Lake Erie Plankton Abundance Study (LEPAS) Methods

Methods for sampling and laboratory analysis of ODW-Sandusky, ODW-Fairport, OEPA, and Canada NWRI samples

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1. Physiochemical data

1.1 Water temperature

A YSI multi-parameter data sonde is used to record vertical temperature profiles at most sample sites. At the very least, surface and bottom water temperatures (°C) are recorded at all sampling sites during sample collection.

1.2 Secchi depth

The Secchi depth (m) of each site is determined by lowering a Secchi disk into the water and recording the deepest depth at which the disk is visible.

1.3 Pigments

Water samples are collected with an integrated tube sampler (2.5 cm diameter) from the surface to twice the Secchi depth and emptied into a clean bucket. One liter of the collected water is poured into a plastic collection bottle and stored on ice in a cooler, protected from the light. Either on board the ship or in the lab, each sample is homogenized by shaking and then vacuum-filtered through a 47 mm GF/C glass fiber filter. If the filter clogs, additional filters are used until the whole liter has been filtered. The filter(s) are then placed in a black plastic 35mm photographic film container, marked with the date, station, and volume filtered, and stored in an iced cooler in the dark. On each sampling date, two blank filters are also prepared, collected by filtering 500 ml of distilled water through the same filtration apparatus, and placed in separate black plastic film containers. All filters are then sent to The Ohio State University laboratory for analysis, where they are stored frozen until analyzed within 30 days.

The frozen filters are analyzed spectrophotometrically for chlorophylls *a*, *b*, *c*, and pheophytin *a* according to a modified version of Standard Methods 10200H (APHA 2005). All analyses are performed in minimal lighting, and samples are stored in the dark when not being handled. Each filter is removed from its film container with tweezers and placed into a 40 ml Pyrex Tenbroeck Tissue Grinder, along with 3 ml of buffered aqueous acetone (preparation: 1 g $MgCO_3$ dissolved in 100 ml distilled water added to 900 ml acetone). The filter is then ground up and the resulting solution poured into a screw-top centrifuge tube. An additional 3-4 ml of aqueous acetone is used to rinse any residue from the grinding equipment and it is then poured into the centrifuge tube with the original solution. The samples are then stored in the dark in a refrigerator to extract for a minimum of 2 hours, but no greater than 24 hours.

After the samples are extracted, they are centrifuged for 20 minutes at 2000 rpm. The centrifuge tubes are returned to the refrigerator to avoid excess light until the samples are filtered (one at a time). The supernatant of each sample is then poured into a 10 ml graduated cylinder and the volume recorded as "extract volume". The solution is then poured into a 40 mL vial and drawn

into a 10 ml syringe. A 25 mm diameter, 0.45 μm pore-size PTFE filter is then placed on the syringe and the solution discharged through the filter into a test tube. The sample is stored in the refrigerator until it is to be analyzed spectrophotometrically. A disposable Pasteur pipet is then used to transfer an individual sample to a spectrophotometer cuvette. The cuvette is placed in a spectrophotometer and the absorbances are recorded at wavelengths of 750, 664, 647, and 630 nm.

To differentiate between chlorophyll *a* and pheophytin *a* content, the sample is then acidified with two drops of 0.1 N HCl. After waiting 90 seconds, absorbances are read at 750 and 665. Between each sample, the graduated cylinder, syringe tube, syringe, pipet, and cuvette are all rinsed with acetone.

The concentration of chlorophyll (mg/L) in the extract volume is then calculated using the following equations. These values are not corrected for pheophytin.

$$Chl\ a = 11.85 \cdot (ABS_{664} - ABS_{750}) - 1.54 \cdot (ABS_{647} - ABS_{750}) - 0.08 \cdot (ABS_{630} - ABS_{750}) \tag{1}$$

$$Chl\ b = 21.03 \cdot (ABS_{647} - ABS_{750}) - 5.43 \cdot (ABS_{664} - ABS_{750}) - 2.66 \cdot (ABS_{630} - ABS_{750}) \tag{2}$$

$$Chl\ c_1 + c_2 = 24.52 \cdot (ABS_{630} - ABS_{750}) - 7.6 \cdot (ABS_{647} - ABS_{750}) - 1.67 \cdot (ABS_{664} - ABS_{750}) \tag{3}$$

The chlorophyll *a* concentration (mg · L^{-1}) in the extract volume, corrected for pheophytin *a*, and the pheophytin *a* concentration (mg · L^{-1}) are then calculated according to the following equations:

$$Chl\ a = 26.7 \cdot [(ABS_{664b} - ABS_{750b}) - (ABS_{665a} - ABS_{750a})] \tag{4}$$

$$Pheo\ a = 26.7 \cdot [1.7 \cdot (ABS_{665a} - ABS_{750a}) - (ABS_{664b} - ABS_{750b})] \tag{5}$$

where
   b = before acid addition
   a = after acid addition

The concentration of each pigment in the whole water sample is calculated with the following formula.

$$C_w = \frac{C_E \cdot EV}{SV \cdot CL} \tag{6}$$

where
   C_w = concentration of pigment (μg · L^{-1}) in the whole lake water sample
   C_E = concentration of pigment (mg · L^{-1}) in the extract volume
   EV = extract volume (mL)
   SV = volume of whole lake water sample filtered (L)

$$CL = \text{width of the cuvette (cm)}$$

After the concentration of each pigment is calculated for all samples, the concentrations found in the blank samples are subtracted from those found in the whole water samples.

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2. Plankton and seston

2.1 Phytoplankton

Water samples are collected with an integrated tube sampler (2.5 cm diameter) from the surface to twice the Secchi depth. Rinse the sampler at the field site from which the sample will be collected, collect the sample and pour it into a clean bucket. Collect 500 mL sample and preserve it in a 1 pint plastic-capped jar with Lugol's solution, using approximately 1 mL of Lugol's for every 100 mL of sample. After analysis and prior to long-term storage, two drops of sucrose formalin are added to each sample to ensure preservation.

All samples are transported to The Ohio State University for analysis and long-term storage. Samples are first condensed by taking a well-mixed 250 mL subsample, transferring it to a graduated cylinder, and allowing it to settle for 7 days. Each sample is then concentrated down to 30 mL by siphoning off liquid from the top with a Pasteur pipet with the tip melted to bend it into a U-shape. The 30 mL sample is swirled and then transferred into a 40 mL vial. We then add 5 mL distilled water is added to the graduated cylinder, swirl it around and pour it into the 40 mL vial. Further subsamples of approximately 0.3-0.5 mL are obtained from the concentrated samples and placed into a 10 mL Utermohl counting chamber. The areas of the chamber bottom and an equatorial diameter of the chamber (cm^2) are measured to allow calculation of the fraction of each sample counted. All phytoplankton genera are identified and counted using a Wild inverted microscope at 400x magnification. Transects are counted until 100 algal units (cells, filaments, or colonies) of the most common taxa are recorded, with a minimum of two transects counted for each sample. Measurements are recorded for the first 20 algal units for each taxon. For filamentous algal taxa, all filament lengths are measured and the cells per filament counted. The filament lengths are then summed and recorded as total filament length and cell number for each taxon. All of the recorded data are entered into a spreadsheet program that calculates the density and biomass of each algal taxon. Table 1 contains a list of all algal genera and general categories that are used in the spreadsheet program. It also contains the critical dimensions that are measured for each algal species or category.

Densities for solitary algal taxa are calculated using the following formula.

$$D = \frac{N \cdot CA \cdot CV}{T \cdot TA \cdot V \cdot SV} \tag{12}$$

where

D = density (cells · mL^{-1})
N = number of cells counted in all transects
CA = Utermohl chamber area in mm^2
CV = concentrated sample volume in mL (usually 35 mL)
T = number of transects counted
TA = transect area in mm^2
V = volume of the sample placed into the chamber (mL)
SV = original sample volume in mL (usually 250 mL)

For most colonial algal taxa, the number of cells in the colony are counted and measured so the above formula is used to calculate the density. However, small-celled *Microcystis*, density is calculated using colony dimensions. The density is then calculated using the formula above, except 'N' is the number of colonies and the units of 'D' are colonies · mL^{-1}.

Densities for the filamentous algal taxa are calculated using the above formula as well, except for the 1995 to 1997 samples. For those samples, the number of cells in each filament was not recorded, so the number of filaments is used for 'N' and the units of 'D' are filaments · mL^{-1}. In order to estimate the density of filamentous algal taxa in cells · mL^{-1}, a conversion factor is determined. Representative samples that contained each filamentous algal taxon are examined and total filament lengths and number of cells in the filament are recorded. The average μm per cell is used as the conversion factor. The following formula is then used to calculate the densities in cells · mL.

$$Cell\ D = \frac{Total\ filament\ length}{Conv} \tag{13}$$

where
$Cell\ D$ = phytoplankton density (cells · mL^{-1})
$Total\ filament\ length$ = total length of all filaments found in the transects
Conv = average cell length for the algal taxon (μm)

The conversion factors for each filamentous algal taxon, except *Oscillatoria* and *Spirulina*, are listed in Table 2. For each sample that contained *Oscillatoria*, the average filament width is used as average cell length in the above formula.

In order to calculate the biomass of each algal taxon, the average individual volume (for cells, filaments, or colonies) is first calculated. Values for each critical dimension are averaged for each taxon and then used to calculate the average individual volume for each taxon. The types of volumetric equations that are used for each taxon are listed in Table 3. The total volume of each taxon (μm^3 · mL^{-1}) is then calculated with the following formula.

$$TV = AIV \cdot D \tag{14}$$

where
TV = phytoplankton volume (μm^3 · mL^{-1})
AIV = average individual volume (μm^3 · cell^{-1}, μm^3 · filament^{-1}, or μm^3 · colony^{-1})

D = phytoplankton density (cells · mL^{-1}, filaments · mL^{-1}, or colonies · mL^{-1})

The phytoplankton volume is then converted to wet biomass using the following formula.

$$BM = TV \cdot \frac{10^3 \; mL}{1 \; L} \cdot \frac{1 \; cm^3}{10^{12} \; \mu m^3} \cdot \frac{10^3 \; mg}{1 \; cm^3} \qquad (15)$$

where

BM = phytoplankton biomass (mg · L^{-1}), assuming a density of 1 g · cm^{-3}
TV = phytoplankton volume (μm^3 · mL^{-1})

After the density and biomass of each algal taxon is calculated, they are summarized into the following categories: Chlorophyta, Chrysophyta, Cryptophyta, Cyanophyta, Pyrrhophyta, Edible Phytoplankton, Inedible Phytoplankton, and Total Phytoplankton. Table 1 indicates which species are edible and which are inedible.

