

**Larval Assessment Sampling Protocol
Using the AbP-2 Backpack Electrofisher
In Great Lakes Streams**

by

The Larval Assessment Task Force of
The Sea Lamprey Control Program

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INTRODUCTION	3
Stream Selection and Priority	4
Sampling Definitions	4
Ranking Surveys	5
Surveys for Other Purposes	5
Scheduling Streams for Larval Assessment.....	7
RANKING SURVEYS SAMPLING TECHNIQUE	8
Reach Selection	8
Site Selection	9
Obtaining Samples of Larval Sea Lamprey Densities	9
Electrofishing.....	10
Data Records	10
Substrate Sampling Plan.....	10
Substrate Classification	11
Substrate Measurement	12
Transect placement.....	12
Interpretation and Analysis	13
APPENDIX A – Streams Classified as Expert Judgment.....	22
Lake Superior.....	22
Lake Michigan	23
Lake Huron.....	23
Lake Erie	24
Lake Ontario.....	25
APPENDIX B – Quantitative Assessment Sampling Technique (QAS)	26
Larval Density Sampling Plan	27
MEMBERS OF THE LARVAL ASSESSMENT TASK FORCE	33

INTRODUCTION

This document describes larval assessment sampling protocols effective April 10, 2016, and describes the purpose and methodology of all sampling conducted with the AbP-2 backpack electrofisher (BPEF) in wadable waters of Great Lakes tributaries for larval sea lampreys. Wadable waters are defined as waters typically less than 0.8 m deep with substrate suitable for wading and water clarity which allows the use of backpack electrofishers as a sampling tool. The primary goal of larval assessment is to guide management actions designed to minimize escapement of juvenile sea lampreys into the Great Lakes. These actions include control through the application of lampricides as well as alternative controls such as barriers.

The intent of this protocol is to establish a detailed plan for sampling with backpack electrofishers and data analysis that is consistently followed by the U.S. Fish and Wildlife Service and Fisheries and Oceans, Canada. Deviations can be made to the protocol when the Larval Assessment Task Force recommends a change based on further study. This protocol will be reviewed after the 2016 field season and refinements can be proposed at that time. The scheduled time for this review is January, 2017.

The primary objectives of this protocol are to: Identify tributaries to the Great Lakes that harbor larval sea lampreys; rank these streams for treatment in a cost-effective manner (based on the cost per kill of larvae ≥ 100 mm in length) on an annual basis, and; recommend appropriate locations for the application of lampricides or placement of barriers. We identified five methods that are critical to meeting these objectives:

- 1) Sample each tributary to the Great Lakes with potential for producing sea lampreys, but with no record of sea lamprey infestation a minimum of once every 10 years.
- 2) Sample each tributary to the Great Lakes with a history of sea lamprey production a minimum of once every 3-5 years to document recruitment, size structure, and distribution of larvae.
- 3) Sample each tributary to the Great Lakes containing a population of larvae that contains individuals ≥ 100 mm in length at the end of the growing season and rank these streams for treatment the following year.
- 4) Sample each tributary to the Great Lakes between 2 to 12 months following treatment to determine if a residual population of larvae is present.
- 5) Sample upstream of barriers to spawning sea lampreys a minimum of once every 3 to 10 years to detect barrier failures and recruitment.

Stream Selection and Priority

The selection of streams for sampling is based on their potential for lampricide treatment the following year. A stream is prioritized for sampling based on the treatment cycle (if any) for that stream, the current timing or placement within that cycle, and its potential for producing larvae ≥ 100 mm in length. Streams are prioritized from 1-6 for sampling using the following criteria (1 ranks highest):

- 1) A stream with a history of a 3-5 year treatment cycle that will harbor a substantial number of larvae ≥ 100 mm in length at the end of the year it is sampled (based on historical survey and treatment data).
- 2) A stream with a variable treatment periodicity and the expectation that it will harbor a substantial number of larvae ≥ 100 mm at the end of the year.
- 3) A stream that has been treated in the current year or a stream that was treated the previous year and has not been sampled since treatment.
- 4) A stream that is likely to be listed for future lampricide treatment and does not harbor larvae ≥ 100 mm at the end of the next year.
- 5) A stream with a history of larval infestation that has never been treated with lampricide.
- 6) A stream with no history of infestation.

Sampling Definitions

Sampling larval sea lampreys provides an index abundance of larvae ≥ 100 mm, documents recruitment, delineates distribution, and supports research. Wadable portions of streams less than 0.8 m and with water clarity usually permitting visibility to this depth are surveyed using AbP-2 backpack electrofishers. Portions of streams deeper than 0.8 m or shallow water with consistently low visibility are surveyed with other gear and are considered in a separate protocol. All electrofishing sampling is conducted in the best larval habitat available at an approximate rate of 60 seconds/m².

Quantitative Assessment Surveys are no longer conducted to estimate abundance of sea lampreys, but can be used for estimating native lamprey populations and for ranking streams on Lake Champlain. Methods specific to Quantitative Assessment Surveys are provided in Appendix B.

Sampling is defined under the two following categories:

Ranking Surveys

Ranking Surveys (RS) are conducted to index the abundance of larval sea lampreys ≥ 100 mm present in a stream at the end of the year of sampling. RS is typically conducted after August 1 to better predict end-of-season size structure, although in some cases, RS may be conducted earlier to minimize macrophyte interference or due to scheduling logistics. RS is conducted in any stream expected to harbor larvae ≥ 100 mm in length by the end of the year of sampling.

The RS technique uses backpack electrofishers to obtain an estimate of larval density, and combines this with measures of habitat area to index abundance of larvae ≥ 100 mm (i.e., estimated # of larvae ≥ 100 mm in the entire reach). Reach-specific estimates of larval habitat are derived from averages of historic annual measures contained in the Empiric Stream Treatment Ranking (ESTR) database (Christie et al. 2003). Target effort is 1800 seconds (30 minutes) total AbP-2 meter time at each sampling site (usually split evenly between two electrofishers).

Streams predicted to produce larvae ≥ 100 mm are selected for RS sampling using any of the following criteria:

- Demonstrated presence based on sampling since last treatment
- Expected presence based on historic treatment cycle and recruitment
- Analyses of lampricide concentrations or anecdotal information from previous treatment suggests treatment was ineffective
- Treatment evaluation surveys indicate significant residual population
- Stream has a history of residual larvae

If Ranking Surveys are conducted and the stream does not rank for treatment it is usually re-evaluated the following year.

