Standard Operating Procedure for Collection and Processing of Drop-Down Camera Images for *Dreissena* spp. and round goby (*Neogobius melanostomus*) monitoring

LG410

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1.0 SCOPE AND APPLICATION

1.1 This standard operating procedure describes a method for collection and processing of drop-down camera images for *Dreissena* spp. and round goby (*Neogobius melanostomus*) monitoring.

2.0 SUMMARY OF METHOD

2.1 During the Great Lakes Biological Monitoring Program's Long-Term Monitoring (LTM) summer survey and Cooperative Science and Monitoring Initiative (CSMI) surveys, all planned Ponar stations (including "*Dreissena* only" stations) are sampled and analyzed for *Dreissena* spp. coverage, as well as *Dreissena* spp. and round goby counts using a drop-down camera. Additional drop-down camera stations may be added while surveys are underway at the discretion of the EPA Chief Scientist. Three video segments (all in one video collection, i.e., three replicates) are taken with a drop-down camera at each designated sampling station in each lake. Each video segment is processed and stored separately.

2.2 At the end of each CSMI survey *Dreissena* coverage (%), counts of adult *Dreissena* spp., counts and size structure of round gobies from still images will be used to generate preliminary lake-wide distribution maps of *Dreissena* coverage, and *Dreissena* and round goby densities. Due to the difficulty of discerning small mussels and gobies in images, this SOP will only pertain to large *Dreissena* spp. (generally >10 mm length) and gobies >15 mm in length in the text unless noted. *Dreissena* counts, densities and percent cover estimations and resulting maps will be considered estimations since the exact *Dreissena* counts, including mussels <10mm in length, will be known only after Ponar samples are processed 12-18 months after the survey. They will, however, provide a rapid and reliable estimation of the abundance of ecologically important large mussels and gobies. The newly settled small mussels omitted in these counts have very high mortality and very low biomass and thus negligible functional role.

3.0 EQUIPMENT

- Drop-down camera (as described in Angradi 2018) consisting of:
  - a steel frame equipped with a scale bar
  - two underwater lights (Suptig Underwater Lights Dive Light 84 LED High Power Dimmable Waterproof LED Video Light Waterproof 164ft (50m))
  - two GoPro cameras (Hero5, one down-looking camera, one oblique- or side-looking camera) in deep-water housings
- Data storage (on-board computer and external hard drives)

- Photoshop CC software (Adobe Inc.)
- Stopwatch or wristwatch to keep track of the time

4.0 ON-STATION DROP-DOWN CAMERA DEPLOYMENT PROCEDURES

4.1 Sample Collection

4.1.1 Lower the equipped drop-down camera frame from the starboard side of *R/V Lake Guardian* down to the lake bottom (Figure 1).