A macro constructed in Microsoft Excel calculates phytoplankton biomass using equations found in Table 4.

2.2 Protozoa

Protozoa water samples are obtained using an integrated tube sampler (2.5 cm diameter). After pouring 10 mL of glutaraldehyde into a 120 mL screw-top plastic jar, the rest of the jar is filled with sample water. All samples are then transported back to The Ohio State University for identification and counting.

2.3 Zooplankton

Zooplankton samples are collected by vertical tows, using a front-weighted zooplankton net (0.5 m diameter, 64 μm mesh) with a General Oceanics 2030RC flow meter and pint jar for collection. A 5-lb weight is tethered to the front of the frame by a 1-meter long line, and the net is allowed to free-fall to near the lake bottom (< 1 m) with the open end pointing downward. When the operator feels the weight hit the bottom, he immediately begins pulling the net back up to avoid collecting mud in the sample. This allows the water column to be sampled as the net is lowered and as it is pulled up. Flow meter numbers are recorded before and after the tow. After each sample is taken, the net is rinsed off from the outside so that all of the zooplankton is deposited in the collection jar. The samples are then filtered through 64 μm mesh sieves, rinsed into one or more 125 mL plastic sample cups, and preserved with concentrated sugar-formalin solution (4 parts plankton sample, 1 part preservative) such that the final concentration is 40 mL formaldehyde · L^{-1} and 40 g sucrose · L^{-1}.

The concentrated sugar-formalin solution is made by dissolving 400 g sucrose in 1 L distilled water and then adding 1 L commercial Formalin (40% formaldehyde by volume). The concentrated solution thus has 200 g sucrose · L^{-1} and 200 ml Formaldehyde/L. Mixing four parts

plankton sample to one part concentrated-sugar formalin solution (e.g., 80 mL of plankton sample with 20 mL concentrated sugar-formalin) thus preserves the sample with 40 mL formaldehyde · L^{-1} (4% formaldehyde or 10% formalin) and 40 g sucrose · L^{-1}. To protect samples from acidic conditions, sodium phosphate buffer and pH indicator (bromothymol blue) are added to each sample after analysis and prior to long-term storage.

All samples are transported to The Ohio State University for analysis and long-term storage. Each sample is diluted with deionized water to a known volume, usually ranging from 500 mL to 3000 mL, depending on the zooplankton density. The diluted volume may be outside this range if the sample contains an extremely small or large amount of zooplankton. After dilution, all of the zooplankters in at least two subsamples of 3-10 mL are identified and counted. Cladocerans and copepods are identified to species while rotifers and dreissenid veligers are identified to genus. Complete subsamples are analyzed until 100 individuals of the most common taxa are recorded. The most common taxa used for this rule cannot be nauplii, rotifers, or veligers, because they commonly have a much higher abundance than the larger cladoceran and copepod taxa. Individuals in each taxon are categorized as either having eggs or not having eggs. Body length measurements are then recorded for the first 20 individuals in each of these categories. If fewer than 20 individuals are found, then measurements are recorded only for the individuals found (e.g. if 10 individuals are found then 10 measurements are recorded). In addition, the number of eggs for all females counted (not just those of the 20 measured) of each taxon and the total numbers of loose eggs are also recorded.

Some species of cladocerans and copepods are considered common and some are considered rare. The eggs attached to females for the common species are recorded separately and grouped together by species. However, for the rare species any eggs found with a female are grouped together as either rare cladoceran eggs or rare copepod eggs. Table 5 provides a list of everything that is counted and whether they are considered common or rare.

After each sample is analyzed, all recorded data are entered into a spreadsheet program that calculates the total volume sampled and the zooplankton densities, biomass, phosphorus and nitrogen excretion rates, filtering rates, and secondary productivities for that sample. The total volume is calculated using the General Oceanics 2030RC flow meter numbers and a net constant. Note that a one-digit change in the meter reading reflects one-tenth of a revolution of the rotor, but for simplicity we consistently refer to these meter readings as revolutions. The net constant is determined by using the following formulas.

$$Distance\ traveled\ (m) = \frac{Diff.in\ counts \cdot Rotor\ constant}{999999} \tag{18}$$

$$Vol.sampled\ (m^3) = \frac{\pi \cdot Net\ diameter^2}{4} \cdot Distance \tag{19}$$

The rotor constant of the flow meters that are used is 26873, and the net diameters are 0.5 m. This gives us the following equation.

$$Vol.sampled\ (m^3) = \frac{\pi \cdot 0.5^2}{4} \cdot \frac{Diff.in\ counts \cdot 26873}{999999} \tag{20}$$

Converting the volume to liters results in the following formula.

$$Lake\ vol.\ sampled\ (L) = Diff.\ in\ counts \cdot 5.2765\ L \cdot revolution^{-1} \tag{21}$$

Based on the above equation, the following formula is used in the spreadsheet to calculate the total volume of water sampled.

$$TV = (M_1 - M_2) \cdot NC \tag{22}$$

where
      TV = Total volume sampled (L)
      M_1 = Flow meter number before vertical tow
      M_2 = Flow meter number after vertical tow
      NC = Net constant (5.2765 L · revolution[-1])

The density of each zooplankton taxon is calculated according to the following formula:

$$D = \frac{N \cdot DV}{SV \cdot TV} \tag{23}$$

where
      D = zooplankton density individuals · L^{-1}
      N = number of individuals counted in the subsamples
      DV = diluted sample volume (mL)
      SV = total subsample volume counted (mL)
      TV = total lake water volume sampled (L)

The dry biomass of each zooplankton taxon is calculated using the following formula.

$$B = D \cdot AIB \tag{24}$$

where
      B = zooplankton biomass (dry weight) μg · L^{-1}
      D = zooplankton density in individuals · L^{-1}
      AIB = average individual biomass in μg · individual[-1]

The average individual biomass for each taxon of cladocerans and copepods, except for *Bythotrephes cederstroemi* and *Cercopagis pengoi*, is determined by calculating the biomass of each individual found in the subsamples and then averaging these biomass values. The biomass of each individual is calculated by using length-weight regression equations from Culver et al, 1985. Three of the equations (*Epischura lacustris, Eurytemora affinis,* and *Limnocalanus macrurus*) were developed by Julie Ritchie for her Undergraduate Honors Thesis in Winter of 2003. Each equation is in the following format.

$$IB = a \cdot L^b \tag{25}$$

where
      IB = dry weight of the individual in (μg)
      a = coefficient

$b =$ coefficient
L = length of the individual (mm)

Table 6 contains a list of the 'a' and 'b' coefficients used for each species of cladocerans and copepods. The same coefficients are used for individuals with and without eggs. Equations have not been developed for all species that are found in Lake Erie, so for those species without established length-weight relationships, equations from similar species are used. For example, the equation for *Daphnia retrocurva* is used for *Daphnia ambigua, Daphnia longiremis, Daphnia parvula, Daphnia pulex, Daphnia schodleri,* and the various chydorids. The equation for *Diacyclops bicuspidatus thomasi* is used for *Cyclops scutifer, Ergasilus* sp., *Eucyclops agilis, Eucyclops speratus, Paracyclops fimbriatus poppei, Tropocyclops prasinus mexicanus*, unidentified cyclopoids, and *Canthocamptus robertcokeri*. In addition, the equation for *Skistodiaptomus oregonensis* is used for *Leptodiaptomus ashlandi, Leptodiaptomus sicilis,* and *Skistodiaptomus reighardi*. The equation for cyclopoid nauplii is also used for harpacticoid nauplii.

The average individual biomass for *Bythotrephes cederstroemi* is calculated using a regression equation from Yan and Pawson, 1998:

$$\log W = 0.939 + 3.066 \cdot \log L \tag{26}$$

where
L = length of the individual from the top of the head to the first tail spine (mm)
W = dry weight of the individual (μg)

Converting to $a \cdot L^b$ format,
$$B\ (\mu g) = 8.6896 \cdot L^{3.066} \tag{27}$$

For *Cercopagis pengoi,* the individual biomass is calculated using a regression equation from Grigorovich et al. (2000):

$$\log W = 0.375 + 2.442 \cdot \log L \tag{28}$$

where
L = length of the individual from the top of the head to the first tail spine (mm)
W = dry weight of the individual (μg)

Converting to aL^b format,
$$B\ (\mu g) = 2.3713 \cdot L^{2.442} \tag{29}$$

Before 2007, dreissenid veliger biomass was estimated using an average individual biomass number (Table 8). However, veliger lengths have been recorded since 2007. Post-2007 biomasses were updated in 11/2020 to be calculated using eq. 2 from Bowen et al. (2018):

$$IB = a \cdot e^{b \cdot L} \tag{30}$$

where

       IB = dry weight of the individual in (μg)
       a = coefficient
       b = coefficient
       L = length of the individual (mm)
       e = base of the natural logarithm.

For calculating the biomass of eggs, rotifers, and veligers (pre-2007), the average individual biomass (AIB) has been predetermined and is listed in Table 8. For veligers, this value is the average of all veligers measured from 2007-2019. These values are used as the average individual biomass in the calculations for all samples.