Surveys for Other Purposes

Additional sampling is conducted to detect the presence of larval sea lampreys, delineate distribution, collect larval specimens, and collect information on the size structure of the larval population. This information is used to set application points for lampricide applications, support ongoing research, measure effectiveness of barriers and to determine when RS should be conducted.

Non-ranking surveys use measures of effort (time) and the catch (number) of larval sea lampreys to provide an index of their relative abundance. Target effort is 2160 seconds on the AbP-2 timer (36 minutes) at each sampling site.

These surveys are classified as evaluation, treatment evaluation, distribution, detection, barrier or biological collection surveys.

Evaluation surveys are conducted to assess relative abundance and larval size structure and are often used to determine when RS is needed. Streams are selected for evaluation surveys when they have a history of recruitment and have not been sampled in 3-5 years, or have not been sampled since their last treatment.

Treatment Evaluation surveys are conducted to assess the relative abundance and size structure of larval sea lampreys that survive lampricide applications. They are often used to determine when RS is needed. Treatment evaluation surveys are conducted on all streams treated with lampricides and are conducted between 2 – 12 months after treatment.

Distribution surveys are conducted to determine the in-stream range of larval infestation prior to lampricide treatment. Typically, surveys start near the uppermost locations of historical distribution or upstream of areas considered for RS. When necessary, distribution surveys will also be conducted to determine the downstream distribution. Two negative sites are recommended up or downstream of the last positive site to determine up and downstream limits of lamprey infestation. Streams are selected for distribution surveys based on the following criteria:

- Any stream scheduled for lampricide treatment that has an unknown infestation length
- Any stream selected for RS that has an unknown infestation limit, unless the RS yielded no larvae that will be ≥ 100 mm in length by the end of the year of sampling

Detection surveys are conducted to determine the presence of sea lamprey larvae. Streams that meet all of the following criteria are selected for detection surveys:

- No history of infestation
- Potential for sea lamprey production based on previous surveys or anecdotal evidence (i.e. improvements to water quality, observations by public, etc.)
- Not surveyed in the last five years

Barrier surveys are conducted to measure the effectiveness of barriers at stopping the upstream migration of spawning sea lampreys. Barrier surveys are conducted upstream of barriers on streams that meet any of the following criteria:

- Presence of spawners
- History of infestation
- Suspicion of barrier failure
- Not surveyed in last 10 years
- Requested by barrier coordinator

Biological Collection surveys are conducted to provide lampreys for research,

management studies and toxicity tests. Streams are selected for biological collection based on either of the following criteria:

- Large numbers of lamprey present and easily collected
- Funded by GLFC-funded researchers, or requested by control agents and feasible within the annual MOA work plan

Use of Non-ranking Surveys in the Ranking Process

In certain circumstances, the data from select non-ranking surveys (i.e., evaluation, treatment evaluation, detection, and barrier surveys) may be utilized in the ranking process. To best estimate the larval population, selection of non-ranking surveys for use in the ranking process must meet the following criteria:

- Number of surveys in a reach are greater than or equal to the minimum number of ranking surveys required for the reach (see below)
- Surveys are conducted within the infested length of the stream
- Selection of individual surveys in a reach can be either:
 - o All inclusive (so long as the surveys meet the second criterion above), or
 - o Selected at random (i.e., including both positive and negative sites within the infested distribution)

Agents should indicate the intent to use these surveys for ranking on the survey form where applicable.

In situations where time allows, and in the event of non-ranking surveys resulting in unexpected numbers of large larvae, RS should be scheduled and conducted according to protocol.

Scheduling Streams for Larval Assessment

Table 1 provides a summary of survey classification, technique and frequency based on the treatment history of a given tributary.

Streams with a history of regular treatment (3-5 year cycle) and streams irregularly treated (mean frequency >5 and <10 years) that are regularly inhabited by sea lampreys will be scheduled for RS the year prior to the expected year of lampricide treatment. This will be based on historical treatment frequencies, the results of evaluation surveys, and/or the year larvae are expected to be ≥ 100 mm in length by the end of the growing season. Tributaries identified as Expert Judgment streams (possessing consistent re-establishment, recruitment, and treatment intervals) are not scheduled for RS surveys unless a residual population is of concern. Appendix A lists all Expert Judgment streams.

Streams with a history of irregular treatment (mean frequency >5 and <10 years) that are not regularly inhabited by sea lampreys are sampled using evaluation surveys no more frequently than once every two years depending on the historical minimum number of years between treatments. If a population is

detected and requires RS, the stream is scheduled for RS at the appropriate time based on the size structure of larvae collected.

Streams with no history of treatment or infestation typically will be sampled once every 5 to 10 years using detection surveys. Ideally, a minimum of 3 sites are examined per stream. Sites are selected in areas where there is a high probability of collecting larvae. If a population is detected that requires RS, the stream is scheduled for RS at the appropriate time based on the size structure of larvae.

Table 1. Summary of stream history, survey classification, technique and frequency for tributaries having the potential to produce sea lampreys in the Great Lakes basin.

Treatment History	Survey Classification	Survey Frequency
3-5 year cycle	RS or Evaluation	2-4 years
Irregular and regularly infested	RS or Evaluation	Minimum number years between treatment
Irregular and irregularly infested	RS or Evaluation	No more frequent than every 2 years
Any stream requiring treatment	Distribution	Same year as RS
Any treated stream	Treatment Evaluation	2 – 12 months post-treatment
No previous infestation	Detection	Every 5+ years
Stream with a barrier	Barrier	As needed
Any stream	Biological Collection	When approved

RANKING SURVEYS SAMPLING TECHNIQUE

Reach Selection

Biological reaches have been established for all streams historically infested with sea lampreys. Reach definitions were originally established as portions of

streams that contained similar densities of larval sea lampreys and could be considered an independent section of the stream for the purpose of lampricide application. Contiguous sections of larger streams have often been divided into more than one reach.

Biological reaches scheduled for RS will be identified prior to each field season.

Site Selection

In each biological reach, RS sites are determined by random selection of two to four survey sites. The number of sites selected for sampling is based on the area of effective Type I larval habitat in each reach (Table 2). The calculation for determining effective Type I larval habitat area is demonstrated in Bullet 3 of the “Interpretation and Analysis” section of this document. The number of reach specific sites are calculated in the ESTR dataload module using the most recent available data and are listed in the “Ranking Surveys Sampling Intensity Calculations” table.

Sites that have historically been problematic in respect to stream conditions or access may be eliminated from consideration

Table 2. Number of RS samples per reach based on effective Type I larval habitat area.