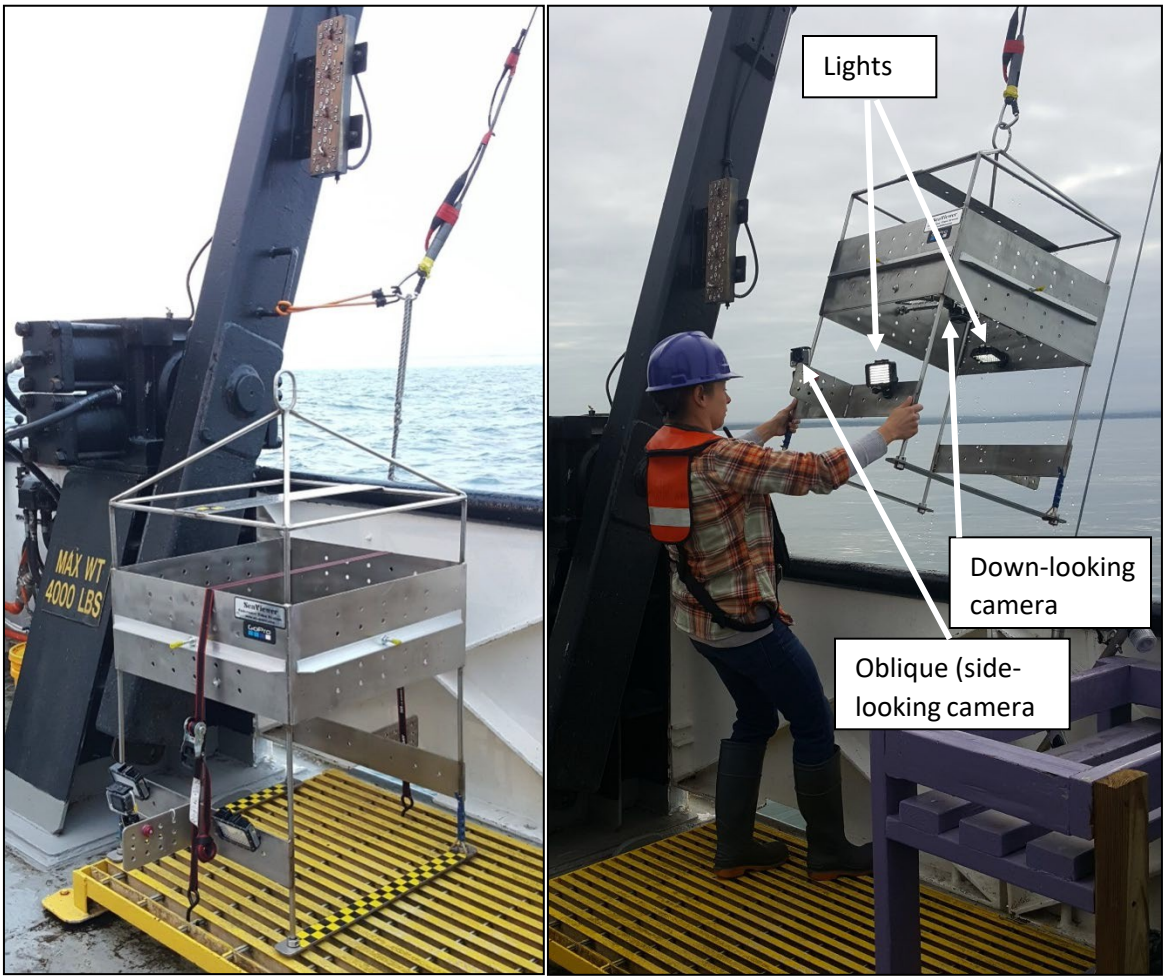

Figure 1. Drop-down camera deployment. Left: drop-down camera frame on deck of R/V *Lake Guardian*; right: camera frame being deployed from starboard side of R/V *Lake Guardian*. Position of cameras and lights are indicated.

4.1.2 The frame should remain on the lake bottom for one minute (this will constitute the first replicate, or RFS). This bottom duration will increase the probability that a clear view (that will be seen later in the video) of the area within the marked scale will be obtained, as any resuspended sediment is allowed to settle or clear from view. Three replicates are taken at each site to calculate the average

Dreissena coverage and densities and round goby densities at the station, to assess the precision of video sampling (the degree of agreement among replicate measurements), and to evaluate the variability of coverage and densities at the station. Taking three replicates is particularly useful due to *Dreissena* and gobies high spatial variability even within a small area (*Dreissena* densities can vary from 0 to 10,0000 individuals/m^2 within a few meters). Multiple replicates can inform about spatial heterogeneity within a sampling station and will be used for statistical analyses within a station (e.g. mean, standard deviation and coefficient of variation). Multiple replicates also allow statistical comparisons among stations, depth intervals, and lake basins.

4.1.3 After one minute, the frame is lifted 1-2m from the bottom. After 30 seconds, the frame is lowered again to remain on the lake bottom for one minute (second replicate - FD1).

4.1.4 Repeat step 4.1.3 (third replicate - FD2).

4.1.5 After the frame is retrieved from the water, videos from both cameras are immediately downloaded to an external hard drive for onboard analysis.

5.0 STORAGE OF ORIGINAL DROP-DOWN VIDEOS

5.1 All drop-down videos will be saved immediately after the end of each survey (GLBMP summer survey, CSMI) to an external hard drive.

5.2 Videos are stored in folders designated for each lake. Each folder should contain subfolders labelled ‘CSMI + year’ and ‘LTM +year’. Each subfolder has an additional folder labelled ‘Drop-down Videos’. Each video is labelled ‘Station ID’_’Survey’_’Sampling Year’_‘Camera View’ (e.g. KM1_CSMI_2019_A). **Note**: A = down-looking view, B = oblique or side-looking view.

6.0 PROCESSING OF DROP-DOWN VIDEOS IN PHOTOSHOP CC FOR SPATIAL COVERAGE ANALYSIS

6.1 Before further analysis all drop-down videos will be reviewed and be classified as acceptable or unacceptable.

An image is considered **acceptable** for analysis if *Dreissena* and round goby presence/absence could be determined with high confidence and, if present, their counts and per cent coverage could be estimated (as the ratio between the area within an image covered by mussels and the area of the entire image). If the video is of poor quality (unclear due to mussels covered by suspended sediments and macrophytes, camera not in focus, insufficient light, high water turbidity, etc.), analysts watch the whole video segment, clip only the best shot, and then process only acceptable images.

