raw files into the version controlled repository. Manual data entry will be conducted via data entry forms with initial quality control measures applied to those fields (e.g. throwing an error if water temp is not between 0-100 degrees Celsius). Data aggregation for all sources will be scripted and automated as much as is feasible.

Code for this project will be developed following standard best practices which include full documentation, code review, and use of a version control system (i.e. git). Collaboration on code development will be facilitated via GitHub.

R will be the primary analytical language; however we will explore others (e.g. python , javascript, c++, etc.) as required. The computational work for this project relies on open source software, and versions of most open source software packages change often. Thus, specifying these *a priori* is not recommended as versions will change. To ensure reproducibility of our work we will include specifications of software and operating system details (e.g. versions of R,

packages, and operating system) for all research products such that others can recreate the computational environment used for our analyses.

Lastly, all code, data, and documents will be managed as a research compendium (e.g. Marwick et al. 2018, <https://doi.org/10.1080/00031305.2017.1375986>). The compendium will be available via GitHub, archived on Zenodo, and will follow standard for research compendia written in the R language. A final README file will outline the file and directory structure and will be completed upon completion of the project.

C. Assessment/Oversight

C1. Assessments and Response Actions

The project leader will be responsible for overall oversight of the project. That leader will also initiate action in response to QA/QC issues. This research project falls into QA Category B. Assessments are not required but may occur at the discretion of management and/or QA staff, in which case they will be discussed, scheduled, and conducted at the convenience of QA manager and the project staff.

C2. Reports to Management

Annual reports will be provided to ORD as a measure of accountability and a barometer of project success.

D. Data Validation and Usability

D1. Data Review, Verification, and Validation

All data produced by analytical equipment will be reviewed for issues upon output. All handwritten data will be inspected and reviewed for issues created when transferring from notebook to database.

D2. Verification and Validation Methods

The inclusion of spikes and duplicates during analyte determination will validate data quality. All analytical output will be reviewed to ensure that QC checks are within the tolerances established in the corresponding methodologies. All manually entered data will be inspected for potential problems (e.g. transpositions).

D3. Reconciliation with User Requirements

Any analytical output that exceeds method tolerances will be rerun on a batch scale and reviewed again upon completion. Any errors found in manually entered data will be verified against the original handwritten data logs and corrected as needed.

Table 1: YSI ProDSS sonde probe specifications

	Range	Accuracy	Resolution
Temperature	-5-70 °C	± 0.2 °C	0.1 °C
Dissolved oxygen	0-50 mg/L	± (0.1 mg/L or 1.0% of reading)	0.01 mg/L
pH	0-14	± 0.2	0.01
Salinity	0-70 ppt	± (0.1 ppt or 1.0% of reading)	0.01 ppt
Conductivity	0-200 mS/cm	± (0.5 % of reading or 0.001 mS/cm)	0.001 mS/cm

Table 2: bbe AlgaeTorch probe specifications

	Range	Resolution
Total Chlorophyll	0-200 ($\mu\text{g chl a/L}$)	0.1 ($\mu\text{g chl a/L}$)
Cyanobacteria	0-200 ($\mu\text{g chl a/L}$)	0.1 ($\mu\text{g chl a/L}$)

Table 3: Coordinates for sampling locations in Warwick, Yawgoo, and Mashapaug ponds

site_id	long	lat
warw_1	-71.4104	41.72556
warw_2	-71.4111	41.72139
warw_3	-71.4145	41.72041
warw_4	-71.414	41.72248
warw_5	-71.4152	41.72512
warw_6	-71.414	41.72675
warw_P	-71.412	41.72267
yawg_1	-71.5737	41.51531
yawg_2	-71.5712	41.51354
yawg_3	-71.5694	41.51009
yawg_4	-71.5706	41.50811
yawg_5	-71.5728	41.50866
yawg_6	-71.5755	41.51285
yawg_P	-71.573	41.51068
mash_1	-71.4353	41.79314
mash_2	-71.4317	41.78974
mash_3	-71.4301	41.79124
mash_4	-71.4319	41.79344
mash_5	-71.4307	41.79772
mash_6	-71.4324	41.79829
mash_P	-71.4333	41.79389