Effective Type I Habitat Area	Number of samples
< 25,000 m ²	2
25,000 m ² to 200,000 m ²	3
> 200,000 m ²	4

Obtaining Samples of Larval Sea Lamprey Densities

Mean density of larval sea lampreys ≥ 100 mm in length is estimated in a biological reach by electrofishing for 1800 seconds (approximately 30 m²) at each of the randomly selected survey sites. The best available larval habitat is sampled at each site. Preferably, 15 m² is sampled upstream and 15 m² is sampled downstream of each access site. However, one hour (clock time) is the maximum amount of time that should usually be spent on-stream (e.g. 30 minutes in each direction). If conditions prevent sampling upstream or downstream from a survey site, all sampling is conducted in one direction from the site.

Best available habitat typically consists of Type I habitat, but if Type I is limited or unavailable, Type II habitat may be included. High quality habitat may contain aquatic vegetation, detritus and/or sticks and is usually in defined depositional

areas or along the edges of current flow. Anaerobic conditions are not typical of high quality habitat.

When young-of-year (YOY) larvae are encountered they are counted and measured, but since they are not included in the calculation of density, effort should be focused on collecting non-YOY larvae.

Electrofishing

Electrofishing is conducted at an approximate rate of 60 seconds/m² during RS (the same rate as all other survey types) as measured by the AbP-2 timer. Electrofishing is not conducted when stream flow is high and water is turbid, as these sampling conditions yield unreliable data. Polarized glasses should be used to maximize electrofishing effectiveness.

All electrofishing surveys will be conducted using the parameters listed in Table 3. These settings will be used without exception until the Larval Assessment Task Force recommends a change based on further study.

Table 3. Standard ABP Backpack Electrofisher Settings (from Weisser and Klar 1990)

SLOW PULSE		FAST PULSE		BURST	VOLT RANGE 100 - 250
RATE	DUTY CYCLE	RATE	DUTY CYCLE		
3 pps	25 %	30 pps	25 %	3:1	125

Data Records

Electrofisher time is recorded to the nearest second. Actual time (effort) electrofished should be recorded even if the effort is other than the desired 1800 seconds. Site-specific water temperature, conductivity, and average water depth in electrofished plots are recorded. All sea lampreys collected at each survey site are identified, counted, measured and recorded. Native lampreys are identified to genus or species, counted and recorded.

Substrate Sampling Plan

Newly infested streams or portions thereof will require quantification of available larval habitat. Regularly infested streams require substrate sampling once every 10 years, or more frequently if in-stream habitat is thought to have been altered appreciably (e.g. barrier removal).

Substrate Classification

- **Type I** substrate: Consists primarily of silt, with sand and detritus as secondary components. The sand fraction is mainly comprised of very fine, fine, and medium sands. Coarse sands, gravel or rubble may be present, but their contribution is minor. Surface cover is often provided by woody debris or aquatic macrophytes. Type I substrates are indicative of depositional hydraulic environments that exist in back eddies, on the inside bends of streams, or behind large permanent or semi-permanent objects, such as boulders, logs, and bridge abutments, where stream velocities are usually < 5cm/s. Type I substrates should be disregarded if they are < 2.54 cm in depth.
- **Type II** substrate: Consists primarily of sand, with particle sizes mostly in the range of medium and coarse sands. Compared with Type I, mean values for silt and detritus decline, while those for gravel and rubble rise. Amounts of woody debris are similar to those for Type I, however macrophytes are few, likely as a consequence of the low organic content in Type II substrates. Type II substrates are found in transitional environments where velocity ranges approximately from 5 to 10cm/s, and is largely unimpeded by frictional forces associated with stream banks, bends in the stream, or upstream objects.
- **Type III** substrate: Consists primarily of hard substrates that deter burrowing, such as gravel, rubble, hardpan clay, or bedrock. Interstices in Type III substrates that contain Type I or Type II material may occasionally harbor larvae, however, these areas will be dismissed if the length (along the transect) is less than the minimum recordable measure (0.1 m). Type III substrates are found in erosional hydraulic environments, such as in riffle areas or in the thalweg of the stream, where velocity (>10cm/s) and bottom characteristics restrict the deposition of fine particles.
- **Type IV** substrate: Unsuitable habitat consisting of dry land.
- **Spawning** habitat consists of substrates of suitable gravel (> 9.0 mm in diameter) with a steady, unidirectional flow and satisfactory velocities (0.5-1.5 m/s). Sand exists as a minor component among interstitial spaces in the gravel.

The burrows of large larvae may reach 15 cm in depth (Applegate 1950). A visual inspection of surface cover reveals only the recent depositional history, and it is therefore necessary during the classification process to probe the substrate to determine whether the underlying strata are consistent with surface observations.

Substrate Measurement

Averages of substrate area contained in the ESTR database (Christie et al. 2003) will be used to index abundance of larvae ≥ 100 mm in length and to determine the number of survey sites to sample when conducting RS. Recognizing that larvae often inhabit Type II habitat and that Type II habitat is not always sampled, larval density in each reach is estimated in Type II habitat. These estimates are either based on the average ratio of previous, stream-specific measures of larval density in Type I and Type II habitat or by using a lake-specific conversion factor by predicting proportional density in Type II.

On streams with no historic measures of substrate (i.e. new producers), substrate is measured the first two times RS is conducted. The description and measurement of substrate transects, and the selection of electrofishing plots will commence only under reliable sampling conditions when stream flow and visibility are conducive to seeing and capturing larvae. Larval substrate, classified as Type I, II, III or IV will be measured along four transects at each of six randomly selected survey sites. Measurement of substrate typically begins from the left bank of the stream when looking upstream and continues across the stream along the transect perpendicular to flow. A metric tape or a laser rangefinder is used to measure each segment of substrate. At each change in habitat greater than 0.1 m, measurements of stream width, average depth of segment and habitat type are recorded.

If a section of a habitat transect is too deep to assess and the use of a boat is impractical, a best estimate of the habitat using information such as water velocity and adjacent shallower portions of the transect may be used. However if this information is deemed insufficient then the entire transect is skipped and a make-up transect is added by going further upstream or downstream using the established transect spacing.

If an area of stream is not connected to the main channel (i.e. ponds separated from the flow by a land bridge) these areas will be included in the habitat measurements only if they are likely to harbour lamprey larvae that would survive the summer. Also, when anoxic (often characterized by sulfuric odours) and/or slime-covered Type I habitat is encountered, these areas should be deemed Type II, given the assumption that these areas will not contain nearly the same larval densities of typical Type I habitat.