Unacceptable videos will be kept and archived but not used for further analysis. However, unacceptable videos will be used to determine whether the reason for an unusable video is controllable and thus can be fixed (lights off, camera settings off) or uncontrollable (algae cover, sediment cover).

6.2 Upload the videos into Photoshop CC for analysis.

6.2.1 Open an Excel file, format as shown in APPENDIX, and insert information about the sample (station, replicate, lake, depth, basin, date).

6.2.2 In Photoshop under 'File' go to 'Open', choose the video from folder, and hit Open. The video will appear in the window as well as a Timeline tool.

6.2.3 As the video contains recorded segments that are not necessary for analysis (e.g. footage of camera frame on deck and being lowered/retrieved through the water column), the Timeline tool allows use of the sliders to select the video segment where the camera frame is on the lakebed and resuspended sediment has been cleared. The image will be used for analysis and represents the first replicate RFS. **Note**: Under some circumstances (silty sediment) the frame will sink immediately into the sediment and thus cause erroneous estimation of *Dreissena* size and counts. In those cases, sliders should be used to stop the video exactly at the moment when the camera frame hits the lakebed, and this image (single frame) will be used for analysis.

6.3 Set the correct measurement scale:

6.3.1 Set the correct measurement scale under '**Image → Analysis → Set Measurement Scale → Custom**

6.3.2 A measurement scale window opens

6.3.3 Under '**Pixel Length**' use the ruler to measure the length of a side of the black or yellow square on the scale bar (Figure 2). The value for pixel length should be 59.

Figure 2. Black or yellow squares on the scale bar of the drop-down camera frame will be used to set the measurement scale in Photoshop.

6.3.4 Under '**Logical Length**' enter **2.45**. This is the length of the black/yellow square on the scale bar in cm.

6.3.5 Under '**Logical Units**' enter **cm**.

6.3.6 Hit '**Save Present**' and name it '**Drop-Down Camera**'

6.4 To crop a still image:

6.4.1 To crop each image to the exact size, go to '**Crop Tool**'

6.4.2 After **Crop** window opens, **right** mouse click, go to '**Use Crop Box Size & Resolution**'

6.4.3 Use 40.8 x 52.8 cm for each still image which will result in a cropped area of 2154 cm^2.

6.5 To highlight *Dreissena* in the still image:

6.5.1 Go to the quick selection tool in the tool bar and select **'Magic Wand Tool'** (this tool selects pixels based on tone and color).

6.5.2 In the tool bar use '**Tolerance**'. This option allows the user to choose/highlight a certain color range, i.e. the lower the tolerance a smaller color range will be highlighted and vice versa. **Note:** A value for tolerance of 5 will work well for drop-down images. A higher tolerance value can be chosen when the color of substrate or *Dreissena* clusters is homogeneous or if the area of mussels or sediment covers most of the still image (Figure 3 left). A lower tolerance should be chosen when the color of substrate or *Dreissena* mussels is heterogeneous or if shadows obscure a clear view of *Dreissena* mussels (Figure 3 right).

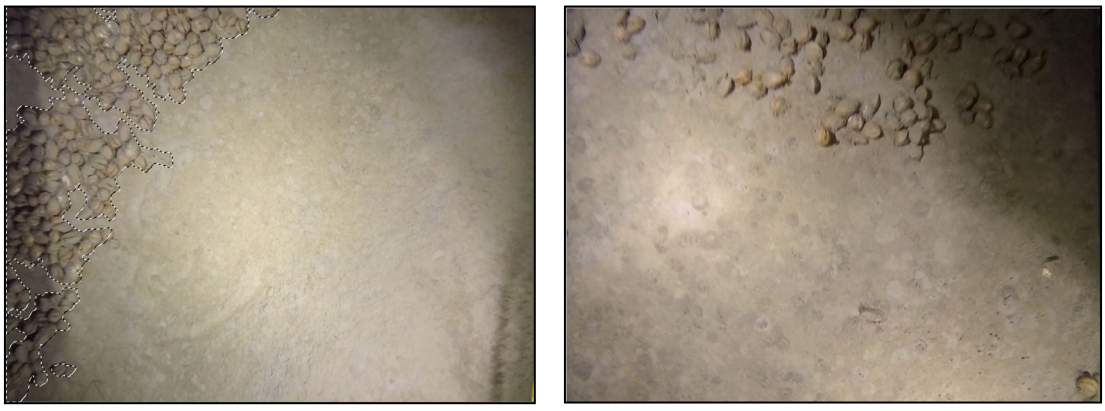

Figure 3. Example of still image with homogenous substrate and clear view of *Dreissena* (left) vs. still image with rather heterogeneous substrate and when shadows obscured the view of *Dreissena* (right).