Figure 1: Regional overview of the three study lakes

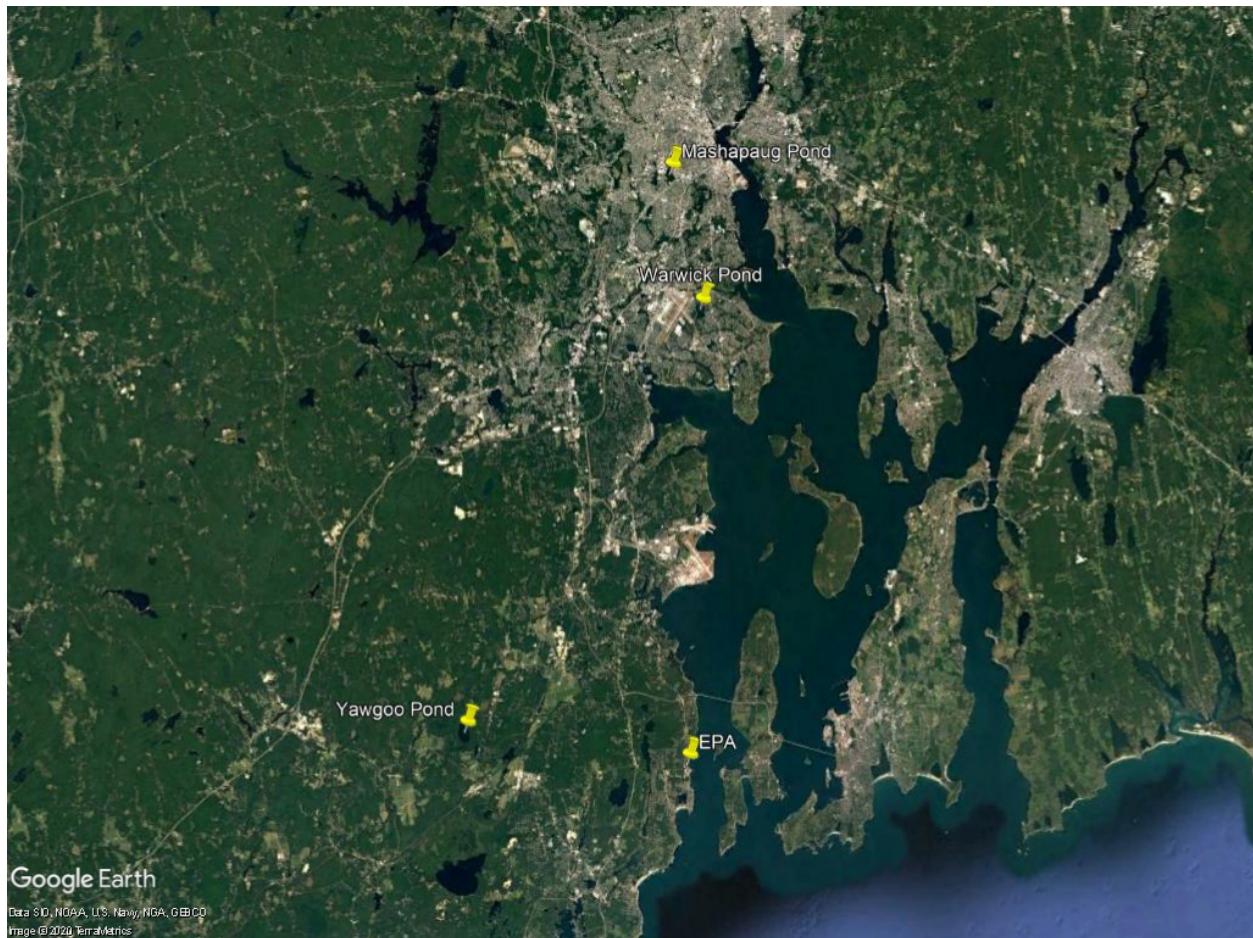
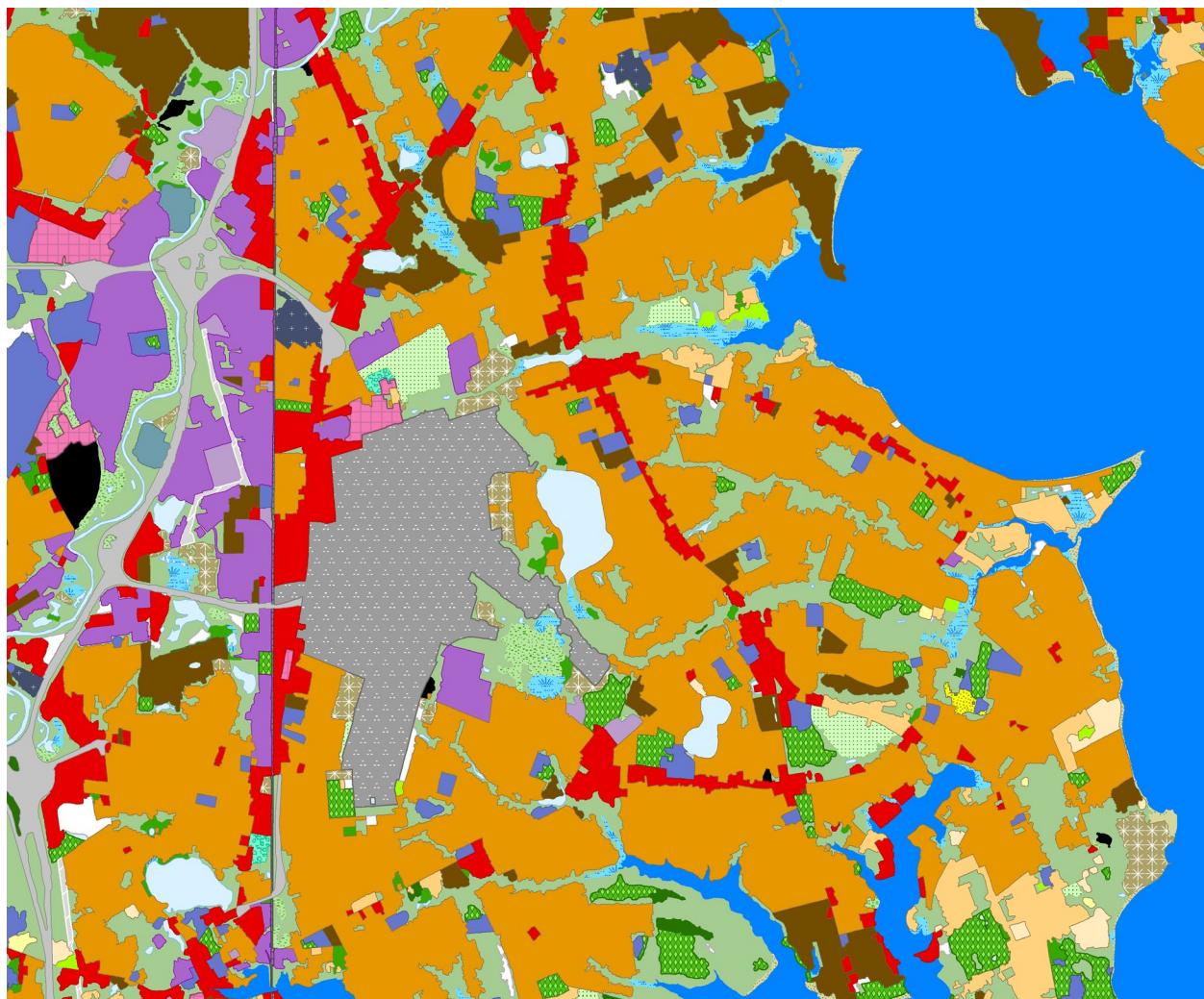