Transect placement

Habitat is measured along four transects (two upstream and two downstream of the survey site) perpendicular to the flow at each of six survey sites. Transects are spaced dependent on the mean stream width (MSW) of the river. For streams with a MSW less than 5 m, transects are spaced at three MSW and for

streams with a MSW of five or more meters, transects are spaced at two MSW. MSW is defined as the width of the first transect at each site (if it appears to be representative of stream width at the site). The first transect begins 40 m upstream or downstream of any in-stream unnatural structure that affects stream hydrology (e.g. bridge abutment). The minimum number of transects measured on any stream is 12.

Special procedures are used when the following conditions are encountered:

- When habitat transects from one survey site overlap with transects from another site, fewer than four transects are sampled.
- When stream length prohibits sampling 24 transects (based on MSW), then fewer than 24 transects are sampled (12 to 24) based on MSW.
- When 12 transects cannot be sampled based on MSW (extremely short streams) then the 12 transects are spaced at equal distances throughout the length of the stream ($\text{length}/12$). The location of the first transect is a random distance between zero and $\text{length}/12$ and subsequent transects are spaced evenly from that point.
- When the reach is less than 9600 m long the 24 transects can be evenly placed throughout the reach.
- When there are fewer than six survey sites available within a reach, habitat transect measurements among sites should be at least 40 m apart.

When the stream is too deep or turbid to describe habitat visually, the survey is rescheduled or habitat is described using a graduated staff or sampling device such as an Ekman or Ponar dredge.

Workshops that emphasize stream substrate classification and RS techniques will be held as needed. The goal of these workshops is to train field personnel to ensure that stream substrates are classified consistently across the Great Lakes Basin. In addition to providing the basis for whole stream estimates of habitat type, substrate classification is important when selecting high quality larval habitat to sample.

Interpretation and Analysis

The following procedures will be used to index the number of sea lamprey larvae ≥ 100 mm in each stream surveyed using RS.

1. Live lengths of sea lamprey larvae are converted to preserved lengths when

necessary using the equation:

$$\text{Preserved length (mm)} = \frac{[\text{live length (mm)} + 1.634]}{1.062}$$

2. The total catch of sea lamprey larvae is adjusted by multiplying by an electrofishing gear correction factor of 2.08. This is based on a 48% efficiency of the sampling equipment.
3. The weighted Type I habitat area of each reach (A) is calculated as:

$$A = L \times W \times (\text{proportion T1} + q(\text{proportion TII}))$$

where L is infested length, W is mean width and q is the ratio of larval densities in Type II to Type I, as determined from historic measures. This ratio (q) is calculated as the average of reach-specific ratios from data collected between 1995 and 2008.

In the event where only Type I larval density data is available, Type II density (d_2) can be determined using lake specific regression data and is calculated as:

$$d_2 = \exp[\beta_{0\text{-lake}} + \beta_{1\text{-lake}} \ln(d_1)]$$

Where $\beta_{0\text{-lake}}$ is the intercept, $\beta_{1\text{-lake}}$ is the slope, and d_1 is the density of larvae found in Type I habitat corrected for gear efficiency. Lake Specific intercepts and slopes are found in Table 4.

Table 4. Estimated intercept (β_0) and slope (β_1) needed for estimating larval density in Type II habitat given the lake basin and the density of Type I habitat.

Lake	β_0	β_1
Superior	-1.356	0.819
Michigan	-1.804	0.740
Huron	-1.173	0.698
Erie	-1.960	0.503
Ontario	-1.317	0.999

4. Excluding any young-of-year larvae, the total number of sea lamprey larvae collected in the reach is divided by the sum of area of larval habitat sampled at all sites. This provides the overall average larval sea lamprey density for the entire reach.

5. The population of larval sea lampreys in each biological reach is estimated by the product of the weighted Type I habitat (m²) and the mean larval density in the reach.
6. Based on the capture date (Julian), length of each sea lamprey larva at capture, and the average daily growth rate for each stream, the length of sea lamprey larvae on the last date of the growing season is estimated, resulting in an adjusted length-frequency distribution for each reach. Growth rates and growing season dates are contained in the ESTR database (Christie et al. 2003).
7. The number of sea lamprey larvae ≥ 100 mm per reach are summed for all reaches contained in a specific chemical option (i.e., stream considered for treatment) resulting in an index of sea lamprey larvae ≥ 100 mm for each chemical option.
8. The summed estimate for sea lamprey larvae ≥ 100 mm is multiplied by the estimate of treatment efficiency (ranging from 75-99%) to produce the estimated number of sea lamprey larvae ≥ 100 mm killed.
9. The cost to conduct the treatment for each chemical option is divided by the number of sea lamprey larvae ≥ 100 mm killed, resulting in a final cost per kill.
10. All chemical options considered for treatment are prioritized based on the cost per kill value derived in step 9.

Ranking Stream Analysis Example:

Ranking surveys were performed on Dirtywater Creek in preparation for potential treatment the following year. Two RS surveys were completed and in both sites, 30 m² of habitat was electrofished. These sea lamprey had 38 days remaining in their growing season (end of growth season – date of capture), and with a growth rate of 0.17 mm per day, were grown to the end of the season (preserved length plus 0.17*38). Dirtywater Creek has a treatment efficiency of 95%.

Preserved Length	Length at End of Growing Season	Preserved Length	Length at End of Growing Season
64	69.5	54	60.5
65	71.5	57	63.5
48	54.5	85	91.5
96	102.5	86	92.5
97	103.5	86	92.5
99	105.5	92	98.5
102	108.5	94	100.5

102	108.5	96	102.5
110	116.5	97	103.5
		127	133.5
		122	128.5
		124	130.5
		126	132.5

The sum of the habitat area sampled is 60m².

2. The gear correction factor is applied to the total catch:

$$2.08 * 22 = 45.76$$

3. The following are the habitat characteristics obtained from historic quantitative habitat measurements (where q = average Type II to Type I sea lamprey ammocoete density from historic measures):

Mean Width	Infested Length	Type I (%)	Type II (%)	q
3.1 m	1800 m	0.18	0.68	0.15

The Type I equivalent habitat area (A) is:

$$1800 * 3.1 * (0.18 + 0.15 * 0.68) = 1573.56 \text{ m}^2$$

4. The average density of sea lamprey larvae at the two sites electrofished is:

$$45.76 / 60 = 0.76 \text{ lampreys/ m}^2$$

The population of sea lamprey larvae in the reach is:

$$0.76 * 1,573.56 = 1,195.91$$

and 59% (13 of 22) of captured larvae are >100 mm at the end of the growing season, so the estimated number of sea lamprey larvae ≥ 100 mm is 706.