6.5.3 In the tool bar on top select '**Sample Size'**. The 'Sample Size' option allows users to highlight adjacent pixels with the same color in the still image. For instance, **Point Sample** highlights the precise color of pixels based on the pixel that was clicked, and thus a smaller area is highlighted (Figure 4 left). The higher the value for **Sample Size** the more pixels are selected to average the color adjacent of the pixel that was clicked, i.e. a larger area of the still image will be highlighted (Figure 4 right). **Note**: A sample size value between **Point Sample** and **3x3** will work well for drop-down images.

Similar to 'Tolerance', a higher Sample Size value can be chosen when the color of substrate or *Dreissena* mussels is homogeneous or if the area of mussels or sediment covers most of the still image. A lower tolerance should be chosen when the color of substrate or *Dreissena* mussels is heterogeneous or if shadows obscure a clear view of *Dreissena* mussels.

Depending on the amount of area that is covered by mussels either highlight the mussels or the sediment. **Note**: In Photoshop CC, while highlighting mussels or sediment, you must either press down '**Shift**' permanently or switch the 'Magic Wand' setting to '**add to selection**' shown by the overlapping solid squares icon (Figure 5).

Figure 4. Area selected in still image by using Point Sample (left) vs. area selected by using a Sample Size of 3x3 pixels (right).

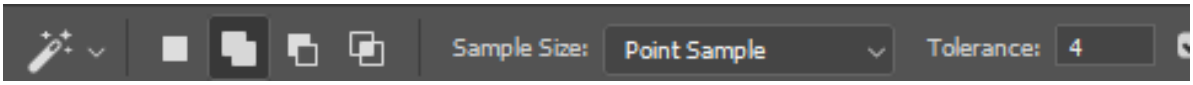

Figure 5. Screen shot showing the 'add to selection' setting selected.

6.5.4 When the mussels or sediment are completely highlighted, select '**Select and Mask**' in the top toolbar to obtain a black and white image (other versions of Photoshop call this function '**Refine Edge**'). All ***Dreissena*** mussels should

appear **black** while the **background** (e.g. sediment) should appear **white** (Figure 6 C).

Figure 6. *Dreissena* in original clipped still image before highlighting (A) with *Dreissena* highlighted (B) and in black and white image after highlighting (C). In highlighted images *Dreissena* appear black and the background appears white. *Dreissena* coverage (%) is calculated by dividing the area covered by mussels (black) by total area of the image.

6.5.5 To measure the area covered by *Dreissena*, right click the mouse, select '**Select Inverse'**. This will highlight only the area covered by mussels.

6.5.6 In the tool bar go to **Image → Analysis → Set Measurement Scale → Drop-Down Camera**

6.5.7 In the tool bar go to **Window → Measurement Log**.

6.5.8 After the Measurement Window opens (Figure 7), hit '**Record Measurement**' to obtain the total area covered by *Dreissena*. In the Excel file record *Dreissena* presence and the measurement of the area covered by *Dreissena* in column "Area *Dreissena*" to calculate the percent coverage.

Figure 7. Screen shot of the Measurement Log with data categories to be transferred to the Excel file in the red box.

6.5.9 Save the original image, image of selected *Dreissena* before converting into black/white, and the converted black/white image in JPEG file format as 'Station ID'_'Survey'_'Sampling Year'_'Camera View'_'Replicate' (e.g. KM1_CSMI_2019_A_RFS).

6.5.10 Repeat the steps 6.5.1 - 6.5.9 for two additional replicates and save images (e.g. KM1_CSMI_2019_A_FD1; KM1_CSMI_2019_A_FD2).