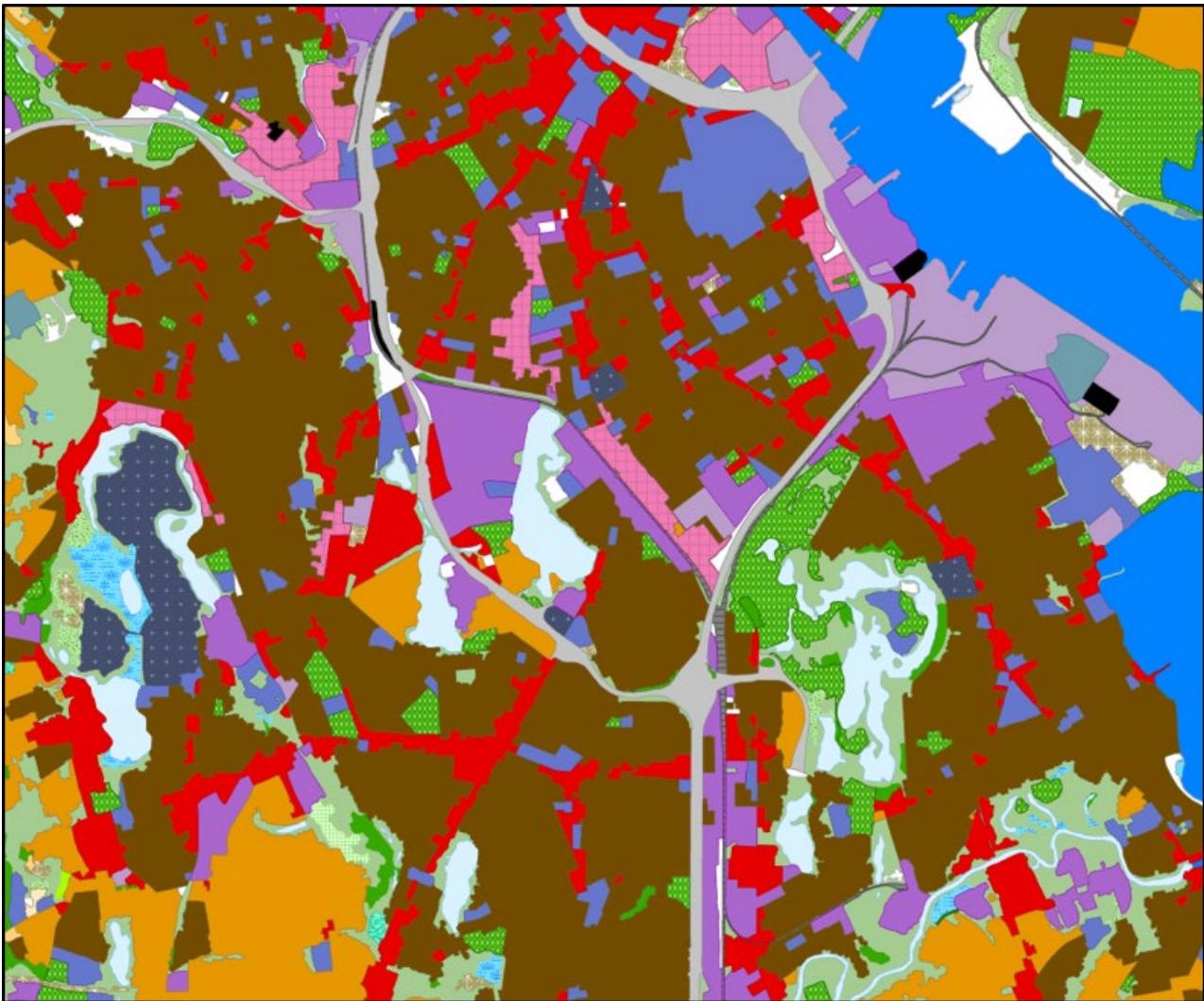