Using the chemical efficiency of 95%, the number of larvae killed is

$$0.95 * 706.67 = 671$$

Dirtywater Creek only has one chemical option; therefore the ranking cost per kill is the total cost of treatment (\$29,500) divided by the larval index:

$$\begin{aligned}\text{Cost per kill} &= \$29,500 / 671 \\ &= \$43.96 \text{ per larvae}\end{aligned}$$

This cost is then used in the rank list, and the top ranking streams (lowest cost per kill) will then qualify for treatment the following year.

Note: rounding should not be used in any steps except for the last calculation.

Guidelines for Selecting Streams for Treatment

Streams are treated based on recent ranking surveys and their resulting rank list position. However, a treatment may be justified by other reasons, including but not limited to: 1) expert judgment; 2) a separate St. Marys River granular Bayluscide plot ranking process; 3) annual treatments; 4) ineffective treatment; 5) deferrals; 6) geographical efficiencies, and; 7) re-infested Lake Erie tributaries. The criteria for selecting streams for treatment are listed below in the order that they appear on the rank list. The order of these criteria does not imply priority. Priority of individual stream treatments is evaluated on a case-by-case basis. Additionally, some streams are excluded from the treatment list for various reasons.

Expert judgment 1 (EJ1): Streams that the Larval Assessment Task Force identifies as meeting specific criteria. These criteria include historical treatments every 3-5 years and consistent records of annual recruitment. Also, catch per unit effort (CPE) surveys must indicate that the first year class immediately following the treatment was re-established.

The streams meeting these criteria are scheduled for treatment based on the last year of treatment, e.g. a stream on a four year cycle that was treated in 2012 will typically be treated in 2016. In the case of a later season treatment, where evaluation surveys indicate that the recruitment during the year of treatment was successfully treated, a delay in treatment may occur to account for the lack of recruitment that year. Typical EJ1 scenarios are as follows:

3 year cycle:

- A) 2012 Treatment → First recruitment after treatment occurs in 2012 → Distribution 2014 → Treatment 2015
- B) 2012 Treatment → First recruitment after treatment occurs in 2013 → Distribution 2015 → Treatment 2016

4 year cycle:

- A) 2012 Treatment → First recruitment after treatment occurs in 2012 → Distribution 2015 → Treatment 2016
- B) 2012 Treatment → First recruitment after treatment occurs in 2013 → Distribution 2016 → Treatment 2017

Expert judgment 2 (EJ2 – Other Criteria): Streams that are scheduled for treatment based on other criteria including, but not limited to:

- Cumulative larval assessment costs exceed treatment costs. Examples are streams that have excessive assessment costs due to remote locations and/or limited access, but are relatively inexpensive to treat due to stream size and personnel requirements. These streams may have been sampled multiple times and continually fall below the rank line.
- High wounding rates in localized areas. Examples include streams that have been sampled and did not rank for treatment, but high localized wounding rates warrant treating as many streams as feasible in areas where wounding is occurring.
- CPE surveys indicate presence of large ammocoetes in streams where Ranking Survey (RS) data are either not available or did not indicate such presence.
- Research conducted on the stream forces treatment.

Expert judgment 3 (EJ3 – Targeted Effort): Streams that are scheduled for treatment based on focused effort directed at a specific suite of streams recommended by the Larval Assessment Task Force and approved by the Sea Lamprey Control Board. This is a set of streams identified for treatment as part of a control strategy independent of the normal ranking process.

St. Marys River Plots: A set of granular Bayluscide plots, ranked first by cost-per-kill of large larvae and then by cost-per-kill of larvae. Base control effort in the St. Marys River is 300 ha, but additional effort can be added depending on funding. Starting in 2014, plots were re-ranked in the same list for a second treatment by applying a predicted 75% kill (from the initial treatment) to the plot's larval estimate.

Annual treatments: Streams treated annually to reduce recruitment to lentic areas. Stream hydraulics in these systems wash larvae downstream into lentic areas that can only be treated by applying granular Bayluscide. Treating the source stream is either more effective or less costly than treating the lentic area after lentic recruitment occurs.

Deferrals: Streams that ranked for treatment the previous year but did not get treated for some reason (e.g., problematic stream conditions, non-target issues).

Geographical efficiency (GE): Streams that did not rank for treatment, but are scheduled for treatment based on both of the following criteria:

- Control crew is treating a ranked stream and staff days are available to treat an infested stream in close proximity.
- CPE surveys demonstrate the presence of large ammocoetes in this nearby, unranked stream.

Lake Erie Tributaries: As recommended by the control agents and agreed to by the SLCB, any Lake Erie tributary that shows recruitment will be treated the third year following the year of first recruitment.

Ranking Surveys (RS): Streams and lentic areas ranked for treatment by indexing the abundance of larval sea lampreys ≥ 100 mm based on ranking surveys conducted the year prior to treatment. Smaller lentic areas are at times combined with lotic treatments, and are sometimes associated with annual treatments.

Emergency Treatments: Larval assessment may find streams, tributaries, or lentic areas that warrant immediate consideration for treatment during the same year in which they are surveyed. However, the treatment Rank List is assembled the year prior to the actual year of treatment. In order to equitably compare any potential chemical options with those surveyed previously, the length data from these new surveys must be modified to reflect the lengths that would have been present if the system had been surveyed the year before. This is done by subtracting the growth that has occurred since the end of the previous season. In ESTR, this is accomplished by running the new data through a different model setting and process outlined here:

- Load any the new survey data into ESTR
- Open Options-Sample Inclusion/Exclusion
- Check: Include the Year-of-Rank-List Data in the Ranking
- Select the chemical option(s) you want to include in the ranking run
- Run ESTR for the year of the original rank list

The above process generates cost-per-kill for the chemical options selected by using length at catch and subtracting daily growth to estimate abundance of larvae >100 mm at the beginning of the current growing season. Chemical option cost-per-kill for large larvae is then compared with the existing Rank List cutoff for staff days. If cost-per-kill is less than the existing cutoff, then lampricide control can add the emergency treatment to their schedule if time and resources

are available.