6.6 To count *Dreissena* spp. in the still images:

6.6.1 Use the same still image where *Dreissena* spp. were detected (step 6.5) and saved (step 6.5.9) to count mussels individually. When the coverage is > 50% divide the still image into a 16-cell grid. To put a grid of 4 x 4 cells over the image go to: **View → Show → Grid**. A default grid will be placed over the images. To change the default, go to **Edit → Preferences → Guides, Grids and Slices**. Go to **Grid → Gridline** every, put 100 and change the unit to percent. Under the "Subdivision" put 4. A grid of 4 x 4 will now be placed over the image. Select randomly three grids, count and record all mussels within each grid. If an individual mussel is >50% within the grid, count it as one mussel. Alternatively, if an individual mussel is <50% in the grid, do not count it. All mussels within three randomly chosen grid cells will be counted.

6.6.2 Repeat the step 6.6.1 two more times and take an average of the three counts. In the Excel file record the average count in column "*Dreissena* count".

6.6.3 To calculate *Dreissena* spp. density in the replicate (individuals/m^2), in the Excel file divide the average *Dreissena* spp. count obtained in the step 6.6.2 by the area of the still image (0.2154 m^2). If the coverage is > 50% and only a part of the still image was used, divide the average *Dreissena* spp. count by the area of the grid used (1/16 of the still image, or 0.0135m^2).

6.6.4 Repeat the steps 6.6.1 - 6.6.3 for two additional replicates.

6.7 To count round gobies (*Neogobius melanostomus*) in the still images:

6.7.1 Use the same still image saved on step 6.5.9. Round gobies could be detected by movement, by shape, size, and the characteristic black spot on the first dorsal fin (Figure 8). To ensure goby presence in the still image, review the whole video segment from the time when the camera frame is approaching the lakebed to make sure that no gobies escaped or moved into the frame from outside during frame landing, and correct the count if that happened.

Figure 8. Counting and measuring round gobies in the drop-down camera frame.

6.7.2 Repeat the step 6.7.1 two more times to make sure all gobies were counted. In the Excel file record the average count in column "Goby count."

6.7.3 To calculate round goby density in the replicate (individuals/m^2), in the Excel file divide the count produced in the previous step (6.7.2) by the area of the still image (0.2154 m^2).

6.7.4 Repeat the steps 6.7.1 - 6.7.3 for FD1 and FD2.

6.8 To measure round goby (*Neogobius melanostomus*) body length:

6.8.1 Use the same still image saved on step 6.5.9.

6.8.2 In the tool bar go to **Image -> Analysis -> Ruler Tool**. **Note**: make sure the measurement scale is set to **Drop-Down Camera**!

6.8.3 Measure each goby by holding the left mouse button pressed and drag it over each goby form the tip of the snout to the end of the tail fin (Figure 8).

6.8.4 Go to **Window -> Measurement Log-> Record Measurement**

6.8.5 In the Measurement Log the value for each length will be found under the column '**Round Goby Length**'. Record the measurement in the Excel file in columns "Goby length". **Note**: Gobies can only be measured individually. Photoshop does not allow multiple measurements at the same time.

6.8.6 Mark each goby in the still image. Go to quick selection tool bar **-> Pencil Tool** (use right mouse click to use a pixel size of 8) and drag pencil over each goby. This will help to recount gobies for quality control.

6.8.7 Save the image in JPEG file format as "Station ID'_'Survey'_'Sampling Year'_'Goby'_'Replicate' (e.g. AK1_CSMI_2019_Goby_RFS).

7.0 TRAINING AND ONBOARD QUALITY CONTROL (QC)

7.1 New analysts are required to receive training by Buffalo State College senior analyst in Photoshop CC software including the appearance and morphology of *Dreissena* mussels and round gobies in underwater images (for instance in some cases small rocks may look like *Dreissena* mussels). During initial training the trainee will highlight *Dreissena* from still images already analyzed by the instructor. Part of the training process includes how to differentiate *Dreissena* mussels from other objects based on their size, visible siphons, color pattern, and shape. Live *Dreissena* could be differentiated by the shape of the shell and the narrow dark gap (opening) between the shell valves posteriorly when filtering. Adults have a triangular shell rounded ventro-posteriorly; the thickest and oldest portion of the shell, the umbo, is pointed and lies anteriorly. Round gobies could be detected by movement and characteristic distinctive black spot on the first dorsal fin (Figure 8). Their eyes are large and protrude slightly from the top of the head and they range in length from 4 to 10 inches (maximum of 9.7 inches or 24.6 cm), increasing as they age.

7.2 For QC purposes at least 10% of randomly selected still images will be recounted by the instructor.

7.2.1 For *Dreissena* coverage: Still images will be 're-digitized' by a second analyst following this SOP. Error percentages in *Dreissena* coverage should be less than 20%. If the error is >20%, both still images will be checked and evaluated to determine the cause of the error and counts repeated.