Figure 2: Land Use and Land Cover of Warwick, Mashapaug, and Yawgoo Ponds





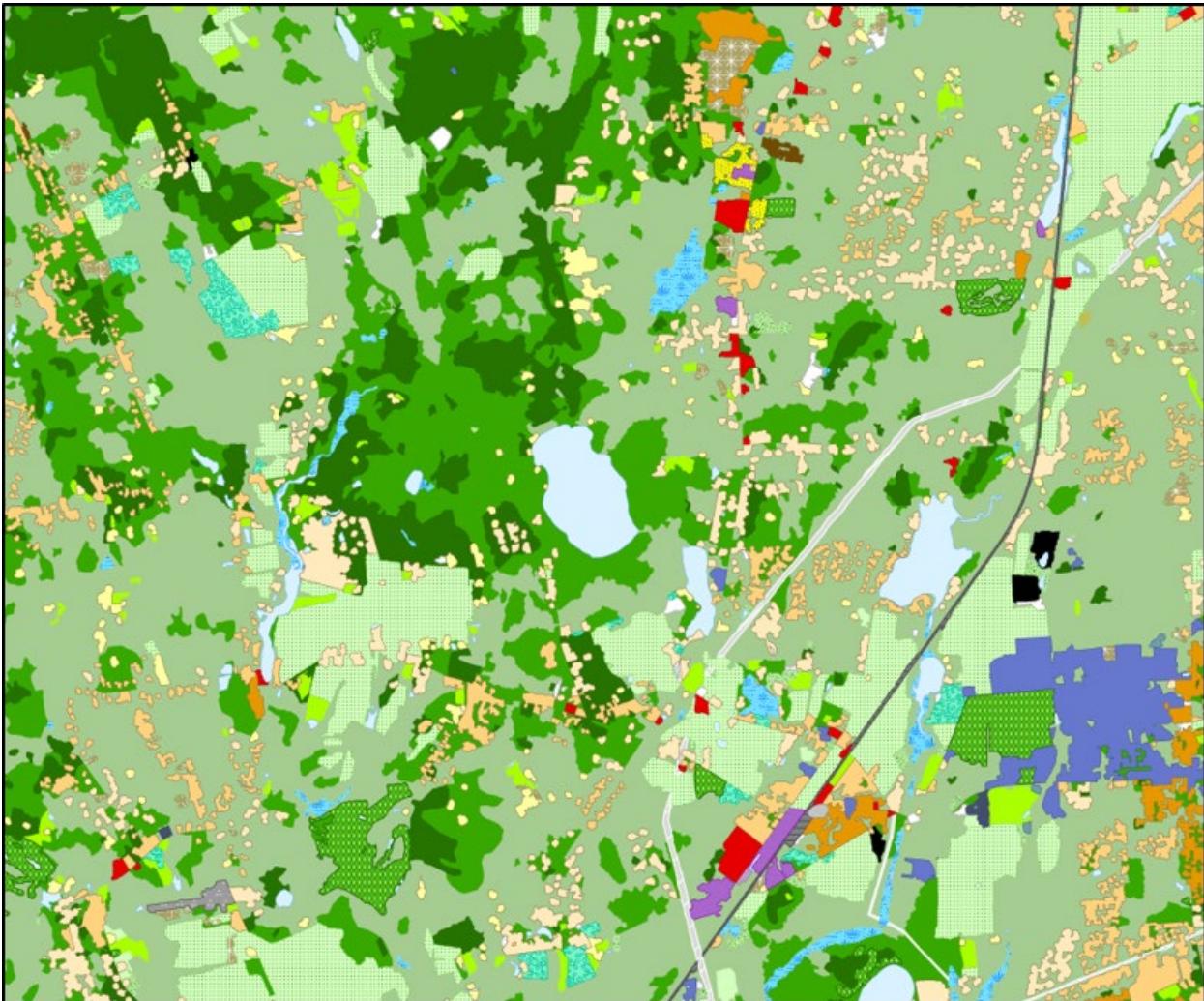