After the biomasses of all individual taxa are calculated, they are summed together in the following categories:

Rotifer Biomass = $\sum$ all rotifers
Cladoceran Biomass = $\sum$ all cladoceran species
Cyclopoid Copepod Biomass = $\sum$ all cyclopoid copepod species
Calanoid Copepod Biomass = $\sum$ all calanoid copepod species
Copepod Biomass = Cyclopoids + Calanoids
Nauplii Biomass = Cyclopoid nauplii + calanoid nauplii + harpacticoid nauplii
Cladoceran Egg Biomass = $\sum$ all cladoceran eggs
Copepod Egg Biomass = $\sum$ all copepod eggs
Crustacean Biomass = cladocerans + copepods + nauplii + cladoceran eggs + copepod eggs
Crust-Naup Biomass = cladocerans + copepods
Total Zooplankton Biomass = crustaceans + rotifers + veligers

Phosphorus and nitrogen excretion rates of each zooplankton taxon are calculated using the following formulas (Wen and Peters, 1994):

$$P = D \cdot AIP \tag{31}$$

$$N = D \cdot AIN \tag{32}$$

where
       P = phosphorus excretion rate in μg P $\cdot$ L^{-1} $\cdot$ day^{-1}
       N = nitrogen excretion rate in μg N $\cdot$ L^{-1} $\cdot$ day^{-1}
       D = zooplankton density in individuals $\cdot$ L^{-1}
       AIP = average individual phosphorus excretion in μg P $\cdot$ day^{-1} $\cdot$ individual^{-1}
       AIN = average individual nitrogen excretion in μg N $\cdot$ day^{-1} $\cdot$ individual^{-1}

The average individual excretion rate for each taxon of cladocerans and copepods is determined by calculating the excretion rate of each individual found in the subsamples and then averaging these excretion rate values. The excretion rate of each individual is calculated by using

regression equations from Wen and Peters, 1994. The regression equations for phosphorus and nitrogen excretion are as follows:

$$E_P = 0.02239 \cdot W^{0.54} \tag{33}$$

$$E_N = 0.04169 \cdot W^{0.67} \tag{34}$$

where
E_P = phosphorus excretion rate (μg P $\cdot$ day^{-1} $\cdot$ individual^{-1})
E_N = nitrogen excretion rate (μg N $\cdot$ day^{-1} $\cdot$ individual^{-1})
W = individual zooplankton dry weight (μg)

After the excretion rates are calculated for each individual taxon, they are summed together into the same categories as the zooplankton biomass.

The average individual filtration rate for each taxon of cladocerans and cyclopoid copepods is determined by calculating the filtration rate of each individual found in the subsamples and then averaging these values. The filtration rate of each individual is calculated by using a regression equation from various sources, but the same generic equation is used:

$$IFR = a \cdot L^b \tag{35}$$

where
IFR = individual filtration rate (mL $\cdot$ day^{-1} $\cdot$ individual^{-1})
a = coefficient
b = coefficient
L = length of the individual (mm)

The filtering rate per liter of each zooplankton taxon is calculated using the following formula.

$$FR_1 = D \cdot AIFR \tag{36}$$

where
F = filtering rate in mL $\cdot$ L^{-1} $\cdot$ day^{-1}
D = zooplankton density in individuals $\cdot$ L^{-1}
$AIFR$ = average individual filtering rate in mL $\cdot$ day^{-1} $\cdot$ individual^{-1}

Table 7 lists the a and b coefficients and the various sources from which the equations came. Equations were not developed for all species that are found in Lake Erie, so for those species without established regressions, equations from similar species are used. The *Daphnia* sp. equation is used for *D. ambigua, D. longiremis, D. parvula, D. pulex, and D. schodleri.* The general cladoceran equation is used for *Alona* sp, *Bosmina longirostris, Bythotrephes cederstroemi, Cercopagis pengoi,* chydorids, *Eubosmina coregoni,* and *Holopedium gibberum.* The Calanoids + Nauplii equation is used for all calanoid copepods and calanoid nauplii.

After the filtering rates are calculated for each individual taxon, the water column filtration rate is then calculated using the following formula.

$$FR_2 = \frac{FR_1 \cdot depth\ (m)}{1000} \qquad (37)$$

where
FR_2 = filtering rate in m^3 · m^{-2} · day^{-1}
FR_1 = filtering rate in mL · L^{-1} · day^{-1}
$depth$ = depth of the water column (m)

Both of the filtering rates are then summed together into the same categories as the zooplankton biomass and excretion rates. The Copepoda equation is used for all copepods and nauplii. The Daphnidae equation is used for *Ceriodaphnia* sp., *D. ambigua, D. galeata mendota, D. longiremis, D. parvula, D. pulex, D. retrocurva,* and *D. schodleri*. The Bosminidae equation is used for *Alona* sp., *Bosmina longirostris,* chydorids, and *Eubosmina coregoni*. The Sididae equation is used for *Diaphanosoma* sp., and *Holopedium gibberum*. Secondary productivity is not calculated for *Bythotrephes cederstroemi, Cercopagis pengoi,* and *Leptodora kindti*.

Secondary productivity is also calculated for each zooplankton species, using regression equations developed by Paul Frost 1997. The basic equation is as follows:

$$P = \alpha \cdot B^\beta \cdot \rho^T \qquad (38)$$

where
P = secondary productivity in μg · L^{-1} · day^{-1}
α = coefficient
B = zooplankton biomass in μg · L^{-1}
β = coefficient
ρ = coefficient
T = water temperature (°C)

Table 9 lists the α, β, and ρ coefficients for each zooplankton group.

2.4 Ichthyoplankton

Larval fish samples are obtained using an ichthyoplankton net with a 0.75 m diameter net with 505 μm mesh size, and General Oceanics 2030RC flow meter. After recording the initial flow meter reading on the field data sheet, the net is towed horizontally, just beneath the water surface, for five minutes to collect the sample. The final flow meter reading is then recorded and the net is rinsed out to ensure that the entire sample is collected. Each sample is then placed in a plastic collection jar and preserved with 50% ethyl alcohol.

Samples are then transported to The Ohio State University for identification and counting. Contents of sample jars are emptied into petri dishes to be examined. All larval fish and fish eggs

are counted and identified to genus (species when possible) under a dissecting microscope. They are then removed from the rest of the zooplankton, algae, and detritus and placed into storage jars containing 95% ethyl alcohol. Ichthyoplankton abundance is calculated using the following formulae:

$$Distance\ traveled\ (m) = \frac{Difference\ in\ counts \cdot Rotor\ constant}{999999} \tag{7}$$

$$Lake\ vol.sampled\ (m^3) = \frac{\pi \cdot Net\ diameter^2}{4} \cdot Distance \tag{8}$$

The rotor constant of the flow meters that are used is 26873, and the net diameters are 0.75 m. This gives us the following equation:

$$Vol.sampled\ (m^3) = \frac{\pi \cdot 0.75^2}{4} \cdot \frac{Difference\ in\ counts \cdot 26873}{999999} \tag{9}$$

Converting the volume to liters results in the following formula.

$$Lake\ vol.sampled\ (L) = Difference\ in\ counts \cdot 11.872\ L \cdot revolution^{-1} \tag{10}$$

$$Ichthyoplankton\ abundance = \frac{Fish\ counted}{11.872 \cdot revolutions} \tag{11}$$

2.5 Seston

Water samples are collected with an integrated tube sampler (2.5 cm diameter) and emptied into a bucket. 250 mL of sample is poured into a 250 mL graduated cylinder. This water is then filtered through a pre-weighed 47 mm diameter 0.45 μm pore size (Millipore HAWG04700) cellulose acetate membrane filter. The filter is placed in a plastic wrapper and labeled with the site, date, and volume filtered. On each sampling date, four blank water samples are obtained by filtering 250 mL of distilled water through the membrane filter.

All samples are then transported to The Ohio State University for analysis. Each sample is dried in an oven at 65° C for 24 hours and then weighed. The amount of seston in the sample is then calculated with the following formula.

$$TS = \frac{(DW - FW) - BW}{V} \tag{16}$$

where
      TS = total seston (mg · L^{-1})
      DW = dry weight of filter and seston (mg)
      FW = pre-weight of filter (mg)

BW = average weight of blank samples (mg)
V = volume of filtered water (L)

Each filter is then saturated with 95% alcohol and burned. The remaining inorganic ash is weighed and used in the following formula to calculate the inorganic content of the seston.

$$AS = \frac{AW - BAW}{V} \tag{17}$$

where
AS = ashed seston (mg $\cdot$ L^{-1})
AW = weight of the seston after burning (mg)
BAW = average weight of the blank samples after burning (mg)
V = volume of filtered water (L)

3. Tables

3.1 Phytoplankton tables

Table 1: All algal genera and general categories, separated by Phylum that are counted when analyzing phytoplankton samples. Also listed for each genus/category is the type of algae (solitary, filamentous, or colonial), whether it is edible or inedible, and what critical dimensions are used for calculating cell volumes.

Phylum	Genus/Category	Algal Type	Edible/Inedible	Critical Dimensions
Chlorophyta	*Actinastrum*	Colonial	Edible	Length, Width
	Ankistrodesmus	Solitary	Edible	Length, Width
	Botryococcus	Colonial	Edible	Diameter
	Carteria	Solitary	Edible	Diameter
	Characium	Solitary	Edible	Length, Width
	Chlamydomonas	Solitary	Edible	Diameter
	Chlorophyte filament	Filament	Inedible	Length, Width
	Closteriopsis	Solitary	Edible	Length, Width
	Closterium	Solitary	Inedible	Length, Width
	Coelastrum	Colonial	Edible	Diameter
	Colonial chlorophyte with sheath	Colonial	Edible	Diameter
	Colonial chlorophyte without sheath	Colonial	Edible	Diameter
	Cosmarium	Solitary	Edible	Diameter

	Crucigenia	Colonial	Edible	Diameter
	Dictyosphaerium	Colonial	Edible	Diameter
	Golenkinia	Solitary	Edible	Diameter
	Gonium	Colonial	Edible	Diameter
	Kirchneriella	Colonial	Edible	Length, Width
	Lagerheimia	Solitary	Edible	Diameter
	Micractinium	Solitary	Inedible	Diameter
	Oocystis	Colonial	Edible	Diameter
	Pandorina	Colonial	Edible	Diameter
	Pediastrum	Colonial	Edible	Diameter
	Quadrigula	Colonial	Edible	Length, Width
	Scenedesmus	Colonial	Edible	Length, Width
	Schroederia	Solitary	Edible	Length, Width
	Solitary Green	Solitary	Edible	Diameter
	Spiny Green	Solitary	Inedible	Diameter
	Staurastrum	Solitary	Edible	Diameter
	Tetraedron	Solitary	Edible	Length, Width
	Treubaria	Solitary	Edible	Diameter
Chrysophyta (Chrysophyceae)	*Dinobryan*	Colonial	Edible	Diameter
	Mallomonas	Solitary	Edible	Length, Width
Chrysophyta (diatoms)	*Asterionella*	Colonial	Edible	Length, Width
	Centric Diatom	Solitary	Edible	Diameter
	Cocconeis	Solitary	Edible	Diameter
	Cyclotella	Solitary	Edible	Diameter
	Cymbella	Solitary	Edible	Length, Width
	Fragilaria	Colonial	Edible	Length, Width
	Gomphonema	Solitary	Edible	Length, Width 1, Width 2
	Gyrosigma	Solitary	Edible	Length, Width
	Aulacoseira (formerly Melosira)	Filament	Inedible	Length, Width
	Navicula	Solitary	Edible	Length, Width
	Pennate Diatom	Solitary	Edible	Length, Width
	Rhizosolenia	Solitary	Edible	Length, Width
	Rhoicosphenia	Solitary	Edible	Length, Width 1, Width 2

	Surirella	Solitary	Edible	Length, Width
	Synedra	Solitary	Edible	Length, Width
	Tabellaria	Colonial	Edible	Length, Width
Cryptophyta	*Chroomonas*	Solitary	Edible	Diameter
	Cryptomonas	Solitary	Edible	Length, Width
	Rhodomonas	Solitary	Edible	Length, Width
Cyanophyta	*Dolichospermum (formerly Anabaena)*	Filament	Inedible	Length, Width
	Aphanizomenon	Filament	Inedible	Length, Width
	Chroococcus	Colonial	Inedible	Diameter
	Merismopedia	Colonial	Inedible	Diameter
	Microcystis	Colonial	Inedible	Diameter
	Narrow Filament	Filament	Inedible	Length, Width
	Oscillatoria	Filament	Inedible	Length, Width
	Pseudanabaena	Filament	Inedible	Length, Width
Pyrrophyta	*Ceratium*	Solitary	Inedible	Length, Spine height, Spine Width
	Gymnodinium	Solitary	Edible	Length, Width
	Peridinium	Solitary	Edible	Length, Width

Table 2: Conversion factors that are used to convert densities of filamentous algae from # filaments/ml to # cells/ml.