7.2.2 Still images with a very low coverage (usually < 5%) can lead to a high percentage of error (e.g. if one analyst obtains 4% *Dreissena* coverage and a second analyst obtains 5% *Dreissena* coverage, the error equals 20%). High error percentages from a low *Dreissena* coverage will be taken into consideration when determining if the sample passes or fails the QC.

7.2.3 For *Dreissena* spp. count and density: Still images will be 're-digitized' by a second analyst following this SOP. Error percentages in *Dreissena* counts should be less than 20%. If the error is >20%, both still images will be checked and evaluated to determine the cause of the error and counts repeated.

7.2.4 For round goby count and density: Gobies in still images will be 're-counted' by a second analyst following this SOP. Error percentages in round goby counts should

be less than 20%. If the error is >20%, both still images will be checked and evaluated to determine the cause of the error and counts repeated.

7.2.5 For round goby body length measurements: Gobies in still images will be 're-measured' by a second analyst following this SOP. Error percentages in round goby body length measurements should be less than 20%. If the error is >20%, both still images will be checked and evaluated to determine the cause of the error and counts repeated.

8.0 GENERATING *DREISSENA* COVERAGE AND PRELIMINARY *DREISSENA* AND ROUND GOBY DENSITY MAPS

8.1 Required Software: ArcGIS version 10.6 or 10.7

8.2 Prerequisites before mapping

8.2.1 Open a **New Project** by selecting **File → New → Blank Map**

8.2.2 Prior to generating the map, download and save Lake Erie Shoreline shapefile from GLAHF.org website (https://www.glahf.org/)

8.2.3 Upload Lake Erie Shoreline shapefile by selecting **File → Add Data**. Select the folder where the shapefile is saved. Click **Add**.

8.2.4 Upload the Excel file with *Dreissena* coverage data into ArcGIS by selecting File **→ Add Data → Add XY Data**. In the field '**Choose a table from the map or browse for another table**' select the Excel file with *Dreissena* coverage data. Under **Coordinate System of Input Coordinates** use Geographic Coordinate System: Name: **GCS_WGS_1984**. The station locations will appear on the map (see Figure 8). **Note:** To extend the extrapolated *Dreissena* coverage map over the entire lake perimeter, coordinates of four extra '**pseudo-stations'** that need to be located beyond the lake must be added to the Excel file (Figure 9).

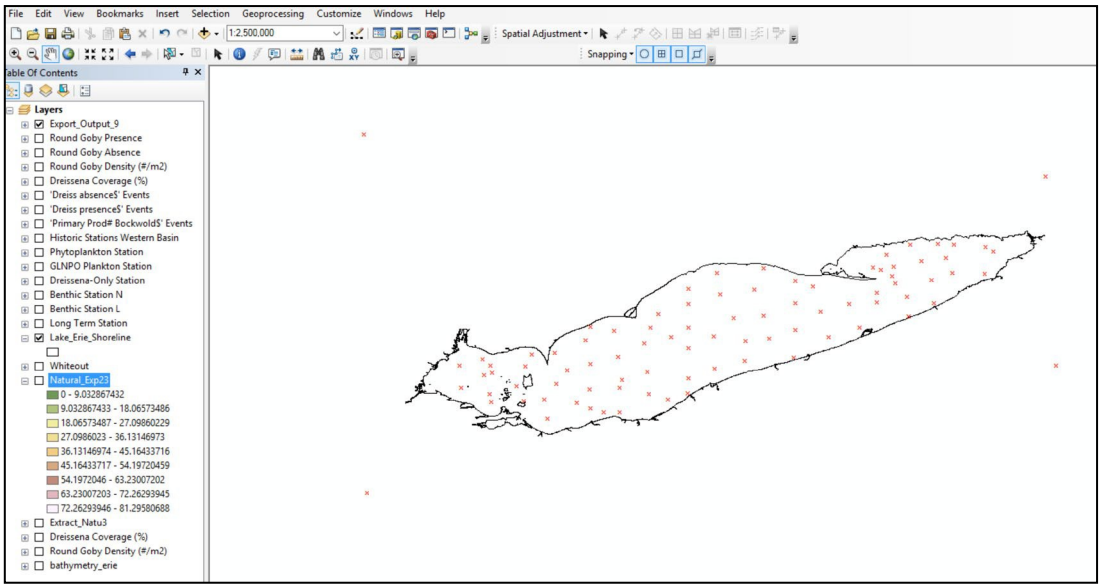

Figure 9. Map of 2019 CSMI Erie stations including four 'pseudo station' locations (red crosses outside the Lake Erie perimeter).