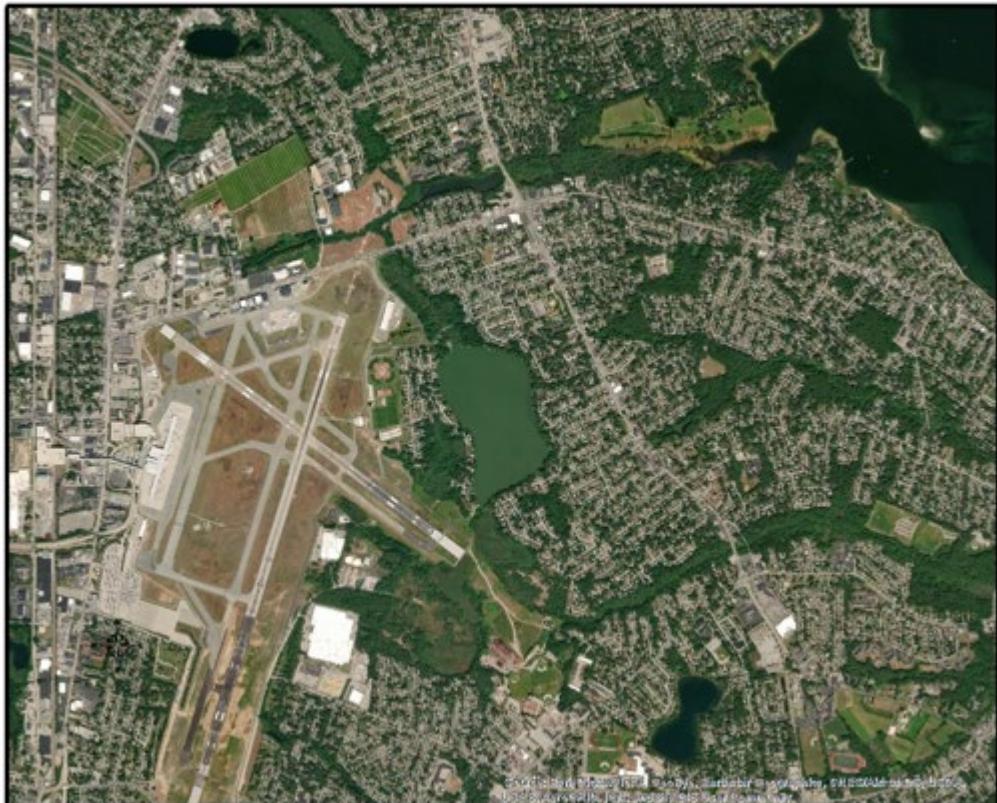
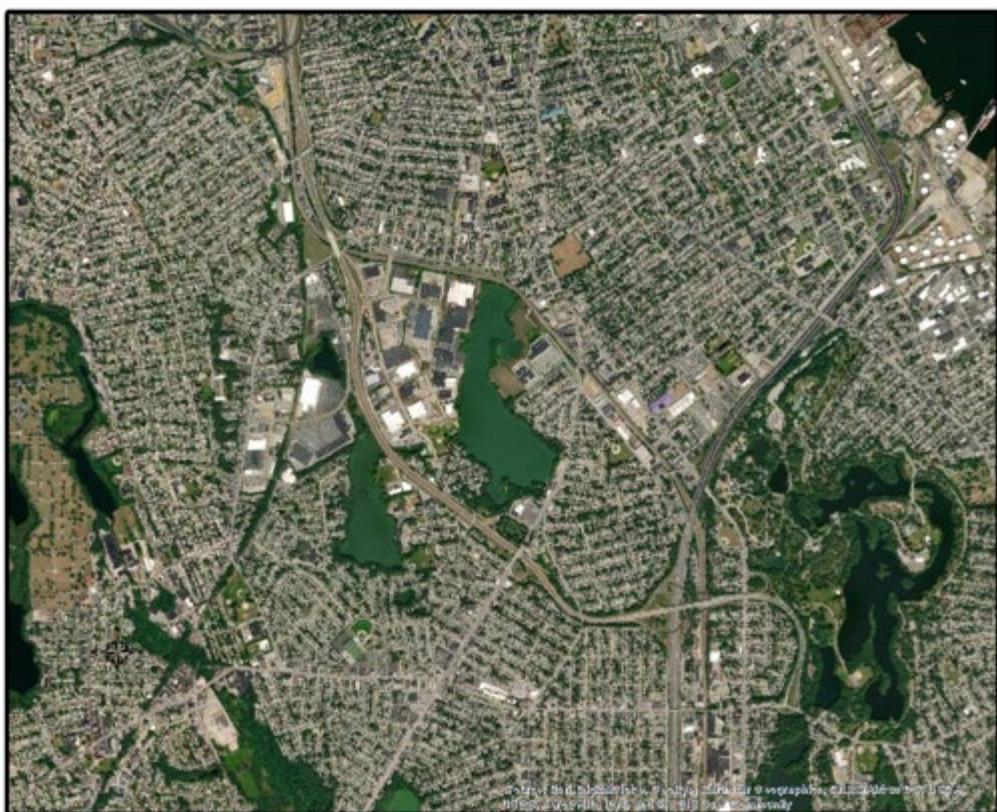