Genus/Category	Conversion Factor (μm/cell)
Dolichospermum	5.61437
Aphanizomenon	2.50452
Chlorophyte Filament	20.93179
Aulacoseira	10.78529
Cyanophyte filament	2.450452

Table 3: Volumetric equations that are used to calculate the cell volume of all phytoplankton genera or categories

Genus/Category	Volumetric Equation
Actinastrum, Chilomonas, Closterium, Cryptomonas, Gymnodinium, Mallomonas, Peridinium, Rhodomonas, Scenedesmus	$(\pi \cdot L \cdot W^2)/6$
Aphanizomenon, Aulacoseira, Chlorophyte Filament, Cyanophyte filament, *Cylindrospermopsis, Dolichospermum, Lyngbya, Oscillatoria, Spirogyra*	$[\pi \cdot W^2 \cdot (TotL/\# \, fil)]/4$

Ankistrodesmus, Characium, Closteriopsis, Kirchneriella, Schroederia, Tetraedron	$(\pi \cdot L \cdot W^2)/12$
Asterionella	$(\pi \cdot L \cdot W^2)/4$
Aphanocapsa, Aphanothece, Carteria, Chlamydomonas, Chroococcus, Chroomonas, Coelastrum, Colonial Chlorophyte with sheath, Colonial Chlorophyte without sheath, *Crucigenia, Dimorphococcus, Dinobryon, Dictyosphaerium, Eutetramorus, Franceia, Golenkinia, Gonium, Lagerheimia, Merismopedia, Microcystis* (colony D 1995-2006; cell D 2007-2010), *Micractinium, Oocystis, Pandorina*, Solitary Green, *Sphaerocystis*, Spiny Green, *Staurastrum, Synura, Treubaria*	$(\pi \cdot D^3)/6$
Centric Diatom, *Cocconeis, Coscinodiscus, Cyclotella, Stephanodiscus*	$(D^2 \cdot \pi \cdot D/2)/4$
Ceratium	$(\pi/12) \cdot [L^3 + (4 \cdot SH \cdot SW^2)]$
Cosmarium	$(D^2 \cdot \pi \cdot D/6)/6$
Cymbella	$W \cdot (W/2) \cdot [L - W + (\pi/4) \cdot W]$
Fragilaria, Gyrosigma, Navicula, Nitzschia, Pennate Diatom, *Opephora, Rhizosolenia, Synedra, Tabellaria*	$L \cdot W \cdot W/2$
Gomphonema, Rhoicosphenia	$(W_2/2) \cdot L \cdot [(W_1 + W_2)/2]$
Pediastrum	$(D^2 \cdot \pi \cdot 0.15)/4$
Spirulina	$(\pi \cdot L^2 \cdot W)/4$
Surirella	$L \cdot (L/2) \cdot [W - L + (\pi/4) \cdot L]$

Table 4: Calculation of cell biovolumes. All phytoplankton biovolume equations represent the average biovolume and are calculated using the average measurements of all individuals of a given taxon that are measured in the transects (B_{cell} = average cell biovolume, B_{col} = average colony biovolume, B_{fil} = average filament biovolume). Total densities (d_{cell}) are calculated using the number of cells counted in the transects ($\#_{cell}$), except for *Microcystis* for which $\#_{col}$ is used. For species that are measured according to colony size or filament size, the density is also calculated using the number of colonies ($\#_{col}$) and filaments ($\#_{fil}$) that are found, giving the density of colonies (d_{col}) or the density of filaments (d_{fil}) in the sample. Total sample biovolumes are calculated by multiplying the average biovolume by the appropriate density ($B_{cell} * d_{cell}$, $B_{col} * d_{col}$, or $B_{fil} * d_{fil}$).

Genus/Category	Items needed for Counting Sheets	Biovolume Equations
Chlorophyta		
Actinastrum (Colonial)	# of cells counted in all transects ($\#_{cell}$) 20 individual cell lengths (L_{cell}) 20 individual cell widths (W_{cell})	$B_{cell} = (\pi \cdot L_{cell} \cdot W_{cell}^2)/6$
Ankistrodesmus (solitary)	# of cells counted in all transects ($\#_{cell}$) 20 individual cell lengths (L_{cell}) 20 individual cell widths (W_{cell})	$B_{cell} = (\pi \cdot L_{cell} \cdot W_{cell}^2)/12$
Carteria (solitary)	# of cells counted in all transects ($\#_{cell}$) 20 individual cell diameters (D_{cell})	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Characium (solitary)	# of cells counted in all transects ($\#_{cell}$) 20 individual cell lengths (L_{cell}) 20 individual cell widths (W_{cell})	$B_{cell} = (\pi \cdot L_{cell} \cdot W_{cell}^2)/12$

Chlamydomonas (solitary)	# of cells counted in all transects (#cell) 20 individual cell diameters (Dcell)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Chlorophyte filament	# of filaments in all transects (#fil) Total length of all filaments (LFilTot) # of cells counted in all transects (#cell) 20 individual filament widths (Wfil)	$B_{fil} = [\pi \cdot W_{fil}^2 \cdot (L_{filTot}/\#_{fil})]/4$
Closteriopsis (solitary)	# of cells counted in all transects (#cell) 20 individual cell lengths (Lcell) 20 individual cell widths (Wcell)	$B_{cell} = (\pi \cdot L_{cell} \cdot W_{cell}^2)/12$
Closterium (solitary)	# of cells counted in all transects (#cell) 20 individual cell lengths (Lcell) 20 individual cell widths (Wcell)	$B_{cell} = (\pi \cdot L_{cell} \cdot W_{cell}^2)/6$
Coelastrum (colonial)	# of cells counted in all transects (#cell) # of colonies in all transects (#col) 20 diameters of colonies (Dcol)	$B_{col} = (\pi \cdot D_{col}^3)/6$
Colonial chlorophyte with sheath	# of cells counted in all transects (#cell) 20 individual cell diameters (Dcell)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Colonial chlorophyte without sheath	# of cells counted in all transects (#cell) 20 individual cell diameters (Dcell)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Crucigenia (colonial)	# of cells counted in all transects (#cell) 20 individual cell diameters (Dcell)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Dictyosphaerium (colonial)	# of cells counted in all transects (#cell) 20 individual cell diameters (Dcell)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Dimorphococcus (solitary)	# of cells counted in all transects (#cell) 20 individual cell diameters (Dcell)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Eutetramorus (colonial)	# of cells counted in all transects (#cell) 20 individual cell diameters (Dcell)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Franceia (colonial)	# of cells counted in all transects (#cell) 20 individual cell diameters (Dcell)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Golenkinia (solitary)	# of cells counted in all transects (#cell) 20 individual cell diameters (Dcell)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Gonium (colonial)	# of cells counted in all transects (#cell) 20 individual cell diameters (Dcell)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Kirchneriella (colonial)	# of cells counted in all transects (#cell) 20 individual cell lengths (Lcell) 20 individual cell widths (Wcell)	$B_{cell} = (\pi \cdot L_{cell} \cdot W_{cell}^2)/12$
Lagerheimia (solitary)	# of cells counted in all transects (#cell) 20 individual cell diameters (Dcell)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Micractinium (colonial)	# of cells counted in all transects (#cell) 20 individual cell diameters (Dcell)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Oocystis (colonial)	# of cells counted in all transects (#cell) 20 individual cell diameters (Dcell)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Pandorina (colonial)	# of cells counted in all transects (#cell) 20 individual cell diameters (Dcell)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Pediastrum (colonial)	# of cells counted in all transects (#cell) # of colonies in all transects (#col) 20 diameters of colonies (Dcol)	$B_{col} = (D_{col}^2 \cdot \pi \cdot 0.15)/4$