Streams excluded for treatment (exclusions): Streams that have been removed from the rank list for one of the following reasons:

- Stream ranked for treatment based on presence of residual larvae collected at one or two locations and survey data and/or treatment reports suggest that larval data are not representative of entire reach (e.g., localized tributary influence, backwater, etc.).
- Stream forecasted to have a high residual population based on past treatments, but treatment data and post treatment surveys suggest that lethal concentrations were maintained throughout the infested area leaving fewer residuals than usual.
- A catastrophic event (e.g., severe flood or drought) occurred after last treatment and survey data indicate that larvae are not present in projected abundance.
- Stream excluded from rank list based on research needs.

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APPENDIX A – Streams Classified as Expert Judgment

Lake Superior

Stream Code	Stream	Treatment Cycle (Years)	Last Treatment	Potential Next Treatment
24	Goulais River	3	2016	2020
52	Batchawana River	4	2016	2020
54	Carp River	4	2016	2020
56	Pancake River	4	2016	2020
116	Gargantua River	4	2013	2018
167	Michipicoten River	4	2016	2020
305	Pic River	6	2013	2019
335	Steel River	4	2016	2020
360	Pays Plat River	5	2015	2020
368	Gravel River	4	2016	2020
374	Cypress River	4	2015	2019
385	Jackfish River	4	2016	2020
392	Nipigon River (Upper Nipigon River)	5	2016	2021
392	Nipigon River (Stillwater Creek)	4	2013	2017
517	Wolf River (Barrier to mouth, TFM)	4	2015	2019
572	Kaministiquia River	3	2016	2019
10022	Tahquamenon River	4	2015	2019
10053	Two Hearted River	4	2016	2020
10064	Sucker River	4	2014	2018
10096	Miners River	4	2016	2020
10126	Chocolay River	4	2016	2020
10156	Little Garlic River	4	2014	2018
10157	Big Garlic River	4	2015	2019
10158	Iron River	4	2016	2020
10159	Salmon Trout River	4	2016	2020
10181	Huron River (gB Stream)	3	2015	2018
10200	Sturgeon River	4	2015	2019
10226	Traverse River	3	2016	2019
10284	Misery River	3	2015	2018
10287	East Sleeping River	4	2016	2019
10289	Firesteel River	4	2016	2020
10295	Ontonagon River	4	2016	2020
10313	Potato River	4	2014	2017
10315	Cranberry River	4	2014	2017
10611	Bad River	3	2014	2017
10679	Brule River	4	2015	2019
10705	Amnicon River	3	2015	2018

Lake Michigan

Stream Code	Stream	Treatment Cycle (Years)	Last Treatment	Potential Next Treatment
10012	Hog Island Creek	4	2017	2021
10014	Black River	4	2017	2021
10023	Millecoquins River	3	2017	2020
10046	Milakokia River	4	2016	2021
10093	Sturgeon River	3	2015	2019
10102	Ogontz River	4	2016	2020
10119	Whitefish River	3	2016	2019
10130	Rapid River	4	2017	2021
10143	Ford River	3	2017	2020
10165	Cedar River	3	2017	2020
10200	Peshtigo River	3	2015	2019
10458	Boyne River	4	2017	2014
10467	Jordan River (Incl. Deer Cr. & Lentic)	4	2015	2015
10519	Platte River (Upper)	3	2017	2015
10519	Platte River (Lower)	3	2017	2015
10523	Betsie River	3	2017	2013
10534	Big Manistee R. (Main, Bear Cr. & Pine Cr.)	3	2016	2013
10534	Little Manistee River (Entire)	3	2015	2015
10560	Lincoln River (Main and N. Branch)	4	2017	2014
10562	Pere Marquette River	3	2017	2015
10577	Pentwater River (N. Branch and Cedar)	4	2016	2013
10591	White River (Above Hesperia)	3	2014	2013
10591	White River (Below Hesperia)	3	2017	2013
10591	White River (N. Branch)	3	2014	2013
10613	Muskegon R. (Main and Bigelow Cr.)	3	2017	2014
10639	Grand River (Crockery Creek)	3	2017	2015

Lake Huron

Stream Code	Stream	Treatment Cycle (Years)	Last Treatment	Potential Next Treatment
3	Root River (Main, Tribs and Estuary)	3	2016	2019
4	Garden River	3	2014	2017
57	Watson Creek	4	2015	2019

88	Thessalon R. (Ottertail to Mouth and Tribs)	3	2014	2017
102	Mississagi R. (from Red Rock GS & Tribs)	3	2013	2017
116	Serpent River: Grassy Creek	3	2016	2019
305	Mindemoya River	4	2015	2017
310	Timber Bay Creek	4	2015	2017
314	Blue Jay Creek	4	2015	2019
606	French River (Wanapitei River)	5	2011	2018
745	Magnetawan River (Mainstream to Byng Inlet)	4	2015	2019
821	Naiscoot River	4	2016	2020
1053	Boyne River	4	2016	2020
1343	Sturgeon River	4	2012	2018
1360	Nottawasaga River (Pine River)	4	2016	2020
1393	Bighead River	3	2015	2018
10089	Pine River	3	2015	2018
10095	Carp River	3	2014	2017
10144	Pigeon River	4	2012	2016
10144	Sturgeon River	4	2012	2016
10144	Maple River	4	2012	2016
10173	Elliot Creek	4	2008	2013
10199	Black Mallard River (Lower)	3	2012	2015
10199	Black Mallard River (Upper)	3	2012	2015
10202	Ocqueoc River (Lower)	4	2012	2013
10210	Schmidt Creek	4	2012	2013
10216	Trout River Lower (Below Barrier)	3	2011	2013
10235	Devils River	3	2011	2014
10255	Au Sable River	3	2010	2013
10271	Tawas Lake River (Cold and Silver creeks)	4	2009	2013
10286	E.AuGres River (Below Barrier)	4	2009	2013
10290	AuGres River (Including Hope Creek)	3	2010	2013
10296	Rifle River (Entire)	3	2011	2014

Lake Erie

Stream Code	Stream	Treatment Cycle (Years)	Last Treatment	Potential Next Treatment
99	Big Otter Creek	3	2013	2017
104	Big Creek	3	2013	2017
10023	Cattaraugus Creek (and Clear Creek)	3	2009	2013
10136	Crooked Creek	3	2009	2013
10153	Conneaut Creek	3	2009	2013