8.3 Mapping Process

8.3.1 In the tool bar select **ArcTool Box → Spatial Analyst Tool → Interpolation → Natural Neighbor**. As for the **Input Point Feature** choose the name of the Excel file that contained the station info. Under **Z value field** choose the column in the Excel file that contains *Dreissena* coverage (%). Under **Output Raster** rename and save the new file that will be generated. Under **Output Cell Size (optional)** leave the default values. Click **Ok**. A new extrapolated layer with *Dreissena* coverage will be generated extending beyond the lake perimeter (Figure 10).

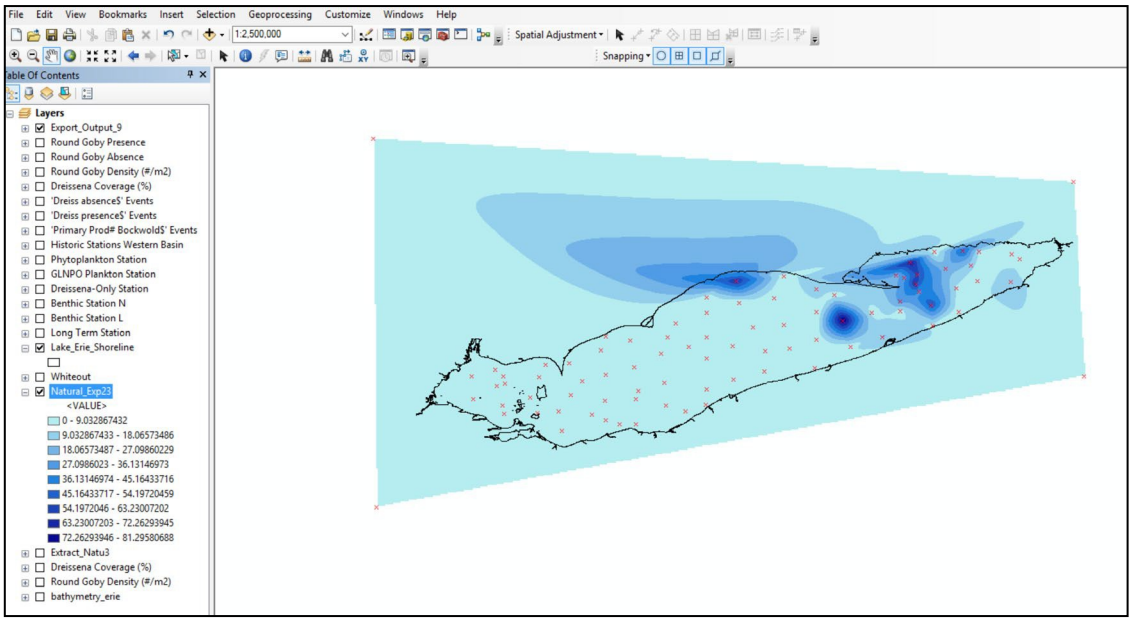

Figure 10: Extrapolated *Dreissena* coverage (%) layer extending over the lake's perimeter.

8.3.2 To clip the new layer to Lake Erie shoreline, select **ArcTool Box → Spatial Analyst Tool → Extract → Extract by Mask**. As for the **Input raster** choose the new extrapolated layer. As for the next field **Input raster or feature mask data** choose Lake Erie Shoreline shapefile. Click **Ok**. After the new **extracted layer** is created, **delete** the **extrapolated layer**.

8.3.3. The extracted layer can now be edited.

8.3.3.1 Rename the layer by **right click** on the layers' name in the **Table of Contents**. Select **Properties → General → Layer Name**. Change the name in the field to "**CSMI 2019 *Dreissena* coverage (%) Lake Erie".**

8.3.3.2 To set the appropriate color range that represent *Dreissena* coverage, double-click on the layers' name in the **Table of Contents**. Select **Properties → Symbology**. Under **Show**, select **Classified**. Under **Class** select **4** then click **Classify**. A new window opens. Under **Break Values** replace the four percent values with **5**, **25**, **50**, and **>75**. These are the per cent ranges that will be presented on the map. To leave stations where *Dreissena* were absent blank, select **Exclusion**. Under **Excluded Values** put **0**. Then select **Legend → Color → White** and under **Label** put **0**. Click Ok. A map of *Dreissena* coverage (%) will be created (Figure 11).