Scenedesmus (colonial)	# of cells counted in all transects (#cell) 20 individual cell lengths (L$_{cell}$) 20 individual cell widths (W$_{cell}$)	$B_{cell} = (\pi \cdot L_{cell} \cdot W_{cell}^2)/6$
Schroederia (solitary)	# of cells counted in all transects (#cell) 20 individual cell lengths (L$_{cell}$) 20 individual cell widths (W$_{cell}$)	$B_{cell} = (\pi \cdot L_{cell} \cdot W_{cell}^2)/12$
Solitary Green (solitary)	# of cells counted in all transects (#cell) 20 individual cell diameters (D$_{cell}$)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Sphaerocystis (colonial)	# of cells counted in all transects (#cell) 20 individual cell diameters (D$_{cell}$)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Spiny Green (solitary)	# of cells counted in all transects (#cell) 20 individual cell diameters (D$_{cell}$)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Spirogyra (filamentous)	# of filaments in all transects (#fill) Total length of all filaments (L$_{FilTot}$) # of cells counted in all transects (#cell) 20 individual filament widths (W$_{fil}$)	$B_{fil} = [\pi \cdot W_{fil}^2 \cdot (L_{filTot}/\#_{fil})]/4$
Staurastrum (solitary)	# of cells counted in all transects (#cell) 20 individual cell diameters (D$_{cell}$)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Synura (colonial)	# of cells counted in all transects (#cell) 20 individual cell diameters (D$_{cell}$)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Tetraedron (solitary)	# of cells counted in all transects (#cell) 20 individual cell lengths (L$_{cell}$) 20 individual cell widths (W$_{cell}$)	$B_{cell} = (\pi \cdot L_{cell} \cdot W_{cell}^2)/12$
Treubaria (solitary)	# of cells counted in all transects (#cell) 20 individual cell diameters (D$_{cell}$)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Chrysophyta (Chrysophyceae)		
Dinobryon (colonial)	# of cells counted in all transects (#cell) 20 individual cell diameters (D$_{cell}$)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Mallomonas (solitary)	# of cells counted in all transects (#cell) 20 individual cell lengths (L$_{cell}$) 20 individual cell widths (W$_{cell}$)	$B_{cell} = (\pi \cdot L_{cell} \cdot W_{cell}^2)/6$
Chrysophyta (diatoms)		
Asterionella (colonial)	# of cells counted in all transects (#cell) 20 individual cell lengths (L$_{cell}$) 20 individual cell widths (W$_{cell}$)	$B_{cell} = (\pi \cdot L_{cell} \cdot W_{cell}^2)/4$
Centric Diatom (solitary)	# of cells counted in all transects (#cell) 20 individual cell diameters (D$_{cell}$)	$B_{cell} = (D_{cell}^2 \cdot \pi \cdot D_{cell}/2)/4$
Cocconeis (solitary)	# of cells counted in all transects (#cell) 20 individual cell diameters (D$_{cell}$)	$B_{cell} = (D_{cell}^2 \cdot \pi \cdot D_{cell}/2)/4$
Coscinodiscus (solitary)	# of cells counted in all transects (#cell) 20 individual cell diameters (D$_{cell}$)	$B_{cell} = (D_{cell}^2 \cdot \pi \cdot D_{cell}/2)/4$
Cyclotella (solitary)	# of cells counted in all transects (#cell) 20 individual cell diameters (D$_{cell}$)	$B_{cell} = (D_{cell}^2 \cdot \pi \cdot D_{cell}/2)/4$
Cymbella (solitary)	# of cells counted in all transects (#cell) 20 individual cell lengths (L$_{cell}$) 20 individual cell widths (W$_{cell}$)	$B_{cell} = W_{cell}^2/2 \cdot [L_{cell} - W_{cell} + (\pi/4) \cdot W_{cell}]$

Fragilaria (colonial)	# of cells counted in all transects (#cell) 20 Length of the cell in each colony (L_cell<col) 20 Width of the cell in each colony (W_cell<col) 20 # of cells/colony (#cell/col)	$B_{cell} = L_{cell} \cdot W_{cell}^2/2$ $(L_{cell} = L_{cell<colTot}/\#_{cell/colTot})$ $(W_{cell} = W_{cell<colTot}/\#_{cell/colTot})$
Gomphonema (solitary)	# of cells counted in all transects (#cell) 20 individual cell lengths (L_cell) 20 individual cell width1's (W1_cell) 20 individual cells width2's (W2_cell)	$B_{cell} = W2_{cell}/2 \cdot L_{cell} \cdot$ $[(W1_{cell} + W2_{cell})/2]$
Gyrosigma (solitary)	# of cells counted in all transects (#cell) 20 individual cell lengths (L_cell) 20 individual cell widths (W_cell)	$B_{cell} = L_{cell} \cdot W_{cell}^2/2$
Aulacoseira (Melosira) (filamentous)	# of filaments in all transects (#fil) Total length of all filaments (L_FilTot) # of cells counted in all transects (#cell) 20 individual filament widths (W_fil)	$B_{fil} = [\pi \cdot W_{fil}^2 \cdot (L_{filTot}/\#_{fil})]/4$
Navicula (solitary)	# of cells counted in all transects (#cell) 20 individual cell lengths (L_cell) 20 individual cell widths (W_cell)	$B_{cell} = L_{cell} \cdot W_{cell}^2/2$
Nitzschia (solitary)	# of cells counted in all transects (#cell) 20 individual cell lengths (L_cell) 20 individual cell widths (W_cell)	$B_{cell} = L_{cell} \cdot W_{cell}^2/2$
Opephora (solitary)	# of cells counted in all transects (#cell) 20 individual cell lengths (L_cell) 20 individual cell widths (W_cell)	$B_{cell} = L_{cell} \cdot W_{cell}^2/2$
Pennate Diatom (solitary)	# of cells counted in all transects (#cell) 20 individual cell lengths (L_cell) 20 individual cell widths (W_cell)	$B_{cell} = L_{cell} \cdot W_{cell}^2/2$
Rhizosolenia (solitary)	# of cells counted in all transects (#cell) 20 individual cell lengths (L_cell) 20 individual cell widths (W_cell)	$B_{cell} = L_{cell} \cdot W_{cell}^2/2$
Rhoicosphenia (solitary)	# of cells counted in all transects (#cell) 20 individual cell lengths (L_cell) 20 individual cell width1's (W1_cell) 20 individual cells width2's (W2_cell)	$B_{cell} = W2_{cell}/2 \cdot L_{cell} \cdot$ $[(W1_{cell} + W2_{cell})/2]$
Stephanodiscus (solitary)	# of cells counted in all transects (#cell) 20 individual cell diameters (D_cell)	$B_{cell} = (D_{cell}^2 \cdot \pi \cdot D_{cell}/2)/4$
Surirella (solitary)	# of cells counted in all transects (#cell) 20 individual cell lengths (L_cell) 20 individual cell widths (W_cell)	$B_{cell} = L_{cell}^2/2 \cdot [W_{cell} - L_{cell} +$ $(\pi/4) \cdot L_{cell}]$
Synedra (solitary)	# of cells counted in all transects (#cell) 20 individual cell lengths (L_cell) 20 individual cell widths (W_cell)	$B_{cell} = L_{cell} \cdot W_{cell}^2/2$
Tabellaria (colonial)	# of cells counted in all transects (#cell) 20 Length of the cell in each colony (L_cell<col) 20 Width of the cell in each colony (W_cell<col) 20 # of cells/colony (#cell/col)	$B_{cell} = L_{cell} \cdot W_{cell}^2/2$ $(L_{cell} = L_{cell<colTot}/\#_{cell/colTot})$ $(W_{cell} = W_{cell<colTot}/\#_{cell/colTot})$

Cryptophyta		
Chilomonas (solitary)	# of cells counted in all transects (#$_{cell}$) 20 individual cell lengths (L$_{cell}$) 20 individual cell widths (W$_{cell}$)	$B_{cell} = (\pi \cdot L_{cell} \cdot W_{cell}^2)/6$
Chroomonas (solitary)	# of cells counted in all transects (#$_{cell}$) 20 individual cell diameters (D$_{cell}$)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Cryptomonas (solitary)	# of cells counted in all transects (#$_{cell}$) 20 individual cell lengths (L$_{cell}$) 20 individual cell widths (W$_{cell}$)	$B_{cell} = (\pi \cdot L_{cell} \cdot W_{cell}^2)/6$
Rhodomonas (solitary)	# of cells counted in all transects (#$_{cell}$) 20 individual cell lengths (L$_{cell}$) 20 individual cell widths (W$_{cell}$)	$B_{cell} = (\pi \cdot L_{cell} \cdot W_{cell}^2)/6$
Cyanophyta		
Dolichospermum (Anabaena) (filamentous)	# of filaments in all transects (#$_{fil}$) Total length of all filaments (L$_{FilTot}$) # of cells counted in all transects (#$_{cell}$) 20 individual filament widths (W$_{fil}$)	$B_{fil} = [\pi \cdot W_{fil}^2 \cdot (L_{filTot}/\#_{fil})]/4$
Aphanizomenon (filamentous)	# of filaments in all transects (#$_{fil}$) Total length of all filaments (L$_{FilTot}$) # of cells counted in all transects (#$_{cell}$) 20 individual filament widths (W$_{fil}$)	$B_{fil} = [\pi \cdot W_{fil}^2 \cdot (L_{filTot}/\#_{fil})]/4$
Aphanocapsa (colonial)	# of cells counted in all transects (#$_{cell}$) 20 individual cell diameters (D$_{cell}$)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Aphanothece (colonial)	# of cells counted in all transects (#$_{cell}$) 20 individual cell diameters (D$_{cell}$)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Cylindrospermopsis (filamentous)	# of filaments in all transects (#$_{fil}$) Total length of all filaments (L$_{FilTot}$) # of cells counted in all transects (#$_{cell}$) 20 individual filament widths (W$_{fil}$)	$B_{fil} = [\pi \cdot W_{fil}^2 \cdot (L_{filTot}/\#_{fil})]/4$
Chroococcus (colonial)	# of cells counted in all transects (#$_{cell}$) 20 Diameter of a cell in each colony (D$_{cell<col}$) 20 # of cells/colony (#$_{cell/col}$)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$ $(D_{cell} = D_{cell<colTot}/\#_{cell/colTot})$
Lyngbya (filamentous)	# of filaments in all transects (#$_{fil}$) Total length of all filaments (L$_{FilTot}$) # of cells counted in all transects (#$_{cell}$) 20 individual filament widths (W$_{fil}$)	$B_{fil} = [\pi \cdot W_{fil}^2 \cdot (L_{filTot}/\#_{fil})]/4$
Merismopedia (colonial)	# of cells counted in all transects (#$_{cell}$) 20 Diameter of a cell in each colony (D$_{cell<col}$) 20 # of cells/colony (#$_{cell/col}$)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$ $(D_{cell} = D_{cell<colTot}/\#_{cell/colTot})$
Microcystis (colonial)	# of colonies in all transects (#$_{col}$) 20 diameters of colonies (D$_{col}$)	$B_{cell} = (\pi \cdot D_{cell}^3)/6$
Cyanophyte Filament (Narrow Filament)	# of filaments in all transects (#$_{fil}$) Total length of all filaments (L$_{FilTot}$) # of cells counted in all transects (#$_{cell}$) 20 individual filament widths (W$_{fil}$)	$B_{fil} = [\pi \cdot W_{fil}^2 \cdot (L_{filTot}/\#_{fil})]/4$
Oscillatoria (filamentous)	# of filaments in all transects (#$_{fil}$) Total length of all filaments (L$_{FilTot}$) # of cells counted in all transects (#$_{cell}$)	$B_{fil} = [\pi \cdot W_{fil}^2 \cdot (L_{filTot}/\#_{fil})]/4$