Lake Ontario

Stream Code	Stream	Treatment Cycle (Years)	Last Treatment	Potential Next Treatment
19	Black River	3	2015	2018
45	South Sandy Creek	3	2016	2017
48	Lindsey Creek	3	2014	2017
50	Little Sandy Creek	3	2016	2017
53	Salmon River	3	2014	2017
54	Grindstone Creek	3	2016	2019
55	Snake Creek	3	2015	2018
58	Little Salmon River	3	2014	2017
60	Catfish Creek	3	2015	2018
66	Oswego River, Fish Creek	3	2016	2019
73	Sterling Creek	3	2015	2018
76	Bronte Creek	3	2016	2019
92	Credit River	3	2016	2019
117	Duffin Creek	3	2015	2018
121	Lynde Creek	3	2015	2018
124	Oshawa Creek	3	2015	2018
125	Farewell Creek	3	2015	2018
131	Bowmanville Creek	3	2014	2017
132	Wilmot Creek	3	2015	2018
163	Salem Creek	3	2015	2018
230	Trent River, Mayhew Creek	3	2015	2018

APPENDIX B – Quantitative Assessment Sampling Technique (QAS)

The QAS technique uses measures of larval density and habitat area to estimate larval abundance. Larval density is measured with backpack electrofishers and habitat is measured along transects at access sites in streams. Effort is consistent per unit of measured area electrofished.

QAS surveys are conducted to estimate abundance of larval sea lampreys and predict the number of transforming sea lampreys a stream will produce the following year. QAS surveys are conducted as late as possible in the field season to better reflect end-of-season larval distribution and size structure. QAS surveys are typically conducted on streams that are treated on cycle (3-5 years), streams that are regularly inhabited with sea lampreys but at low densities, and in streams suspected of harboring significant numbers of residual lampreys.

Candidate streams are selected for QAS from those streams that are expected to produce transformers the following year, using the following criteria:

- Demonstrated presence based on survey since last treatment.
- Expected based on historic treatment cycle and recruitment.
- Treatment crew suggests last treatment was ineffective.
- Stream has a history of residual larvae.

Expected based on model projections (ESTR).

Sampling frequency

QAS will be conducted the year before transformation is expected to occur from a reestablished larval population or when transformation from a residual population requires remedial action. If QAS is conducted and the stream does not rank for treatment it is reevaluated for larval survey the following year.

Reach selection

Biological reaches will be identified prior to each field season in all locations to be surveyed using the QAS technique. Biological reaches may be subsampled (each portion receives the minimum base effort of 12 type I and 6 type II plots) to determine if a section of stream within the reach may be a reach unto itself. If the variance of larval density is significantly different from that of other reaches of the same stream it can be considered as a separate biological reach and sampled as such in subsequent years.

Site selection

In each biological reach, QAS sites are determined by random selection of 6 sites from a list of locations with suitable access that are spaced a minimum of 800 m apart. Sites with suitable access are numbered (e.g., 1 to 10) and a random method is used to select the 6 sites. Exceptions include:

- When less than 6 access sites are available within a biological reach, the number of plots (e.g., 12-type I) is divided equally among the available access sites.
- When the number of plots can not be distributed equally among access sites the remainder is distributed randomly. At least one site is sampled in each regularly infested, major tributary of each biological reach.
- When a stream is less than 9600 m in length, 12 evenly spaced type I plots may be sampled.
- Streams with limited access may be stratified by length and systematically sampled by length strata to ensure representation throughout the infested area. In these instances, the location of the first plot will be selected randomly.

Larval Density Sampling Plan

Plot selection and size

Mean density of larval sea lampreys is estimated in a biological reach by electrofishing a minimum of 12 type I and 6 type II plots (2 type I plots at each of 6 access sites, and 2 type II plots at 3 sites randomly selected from 6 access sites; preferably one plot upstream and one plot downstream of each access site. If conditions prevent sampling upstream or downstream from an access point, both type I (and type II) plots can be obtained in the same direction from the access site, but no two type I plots should be less than 40 m apart.

A plot consists of 15 m² of type I or type II habitat. Habitat is sampled as it is encountered beginning with the first available habitat up or downstream of the starting point. If the first available habitat encountered is not a 15 m² contiguous area, then sub-plots of a minimum 1 m² will be sampled and summed until 15 m² has been sampled. This process is repeated for type II plots. Type I plots are sampled first and then type II plots are completed. In the rare case that an inadequate amount of type I habitat is available in a given stream, type II plots will be sampled following the guidelines established for sampling type I habitat up to the maximum of 20 plots. Plots will be measured using any device that is graduated in 0.1 m increments and the perimeter of each plot will be demarcated with stakes.

Mean depth of each plot or sub-plot (regardless of habitat type) is calculated and recorded as the mean of 3 depth measurements (minimum, mid, and max). When sub-plots are electrofished, the average depth of the plot is calculated as the mean of the sub-plots. All measurements are made and recorded to the nearest 0.1 m using a device that is graduated in 0.1 m increments.

The smallest dimension (length or width) on any plot is 0.3 m. A plot may consist of less than 15 m² of type I habitat when either of the following circumstances are encountered:

- When historical survey and treatment data indicate that larval densities are expected to exceed 5 larvae/m², the plot size can be reduced to 5 m² or;
- When field personnel have electrofished all type I habitat within 400 m from the beginning of the site. In this case, the plot is equal to the amount of available type I habitat (less than 15 m²). If less than 1 m² of type I habitat is encountered, the entire plot can be obtained in the opposite direction adjacent to the other plot following the guideline of 40 m spacing). If less than 1 m² of type I habitat is encountered next to the adjacent plot, the plot is relocated to the nearest unsampled access site (new access site not previously chosen).

Our goal is to collect a total of at least 100 sea lampreys age 1 and older from the 12 type I plots. When YOY larvae are encountered, they are counted and measured for length, but are not included in the calculation of density with older larvae. If a total of 100 sea lampreys is not collected from the 12 type I plots, additional sites (randomly selected) may be sampled up to a maximum of 10 total sites (20 type I plots) for the reach or until 100 larval sea lampreys are collected, whichever occurs first.

Type II sites/plots are sampled at the rate of one half the number completed for type I sampling (e.g., if 8 sites/16 type I plots are completed, then 4 sites/8 type II plots must be completed.) Sites where type II plots are sampled are a randomly selected subset of the type I sites. There is no goal for the number of larvae collected in type II plots. Collections of larvae from type I and II habitats remain separate.

The decision to sample additional plots will be made by the crew leader and is based on the number and size structure of the larvae collected in the first 12 type I plots (e.g. if very few larvae or no larvae of a transformable size have been collected in the first 12 plots, the crew leader may decide not to sample additional sites). If additional sites are sampled, the sites are randomly selected from a list of all access sites in each biological reach infested with sea lampreys. Habitat is measured at all sites, including the additional access sites.