Figure 3: Aerial View of Warwick, Mashapaug, and Yawgoo Ponds

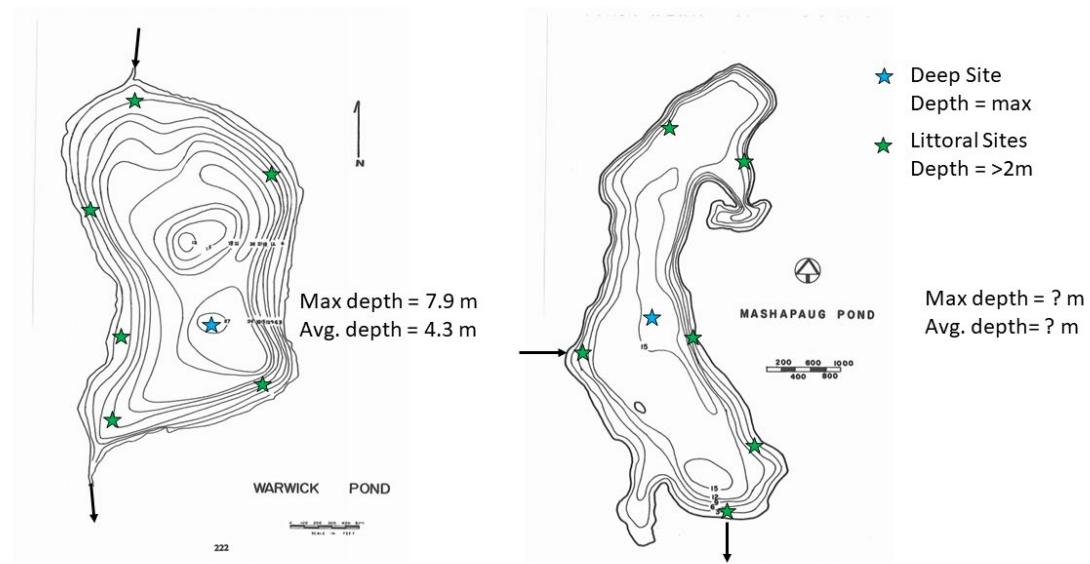




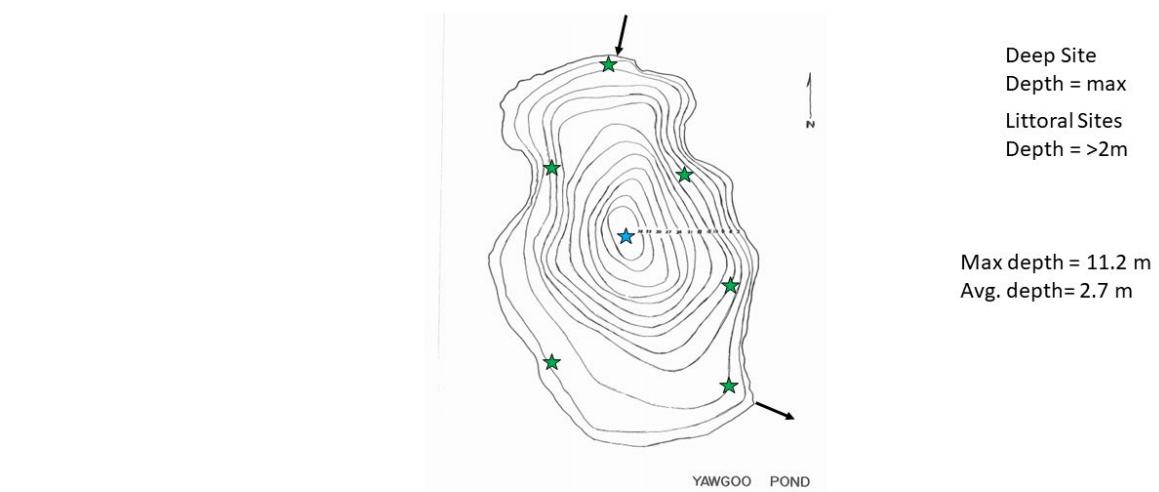


Dataset: NLCD, DigitalGlobe, Esri, Esri, HERE, Mapbox, USGS, USDA, USGS, AerialPhoto, IGN, and Geo_OGI User's Community

Figure 4: Bathymetry of Warwick, Mashapaug, and Yawgoo Ponds



Bathymetry maps and basic limnology from Guthrie and Stoligis 1977



Bathymetry maps and basic limnology from Guthrie and Stoligis 1977

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Appendix – SOPs, LOPs, and Literature

- Kasinak, J-M, B. Holt, M. Chislock, and A. Wilson. 2015. Benchtop Fluorometry of Phycocyanin as a Rapid Approach for Estimating Cyanobacterial Biovolume. *Journal of Plankton Research* 37: 248-257.
- USEPA. 1993. Method 350.1 Determination of Ammonia Nitrogen by Semi-Automated Colorimetry. U.S. Environmental Protection Agency, Cincinnati, OH.
- USEPA. 1993. Method 353.2 Determination of Nitrate-Nitrite Nitrogen by Automated Colorimetry. U.S. Environmental Protection Agency, Cincinnati, OH.
- USEPA. 1993. Method 365.1 Determination of Phosphorus by Semi-Automated Colorimetry. U.S. Environmental Protection Agency, Cincinnati, OH.
- USEPA. 2011. 2012 National Lakes Assessment, Field Operations Manual. EPA 841-B-11-003. U.S. Environmental Protection Agency, Washington, D.C.
- USEPA. 2012. 2012 National Lakes Assessment, Laboratory Operations Manual. EPA 841-B-11-004. U.S. Environmental Protection Agency, Washington, D.C.
- USEPA. 2013. Nutrient Analysis by the Astoria-Pacific Astoria2 Micro-Segmented Flow Autoanalyzer. Atlantic Ecology Division, Narragansett, RI.
- USEPA. 2016. Method 546 Determination of Total Microcystins and Nodularins in Drinking Water and Ambient Water by Adda Enzyme-Linked Immunosorbent Assay. U.S. Environmental Protection Agency, Cincinnati, OH.
- USEPA. 2016. Non-Acid Determination of Chlorophyll *a* Using a Turner Designs Trilogy Fluorometer. Atlantic Ecology Division, Narragansett, RI.