	20 individual filament widths (W_{fil})	
Spirulina (filamentous)	# of filaments in all transects (#$_{fill}$) Total length of all filaments (L_{FilTot}) # of cells counted in all transects (#$_{cell}$) 20 individual filament widths (W_{fil})	$B_{fil} = [\pi \cdot W_{fil}^2 \cdot (L_{filTot}/\#_{fil})]/4$
Pyrrhophyta		
Ceratium (solitary)	# of cells counted in all transects (#$_{cell}$) 20 individual cell widths (W_{cell}) 20 individual spine heights (SH_{cell}) 20 individual spine widths (SW_{cell})	$B_{cell} = \left(\frac{\pi}{12}\right) \cdot [L_{cell}^3 + (4 \cdot SH_{cell} \cdot SW_{cell}^2)]$
Gymnodinium (solitary)	# of cells counted in all transects (#$_{cell}$) 20 individual cell lengths (L_{cell}) 20 individual cell widths (W_{cell})	$B_{cell} = (\pi \cdot L_{cell} \cdot W_{cell}^2)/6$
Peridinium (solitary)	# of cells counted in all transects (#$_{cell}$) 20 individual cell lengths (L_{cell}) 20 individual cell widths (W_{cell})	$B_{cell} = (\pi \cdot L_{cell} \cdot W_{cell}^2)/6$

3.2 Zooplankton tables

Table 5: All zooplankton species or general categories that are counted during the analysis of zooplankton samples. Also listed is whether the species or category is considered common or rare.

	Species	**Commonality**
Cladocerans	*Bosmina longirostris*	Common
	Bythotrephes cederstroemi	Rare
	Cercopagis pengoi	Rare
	Ceriodaphnia sp.	Common
	Chydorids	Rare
	Daphnia ambigua	Rare
	Daphnia galeata mendota	Common
	Daphnia longiremis	Rare
	Daphnia parvula	Rare
	Daphnia pulex	Rare
	Daphnia retrocurva	Common
	Daphnia schodleri	Rare
	Diaphanosoma sp.	Common
	Eubosmina coregoni	Common
	Leptodora kindti	Common
Cyclopoid Copepods	*Acanthocyclops vernalis*	Common
	Cyclops scutifer	Rare
	Diacyclops bicuspidatus thomasi	Common
	Ergasilus sp.	Rare
	Eucyclops agilis	Rare
	Eucyclops speratus	Rare
	Mesocyclops edax	Common
	Paracyclops fimbriatus poppei	Rare
	Tropocyclops prasinus mexicanus	Rare
	Unidentified Cyclopoids	
	Cyclopoid Nauplii	

Calanoid Copepods	*Epischura lacustris*	Common
	Eurytemora affinis	Common
	Leptodiaptomus ashlandi	Common
	Leptodiaptomus minutus	Common
	Leptodiaptomus sicilis	Common
	Leptodiaptomus siciloides	Common
	Limnocalanus macrurus	Rare
	Skistodiaptomus oregonensis	Common
	Skistodiaptomus reighardi	Rare
	Unidentified Calanoids	
	Calanoid Nauplii	
Rotifers/Veligers	*Asplanchna* sp.	
	Brachionus sp.	
	Dreissena veligers	
	Kellicottia sp.	
	Keratella sp.	
	Ploeosoma sp.	
	Polyarthra sp.	
	Unidentified Rotifers	

Table 6: 'a' and 'b' coefficients that are used in the generic length-weight regression equation, $W = a \cdot L^b$, for the calculation of zooplankton biomass.

	Species	**'a' coefficient**	**'b' coefficient**
Cladocerans	*Alona* spp.	3.7847	2.6807
	Bosmina longirostris	17.7369	2.2291
	Ceriodaphnia sp.	4.0216	1.9763
	Chydorus sphaericus	14.0793	1.9796
	Daphnia ambigua, *D. longiremis*, *D. lumholtzi*, *D. parvula*, *D. pulex*, *D. retrocurva*, *D. schodleri*, Unidentified Daphnidae, Unidentified Cladocerans	3.7847	2.6807
	Daphnia galeata mendota	7.4997	1.5644
	Diaphanosoma spp.	5.0713	1.0456
	Eubosmina coregoni	21.9128	2.3371
	Ilyocryptus spp.	399.9	7.942
	Leptodora kindti	1.5605	1.873
	Moina spp.	6.61	2.57
	Scapholeberis (Dumont *et al.* 1975*)*	11.204436	2.70
	Simocephalus (Dumont *et al.* 1975*)*	7.43	3.28
	Cercopagis pengoi	2.3713	2.442
Cyclopoid Copepods	*Acanthocyclops vernalis*	7.0729	2.5563
	Cyclops scutifer, Diacyclops bicuspidatus thomasi, Ergasilus sp., *Eucyclops agilis, E. speratus, Paracyclops fimbriatus poppei, Tropocyclops prasinus mexicanus,* Unidentified cyclopoids	5.6713	1.9347
	Mesocyclops edax	6.6586	2.8945
	Cyclopoid Nauplii	2.5968	1.6349

Calanoid Copepods	*Epischura lacustris*	6.4115	1.7713
	Eurytemora affinis	5.8538	1.8165
	Leptodiaptomus ashlandi, Leptodiaptomus sicilis, Skistodiaptomus oregonensis, S. pallidus, Skistodiaptomus reighardi, Onychodiaptomus birgei	6.1927	1.9604
	Leptodiaptomus minutus	7.3614	3.8564
	Leptodiaptomus siciloides	5.8853	3.8498
	Limnocalanus macrurus	7.9677	1.3691
	Unidentified Calanoids	4.5921	1.7034
	Calanoid Nauplii	3.0093	1.7064
Harpacticoid Copepods	*Canthocamptus robertcokeri*	5.6713	1.9347
	Harpacticoid Nauplii	2.5968	1.6349
Dreissena veligers	Veligers	0.1107	12.123

Table 7: 'a' and 'b' coefficients that are used in the generic filtering rate equation, $F = a * L^b$, for the calculation of zooplankton filtering rates. Veliger FR (mL/L/day) is a linear function of L(mm)

Species	'a' coefficient	'b' coefficient	Source
Calanoids + Nauplii	3.02	2.21	Mourelatos et al. 1989
Ceriodaphnia sp.	23.17	2.13	Mourelatos et al. 1989
Daphnia galeata mendota	3.837	1.343	Wu 1991
Daphnia retrocurva	3.565	-0.674	Wu 1991
Daphnia spp.	7.36	2.23	Mourelatos et al. 1989
Diaphanosoma spp.	8.71	2.39	Mourelatos et al. 1989
General Cladoceran	11.695	2.48	Knoechel and Holtby 1986
Dreissena veligers	ug = -0.0602+0.714L (mm)		Pontius 2000

Table 8: Average individual biomasses used in the calculation of egg, rotifer, and veligers biomass values.

	Type of Egg/Rotifer/Veliger	Average Individual Biomass (µg/L)
Cladoceran eggs	*Bosmina longirostris* eggs	0.51
	Ceriodaphnia sp., *Daphnia retrocurva, D. galeata mendota,* Loose Daphnia eggs, Rare Cladoceran eggs	0.5887
	Diaphanosoma sp. Eggs (Dumont et al.1975)	0.67
	Eubosmina coregoni eggs	1.0953
	Leptodora kindti eggs	1.829
	Loose Cladoceran eggs	1.0153
	Loose Bosmina eggs	0.51
Copepod eggs	*Acanthocyclops vernalis* eggs	0.1143
	Diacyclops bicuspidatus thomasi eggs	0.168

	Eurytemora affinis, Leptodiaptomus ashlandi, L. minutus, L. sicilis, L. siciloides, Skistodiaptomus oregonensis eggs	0.1155
	Mesocyclops edax eggs	0.175
	Loose Copepod eggs, Rare Copepod eggs	0.1149
Rotifers	*Asplanchna* sp.	0.16
	Brachionus sp. (Dumont et al. 1975)	0.2
	Kellicottia sp.	0.062
	Keratella sp. (Dumont et al. 1975)	0.11
	Ploeosoma sp.	0.612
	Polyarthra sp. (Dumont et al. 1975)	0.74
	Unidentified Rotifers	0.439
Veligers	Dreissenidae spp.	0.259

* Based on L-W regressions (2007-2019) using coefficients in Table 6. These coefficients are from Bowen et al., 2018. Mean is back-transformed mean of log-biomass, given log-normal distribution.

Table 9: 'α', 'β', and 'ρ' coefficients that are used in the generic secondary productivity equation, $P = α * B^β * ρ^T$, for the calculation of zooplankton secondary productivities.

Group	'α' coefficient	'β' coefficient	'ρ' coefficient
Copepoda	0.0432	0.8878	1.1010
Daphnidae	0.0532	0.9634	1.1073
Bosminidae	0.0583	1.0133	1.0643
Sididae	0.0042	0.9587	1.1757

Table 10: Veliger length-weight data from Bowen et al. (2018). Data obtained by D. O'Donnell from K. Bowen on 11/13/2020.

Length (μm)	Length (mm)	Weight (μg)
103	0.103	0.12
106	0.106	0.14
107	0.107	0.16
135	0.135	0.33
140	0.140	0.30
165	0.165	0.38
170	0.170	0.48
180	0.180	0.61
198	0.198	0.85
200	0.200	0.87
220	0.220	1.14
225	0.225	1.29
230	0.230	1.37
245	0.245	1.65
250	0.250	1.88
253	0.253	2.33
258	0.258	2.46
282	0.282	3.48

283	0.283	3.32

Box 1: R code for fitting eq. 2 from Bowen et al. (2018) to the data in Table 12. The R package "nlme" is required to fit this nonlinear model.

```
fitBowen <- function(x,a,b){
  a*exp(x*b)
}

NLS.fit <- nls(Weight_ug ~ a*exp(Length_mm*b), data=bowen, start=list(a=1,b=1))

summary(NLS.fit)

Formula: Weight_ug ~ a * exp(Length_mm * b)

Parameters:
 Estimate  Std. Error  t-value  Pr(>|t|)
a 0.027252  0.003727    7.312    4.54e-07 ***
b 17.130356 0.525419   32.603    < 2e-16 ***
---
Signif. Codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.1068 on 20 degrees of freedom

Number of iterations to convergence: 9
Achieved convergence tolerance: 9.963e-06
```

4. Counting sheets

Counting sheets used to record data during the microscope analysis of phytoplankton and zooplankton samples are designed to record all the data needed to perform the calculations to convert laboratory data to their corresponding values in the lake (e.g., mg zooplankton biomass · L^{-1}). Counting sheets are attached for phytoplankton (Table 11) and zooplankton (Table 12).