Electrofishing

Electrofishing is conducted at a rate of 1.5 minute/m² as measured by the AbP-2 clock. The operator electrofishes so that the entire area is covered equally in one pass during the allotted time. Electrofishing is not conducted when stream flow is high and water is turbid (un-reliable). If conditions are reliable, density measurements are made even when conditions (e.g. depth) preclude the measurement of habitat transects.

All electrofishing surveys will be conducted using the parameters listed in Table 2.

Data Records

The specific conductivity of the water is measured once at each access location and recorded on the data form. Plot area is measured using any device that is graduated in 0.1 m increments and recorded to the nearest 0.1 m² and electrofisher meter time is recorded to the nearest second. Actual time and area electrofished should be recorded on the data record even if the shocking rate is other than the desired 1.5 minute/m². All lampreys collected in each plot (total plot area) are identified, counted, measured and recorded.

Substrate Measurement

Substrate is measured each time a QAS survey is conducted with the following exception: based on an analysis by the Program statistician of the variation of previously collected substrate data, a reach may no longer require sampling because of its low sampling variation (consult work plan). The description and measurement of substrate transects, and the selection of electrofishing plots will commence only under reliable sampling conditions. If conditions are reliable, substrate transects are described and measured even when circumstances preclude density sampling (e.g. <1m² of Type I substrate). Larval substrate, classified as Type I, II, or III, will be measured along transects at 6 of the 6-10 access sites. Measurement of substrate typically begins from the left bank of the stream and continues across the stream along the transect. A metric tape or an electronic or laser distance measuring device is used to measure each segment of substrate. At each change in habitat (type I, II, and III) greater than 0.1 m,

measurements of stream width, average depth of segment and habitat type are recorded. In addition, the distance along the transect is recorded each time the water depth is 0.8 m (the point where depth is changes from shallow to deep with respect to backpack electrofishing).

Transect placement

Habitat is measured along 4 transects (2 upstream and 2 downstream of the access site) perpendicular to the bank at the first 6 access sites selected for QAS. Transects are spaced dependent on the mean stream width (MSW) of the river. For streams with a MSW less than 5 meters, transects are spaced at 3 MSW and for streams with a MSW of 5 or more meters, transects are spaced at 2 MSW. MSW is defined as the width of the first transect at each site (if it appears to be representative of stream width at the site). Differential spacing of transects dependent on MSW is required to achieve the target of 95% confidence. The first transect begins 40 m upstream or downstream of any in-stream man made structure that affects stream hydrology. The minimum number of transects sampled on any stream is 12.

Special procedures are used when the following conditions are encountered:

- When habitat transects from one QAS site overlap with transects from another site, fewer transects than 4 are sampled.
- When stream length prohibits sampling 24 transects (based on mean stream width), then fewer than 24 transects are sampled (12 to 24) based on mean steam width.
- When 12 transects can not be sampled based on mean stream width (extremely short streams) then the 12 transects are spaced at equal distances throughout the length of the stream ($\text{length}/12$). The location of the first transect is a random distance between 0 and $\text{length}/12$ and subsequent transects are spaced evenly from that point.
- When the reach is less than 9600 m long the 24 transects can be evenly placed throughout the reach.
- When more than 2 type 1 plots are sampled at an access site (e.g. when there are fewer than 6 access sites available for survey within a reach), no two sets of habitat transect measurements should be less than 40m apart.

When the stream is too deep or turbid to describe habitat visually, the survey is rescheduled or habitat is described using a probe pipe or sampling device such as an Ekman or Ponar dredge.

Interpretation and Analysis

In applying these methods of interpretation and analysis, the goal is to rank candidate streams the year prior to treatment by predicting transformer production for the year of treatment. The following procedures were used to predict the number of transforming sea lamprey larvae in each stream surveyed using QAS in 2005 and was used to estimate abundance of transformers in 2006. Refinements will be added as they become available.

1. The total catch of larvae is adjusted by applying an electrofishing gear correction factor that adjusts for the effects of water depth, larval size and density, and stream conductance. The gear correction factor is based on the results from Steeves (Master's Thesis, 2002). The density of larvae in each biological reach is adjusted by dividing the adjusted catch by the total area sampled (m^2).
2. The population of larval sea lampreys in each biological reach is estimated by the product of the total habitat area (m^2) and the mean larval density measured in the reach. Total habitat is defined as the amount of type I habitat estimated from transects plus the amount of type II habitat estimated from transects. Densities are calculated separately for both type I and Type II habitats.
3. Based on the capture date (Julian), length of each larva at capture and the average daily growth rate for each stream, larvae are grown and their lengths adjusted to the last date of the growth season, resulting in an adjusted length-frequency distribution for each reach. Number of days in the growth season varies depending on the geographic location of the stream.
4. The percent of larvae at each length (mm) in the collection is calculated as a proportion of the adjusted number at each length to the total number of larvae in the adjusted length-frequency collection (all lengths) for type I habitat. The percentage of larvae for each length (mm) is then multiplied by the population estimate for the reach, resulting in an estimate of the number of larvae in the population for each length (mm) represented in the collection for type I habitat. This calculation is repeated for type II habitat.
5. Two probability of transformation curves, one for tributaries of the upper lakes and one for tributaries of the lower lakes, are used to estimate the number of larvae/size that will transform the year following. The transformation curves were developed using a maximum likelihood binary logistic regression model. The appropriate curve is applied to the length-frequency distribution of the estimated population in each reach. This resulted in an estimate of the number of transformers in each biological reach.

6. If quantitative surveys are conducted in more than one biological reach of any given tributary, individual population estimates are calculated based on the adjusted catch, adjusted density and total habitat area within each infested reach for both type I and type II habitats. Larvae are grown and the number of transformers is estimated for each reach. Respective estimates of larvae and transformers for streams with more than one biological reach are then calculated as the sum of the estimates for each reach.

Example: if stream A had 3 biological reaches, an estimate of the number larvae and transformers is made for each respective reach. The sum of these estimates provides an estimate for the entire stream.

7. A final list of streams that will produce transformers is developed for all tributaries for which estimates of transformer production were estimated. Cost effectiveness of a lampricide application on each stream is calculated by dividing the individual stream treatment cost by the number of transformers the stream is predicted to produce. The result is a predicted cost/transformer killed for each stream. The final list of streams is ranked in ascending order based on the cost/transformer killed. This list is used to select streams for treatment.

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