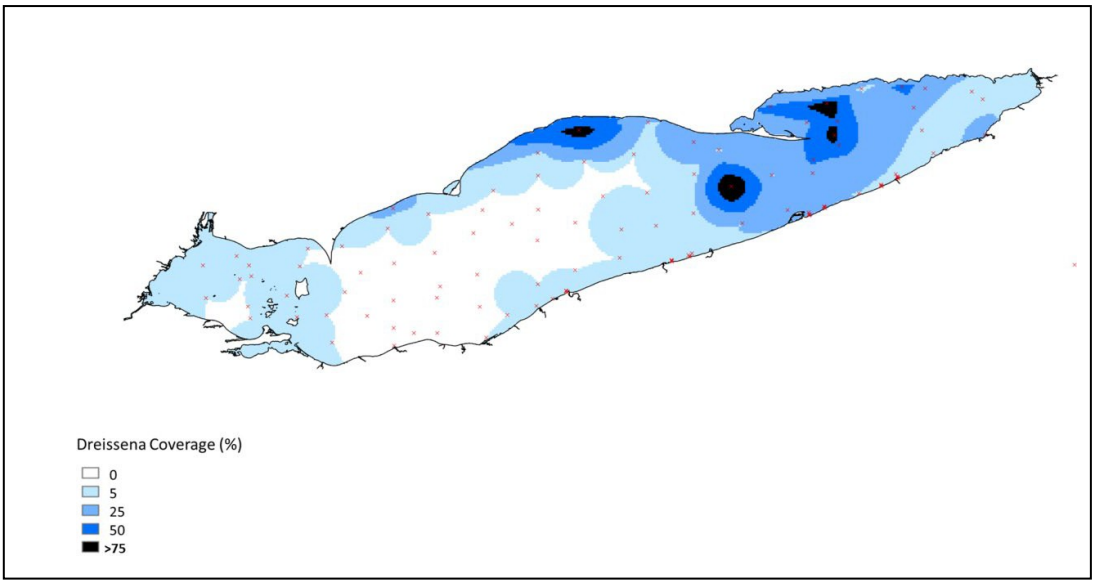

Figure 11. *Dreissena r. bugensis* coverage (%) in Lake Erie based on 2019 CSMI survey data. The coverage classes are: 0%, <5%, 5% to <25%, 25% to <50%, 50% to <75%, and >75%.

8.4 To produce preliminary *Dreissena* spp. density map, repeat steps 8.2.5 – 8.3.3.2 using Excel file with *Dreissena* spp. densities.

8.5 To produce round goby density map, repeat steps 8.2.5 – 8.3.3.2 using Excel file with round goby densities.

REFERENCES CITED

Angradi, T.R. 2018. A field observation of rotational feeding by *Neogobius melanostomus.* Fishes. 3(1), 5; doi:10.3390/fishes3010005

Great Lakes Aquatic Habitat Framework (GLAHF). https://www.glahf.org/data/.

APPENDIX

Example of Excel file for recording video data.

Station	Replicate	Lake	Depth (m)	Basin	Date	Time	Notes	Dressena present (1) absent (0)	Area Frame (cm^2)	Area Dreiss (cm^2)	Dreiss Coverage (%) in still image	Dreissena count	Dreiss Density (m^2)	Goby count (numbers in still image)	Goby length (cm)										Density Goby (numbers/m^2)
															1	2	3	4	5	6	7	8	9	10	
932	RFSa*	Erie	23	Eastern	20190721	13:30		1	2154	1737	80.6	206	956.4	3		x		x	x						13.9
	FD1a	Erie	23	Eastern	20190721	13:30		1	2154	1984	92.1	239	1109.6	4		x	x			x	x				18.6
	FD2a	Erie	23	Eastern	20190721	13:30		0	2154	0	0.0	0	0.0	2			x	x							9.3
	RFSb**,$	Erie	23	Eastern	20190721	16:00		1	2154	n/a	n/a	n/a	n/a	6	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	FD1b	Erie	23	Eastern	20190721	16:00		1	2154	n/a	n/a	n/a	n/a	5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
	FD2b	Erie	23	Eastern	20190721	16:00		0	2154	n/a	n/a	n/a	n/a	4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

*a = down-looking camera view

**b = obliquie camera view

$ = for oblique camera view, no estimates of *Dreissena* coverage, goby length measurements and goby densities possible