Table 11: Phytoplankton Counting Sheet

Phytoplankton Counting Sheet

Counter Name:	Chamber Area (mm^2):	Project I.D.	
Sample Location:	Transect area (mm^2):	Counting time:	
Date Sampled:	Volume counted (mL):	Comments:	
Date Counted:	Conc. Volume (mL):		
Microscope #:	Pre-Conc. Volume (mL):		
um/ocular unit	Number of transects:	Entered:	Checked:

Chlorophyta	Num	Count	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Actinastrum		L_{cell}																					
		W_{cell}																					
Ankistrodesmus		L_{cell}																					
		W_{cell}																					
Carteria		D																					
Chlamydomonas		D																					
Chlorophyta Fil Fil #		W_{fil}																					
Total Length		L_{fil}																					
Total Cell #		C#																					
Coelastrum Col. #		D_{col}																					
Total Cell #		C#																					
Colonial Chlor. w/sh		D_{cell}																					
Colonial Chlor. w/o sh		D_{cell}																					
Cosmarium		D_{cell}																					
Crucigenia		D_{cell}																					
Eudorina		D_{cell}																					
Micractinium		D_{cell}																					
Oocystis		D_{cell}																					
Pediastrum Col. #		D_{col}																					
Total Cell #		C#																					
Scenedesmus		L																					
		W																					
Schroederia		L																					
		W																					
Solitary Green		D																					
Spiny Green		D																					
Cryptophyta																							
Chroomonas (<11 um)		D																					
Cryptomonas (>21 um)		L																					
		W																					
Rhodomonas (11-21 um)		L																					
		W																					
Chrysophyta (Chrysophyceae)																							
Dinobryon		D_{cell}																					
Mallomonas		L																					
		W																					
Bacillariophyta (Diatoms)																							
Asterionella		L																					
		W																					
Aulacoseira Fil #		W_{fil}																					
(Melosira) Total Length		L_{fil}																					
Total Cell #		C#																					
Centric Diatom		D																					
Cyclotella		D																					
Fragilaria Cell #		L_{cell}																					
		W_{cell}																					
		C#																					
Navicula		L																					
		W																					

	Num	Count	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Pennate Diatom		L																					
		W																					
Synedra		L																					
		W																					
Cyanophyta																							
Aphanizomenon *Fil #*		W_{fil}																					
Total Length		L_{fil}																					
Total Cell #		C#																					
Aphanocapsa [b] Col #		D_{col}																					
Total Cell # [c]		C#																					
		I#																					
Aphanathece [b] *Col #*		D_{col}																					
Total Cell # [c]		C#																					
		I#																					
Chroococcus *Cell #*		D																					
		C#																					
Cyanophyte coccoid [a]		D_{cell}																					
Dolichospermum *Fil #*		W_{fil}																					
(Anabaena) *Total Length*		L_{fil}																					
Total Cell #		C#																					
Merismopedia *Cell #*		D																					
		C#																					
Microcystis [b] *Col #*		D_{col}																					
(small cell < 3 um)		D_{cell}																					
		I#																					
Microcystis [b] *Col #*		D_{col}																					
(large cell ≥ 3 um)		D_{cell}																					
		I#																					
Pyrrhophyta																							
Gymnodinium		L																					
		W																					
Peridinium		L																					
		W																					
Rare Species																							

Notes:

[a] If over one hundred cells are counted by the end of the first transect, a ratio is established. The ratio is recorded in [cell#/transect#].

[b] Dimensions are recorded in μm, NOT ocular units

[c] Total cells in all transects counted

Table 12: Zooplankton counting sheet.

Zooplankton Counting Sheet

Counter Name:		Subsamp Vol (ml)			Project (i.d.)		
Sample Location:		Diluted Vol (ml)			Counting Time		
Date Sampled:		Meter 1			Entered		
Date Counted:		Meter 2			Checked		
Microscope #:		Depth (m)			Sample Cups (#)		
mm/ocular unit:		Temperature (C)			Comments		

TAXON

	Number	Length of individual																			
Cladocerans	Counted	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Bos. long. w/																					
Bos. long. w/o																					
Dap. gal. m. w/																					
Dap. gal. m. w/o																					
Dap. retro. w/																					
Dap. retro. w/o																					
Diaph. sp. w/																					
Diaph. sp. w/o																					
Eubos. cor. w/																					
Eubos. cor w/o																					
Leptodora k. w/																					
Leptodora k. w/o																					
eggs																					
Bos. long.																					
Dap. gal. m.																					
Dap. ret.																					
Diaph.																					
Eubos.																					
Lepto.																					
Loose eggs (Bos.)																					
Loose eggs (Clad.)																					
Loose eggs (Dap.)																					
Copepods																					
A. vernalis w/																					
A. vernalis w/o																					
D. thomasi w/																					
D. thomasi w/o																					
Epischura																					
E. affinis w/																					
E. affinis w/o																					
L. ashlandi w/																					
L. ashlandi w/o																					
L. minutus w/																					
L. minutus w/o																					
L. sicilis w/																					
L. sicilis w/o																					
L. siciloides w/																					
L. siciloides w/o																					

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Mesocyclops spp. w/																				
Mesocyclops spp. w/o																				
S. oregonensis w/																				
S. oregonensis w/o																				
Diaptomidae, immature																				
Temoridae, immature																				
Cyclopidae, immature																				
Calanoid nauplii																				
Cyclopoid nauplii																				

eggs

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
A. vernalis																				
D. thomasi																				
E. affinis																				
L. ashlandi																				
L. minutus																				
L. sicilis																				
L. siciloides																				
Mesocyclops spp.																				
S. oregonensis																				
Loose eggs (copepods)																				

Miscellaneous

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Asplanchna																				
Brachionus																				
Dreissena Veligers																				
Kellicottia																				
Keratella																				
Ploesoma																				
Polyarthra																				
Unid. Rotifers																				

Harpacticoids

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Cantho. sp. w/																				
Cantho sp. w/o																				
Cantho naupli																				

Rare Taxon

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20

5. Phytoplankton and zooplankton Excel macro instructions (Forage Task Group)

Phytoplankton and Zooplankton counts are converted to density (individuals · L^{-1}), biomass, excretion rates, and productivity rates in the lake using a series of MicroSoft Excel data files and associated macros. In this section, specific instruction for transferring information from the original counting sheets (Tables 10 and 11) to Excel files and running the macros are presented. Procedures for the phytoplankton and zooplankton samples are very similar, except for the kinds of data entered. Accordingly, we have prepared one generic set of instructions below, altered for specifics for the two groups of samples when required. To prepare excel files ready to enter calibrated data into MS Access requires the original counting sheets and 5 kinds of Excel files:

5.1 Excel files involved

1) *Phyto Blank Data File* or *ZP Blank Data File*
2) *Phyto data Entry Macro* or *ZP Data Entry Macro*
3) *SortData*
4) *Check Phyto Data Macro* or *Check ZP Data Macro*
5) *Convert Phyto to Access Macro* or *Convert ZP to Access Macro*

5.2 Procedure

5.2.1 Data Entry

a) Copy Blank Data File and rename (e.g., as *LEPAS Forage Task Group 2015*).

b) Open Blank Data File (*LEPAS Forage Task Group 2015* file).

c) Open Data Entry Macro and enable macro for use (Both files must be open).

d) Enter content for first counting sheet, press **Control E** to cause macro to place data in row 6 of *LEPAS Forage Task Group 2015* file.

e) Enter next counting sheet content, press **Control E** to cause macro to place the new data in row 6 and move previous data down one line (row 7). Continue until ALL samples are entered.

f) Save progress you are making by saving *LEPAS Forage Task Group 2015* file, preferably after every 10 samples are entered. Save all progress when you are done.

g) Additional samples' data can be entered subsequently by reopening *LEPAS Forage Task Group 2015* and repeating above steps.

5.2.2 Sort data by sampling date and station

a) After all the Data have been entered, the file *LEPAS Forage Task Group 2015* is ready to be sorted by Sampling Date and Station.

b) Open data file (*LEPAS Forage Task Group 2015*) and SortData macro (Both files must be open).

c) Enable SortData macro.

d) Determine the last row number of *LEPAS Forage Task Group 2015*, e.g, row 112.

e) Press **Control D** and enter the row number (e.g., 112) when prompted.

f) Save sorted *LEPAS Forage Task Group 2015* and rename as *LEPAS Forage Task Group 2015 sorted.*

g) Physically sort the Counting Sheets to match the sequence of data in the *LEPAS Forage Task Group 2015 sorted* file and write the corresponding row number in *LEPAS Forage Task Group 2015 sorted* on the upper right corner of each counting sheet.

5.2.3 Check Data

a) Open *LEPAS Forage Task Group 2015 sorted* file.

b) Open Check Data Macro and enable the macro (both files must be open).

c) Press **Control G** which gets the data for a single row (e.g., row 6) that is to be checked.

d) Compare screen display for first sample with the counting sheet. Assure that the following data have been entered into *LEPAS Forage Task Group 2012 sorted* and are correct, or calculations in the Check Macro will fail:

Phytoplankton: Sample Station, Sample Date, Date Counted, Microscope #, micrometers/ocular unit, total chamber area, transect area, volume of concentrated sample counted, Pre-concentrated Volume (e.g., 250 ml), number of transects counted.

Zooplankton: Sample Location, Date Sampled, Date Counted, Microscope #, mm/ocular unit, Subsample Volume counted (ml), Diluted Volume (ml), First and Last Meter Readings, Station Depth, Average water column temperature.

Compare the number counted and the measurements on the screen with the data on the counting sheet for each taxon and correct any errors. The number in the number of measurements column MUST match the number of measurements entered.

e) Once the entire counting sheet has been checked against the displayed data, press **Control P, and CAREFULLY** type in the row number that you are checking to place the data **back into the row from which it came**, or you will lose the data in the row into which you placed your checked data.

f) Repeat this process for each row (i.e., each counting sheet) until you have checked the entire *LEPAS Forage Task Group 2015 sorted* file. As above, however, you can save your progress (as *LEPAS Forage Task Group 2015 sorted and partially checked*) until you have completed the whole group of samples.

g) Once you have completed this process, resave and rename the file as *LEPAS Forage Task Group 2015 sorted and checked*.

<u>5.2.4 Convert data to prepare for use in ACCESS data base management</u>

a) Open *LEPAS Forage Task Group 2015 sorted and checked* file.

b) Open Convert PP/ZP to Access macro and enable the macro.

c) Press **Control X,** and as above, enter the row number of the last row of data when prompted (e.g., 112). This will convert the measurements from ocular units to (microns in PP, and mm in ZP) and place the results in the M and L tabs with the measurements (e.g., from row 6 to 112 in the example).

d) Save *LEPAS Forage Task Group 2015 sorted and checked* as *LEPAS Forage Task Group 2015_x*.

5.3 Phytoplankton and zooplankton Excel data file formats

The Microsoft Excel files produced with these macros generate relatively large spread sheets containing the initial data from individual sample analyses, the abundance (#/L) and biomass data, as well as many additional calculations. To permit use of this information by the Ohio Division of Wildlife and others, we provide here a summary of the arrangement of information in individual sheets in these files.

<u>5.3.1 Phytoplankton sheets</u>

Sheet 1: Entry. A sheet in this format is used for each sample to allow entry of the name of the analyst, the location from which the sample comes, the date sampled, the date the sample was counted, the identification number of the microscope used, the calibration of the ocular micrometer (number of micrometers per ocular unit in the eyepiece), the area of the bottom of the 10 ml counting chamber used to hold the sample on the microscope, the area of a transect counted (usually the width of a field of view at 400x times the width of the counting chamber), the volume of concentrated sample transferred to the counting chamber (usually 0.2 to 0.6 ml),

the volume of the concentrated sample in the vial (usually 35 ml), the volume of lake water originally settled in the cylinder (usually 250 ml), and the number of transects (the number of passes across the counting chamber required to achieve the desired number of cells counted of the most abundant taxa (>100 cells each)). The remainder of the sheet provides space for entering the number of cells, colonies, or filaments counted in the transects examined, and up to 20 sets of measurements with the ocular micrometer for each taxon to provide the cell volume estimates. If very few cells are encountered of a rare taxon, fewer than 20 sets of measurements are taken.

Sheets 2-12: NumCount and M1 through M10. These sheets summarize the basic counts and measurements for each sample counted, one line per sample. Hence an entire year's microscope analyses can be summarized on these sheets and checked against the original counting sheets for accuracy. The ocular micrometer measurements associated with these counts are summarized on sheets M1 through M10. Although the macros in Sheet 13 (below) use the ocular micrometer-to-millimeter conversions to calculate biomass, Sheets M1 through M10 show the original numbers of ocular micrometer units obtained in the microscope measurements. Once all data are entered, the NumCount and M1 through M10 files can be sorted by date and sampling station using the "Sort" program.

Sheet 13: Dens. Using the information on the NumCount sheet, the next set of macros calculates the density of each taxon in number/mL in the original lake water sampled.

Sheet 14: Biom. Using the information on the NumCount sheet and the measurements on sheets M1 through M10, the macros calculate the volume of each cell, colony, or filament from as many as 20 cells, colonies, or filaments for each taxon in each sample. Because every microscope and ocular micrometer have slightly different magnifications and distances between the marks on the micrometer, each microscope and ocular micrometer combination is calibrated against a stage micrometer to enable the conversion of ocular micrometer counts to microns or millimeters. The macros then compute the average volume of the cell, colony, or filament in each sample and then multiplies it by the number of cells, colonies, or filaments/mL from the Dens sheet, providing an individual estimate of the biomass for each taxon in each sample in mg wet weight/L, assuming a density of 1 g/cm^3 for phytoplankton tissue.

5.3.2 Zooplankton sheets

Zooplankton sample calculations are performed in Excel files with macros similar to those described above for phytoplankton, modified for the slight differences between how samples are handled for zooplankton. For example, concentrated samples from the field are diluted with deionized water to from 30 to 3000 ml to provide an appropriate concentration for subsampling.

Sheet 1: Entry. This sheet is used to allow entry (for one sample at a time) of the name of the analyst, the location from which the sample comes, the date sampled, the date the sample was counted, the identification number of the microscope used, the calibration of the ocular micrometer (number of millimeters per ocular unit in the eyepiece), the subsample volume transferred to the counting chamber (usually 6 to 20 ml), the volume of the diluted sample from

which the subsample is drawn (usually 30 to 3000 ml), the initial reading on the plankton net's flow meter, the final reading on the plankton net's flow meter, the depth of the water column at the sampling site (m), and the average water temperature of the water column ($^{\circ}$C). The remainder of the sheet provides space for entering the number of individuals of each taxon (with or without eggs attached) and up to 20 sets of measurements of specimen length in ocular micrometer units for each taxon to provide individual biomass estimates. If very few specimens are encountered of a rare taxon, fewer than 20 sets of measurements are taken.

Sheets 2-10: NumCount and L1 through L8. These sheets summarize the basic counts and measurements for each sample counted, one line per sample. Hence an entire year's microscope analyses can be summarized on these sheets and checked against the original counting sheets for accuracy. The ocular micrometer specimen length measurements associated with these counts are summarized on sheets L1 through L8. Although the macros in Sheet 12 (below) use the ocular micrometer-to-millimeter conversions to calculate biomass, Sheets L1 through L8 show the original numbers of ocular micrometer units obtained in the microscope length measurements. Once all data are entered, the NumCount and L1 through L8 files can be sorted by date and sampling station using the "Sort" program.

Sheet 11: Dens. Using the information on the NumCount sheet, the next set of macros calculates the density of each taxon in numbers/L in the original lake water sampled at a specific site on a specific date.

Sheet 12: Biom. Using the information on the NumCount sheet and the measurements on sheets L1 through L8, the macros calculate the volume of each zooplankter from as many as 20 specimens for each taxon in each sample. Because every microscope and ocular micrometer pair has slightly different magnifications and distances between the marks on the micrometer, each microscope and ocular micrometer combination is calibrated against a stage micrometer to enable the conversion of ocular micrometer counts to millimeters. Each individual length is then entered into a Length/Biomass equation in the form of $B = aL^b$ to obtain the specimen's biomass (Culver et al. 1985). Macros then compute the average biomass (μg dry weight) of each taxon in each sample from the individual biomass of each measured specimen in each sample and then multiply it by the number of specimens/L from the Dens sheet, providing an individual estimate of the biomass for each taxon in each sample in μg dry weight/L.

Sheets 13 and 14: PE and NE. Using the biomass data from Sheet 12, sheets 13 and 14 calculate the zooplankton excretion rates of phosphorus and nitrogen (μg P/L/day and μg N/L/day) from regressions in Wen and Peters (1994).

Sheets 15 and 16: FR1 and FR2. Using the size measurements and Density data, FR1 calculates the filtering rates of zooplankters in mL filtered/L of lake water/day, using regressions from Knoechel and Holtby (1986). In FR2, the data are changed to filtering rates for the water column, in m^3/m^2/day. Applying the filtering rates to the phytoplankton biomass estimates for that site and date provides an estimate of the grazing rate of the zooplankton on the phytoplankton (mg/L/day or mg/m^2/day).

Sheet 17: Prod. Because zooplankton grow and reproduce rapidly, we have developed methods to estimate the turnover rate of biomass (secondary productivity, µg dry weight/L/day) using biomass estimates, growth and egg development times as a function of temperature (Frost 1977). These calculations have been used to compare zooplankton biomass production as food relative to their consumption rate by planktivorous fish.

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6. 2019 LEPAS zooplankton database overhaul

In 2019, a number of issues were identified with the zooplankton portion of the LEPAS database, and D. O'Donnell conducted a comprehensive overhaul of the database to address these issues. Exact steps taken in this effort are extensively documented in the RMarkdown notebooks used to overhaul the database, which can be found on the AEL server in smb://research/units/ael/Projects/FADX09_LEPAS/1-LEPAS_methods/Zooplankton_overhaul_code_2019.

The principle issue addressed was that the size cutoff delineating the "mature" and "immature" copepod categories was increased by LEPAS staff beginning in 2014 for some species, and in 2016 for some others. Those that changed in 2014 were *Eurytemora carolleeae*, *Leptodiaptomus ashlandi*, *L. minutus*, *Skistodiaptomus oregonensis*, Calanoida spp., unclassified and Cyclopoida spp., unclassified; those that changed in 2016 were *Acanthocyclops vernalis*, *Diacyclops thomasi*, *Epischura lacustris*, *Leptodiaptomus sicilis*, *L. sicilloides* and *Mesocyclops edax*. All issues addressed are described below.

1) To create size categories that were consistent from 1995 to 2019, we designated the smallest 1% of lengths across all years as immature (now called "younger" within LEPAS), and the remaining 99% as "older". Using the proportions of lengths above and below this threshold for each sample, we reapportioned counts and biomasses to "older" and "younger" categories for the whole LEPAS time series.

2) The "Genus_sp_lifestage" column in the zooplankton tables contained copepod taxonomic categories that were inconsistent over time in which species they contained and in their demography. For example, some short-lived or discontinued categories contained both immature and mature copepods, but when these categories were discontinued, matures and immatures were placed in new categories, making long-term time series analysis impossible. A new column called "Genus_sp_95toPresent" was thus added to zooplankton tables in which these disparate/inconsistent categories are regrouped to be consistent throughout the time series (1995-2020).

3) There was a wide variety of entry errors, including (but not limited to) typos, mislabeled taxa, mislabeled size classes, misspellings, inconsistent capitalizations, misplaced decimal points, inconsistent size or taxonomic classifications, nonsensical counts or biomass estimates, incorrect/mismatched conversion coefficients, duplicate entries, and

mislabeled sample dates, locations, sample IDs. All such errors encountered during the overhaul were rectified, however some minor errors may still exist.

4) The R code and data pipeline originally written by Dr. Josh Stone for processing and entering raw data into the database had to be updated to account for the changes made to the database during the overhaul. The updated data entry code and the associated RMarkdown front-end (for use by technicians) can also be found in smb://research/units/ael/Projects/FADX09_LEPAS/7-Data Entry/Zooplankton_2020 and also in the "Zooplankton_overhaul_code_2019" folder linked above.

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7. References

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