



Edwards Aquifer Habitat Conservation Plan

Comal Springs Riffle Beetle Work Group Report



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Background: Development of the Comal Springs riffle beetle work group

The Comal Springs Riffle Beetle (*Heterelmis comalensis*) is a federally endangered species and among the eleven covered species managed under the Edwards Aquifer Habitat Conservation Plan (EAHCP). The Comal Springs riffle beetle (CSRB) is primarily encountered in the Comal springs system and is highly associated with springs and spring orifices. They are known to occur in areas of flowing water over gravel and cobble substrates. Several program areas within the EAHCP are committed to protecting the CSRB through monitoring, research, and refugia.

The EAHCP underwent a multi-year review by the National Academies of Sciences (NAS) which sought to determine whether the Conservation Measures prescribed in the EAHCP would achieve Biological Objectives which in turn would achieve species Long-Term Biological Goals (LTBGs). During review of the CSRB with NAS, it became apparent there were issues with the overall knowledge of the species and monitoring of the beetle that would make it difficult for NAS to determine whether LTBGs could be achieved. In the third and final consensus report from NAS, the committee was “*unable to determine*” whether the conservation measures surrounding the CSRB would be able to achieve the Biological Objectives for the beetle. It should be noted, the committee determined the Biological Objectives are “*somewhat likely*” to meet the LTBGs for the CSRB.

In response to the issues highlighted during the three-part review of the EAHCP by NAS and issues expressed by members of the EAHCP Science Committee and Stakeholder groups, a work group was formed in early 2018 to address concerns around a specific set of questions regarding CSRB activities contained within the EAHCP. The charge of the Comal Springs riffle beetle workgroup (CSRBWG) focused on three primary areas: 1) sampling methodology, 2) field activities (Biomonitoring, refugia collections and Applied Research), and 3) EAHCP LTBGs. The specific set of questions from the charge are shown below in Table 1, the full version of the CRBWG Charge can be found in Appendix A.

Table 1. Charge questions tasked to the CSRBWG.

Charge Topic	Topic Question
Cotton lure sampling methodology	Is the current cotton lure sampling methodology an appropriate means to monitor abundance at a locale?
	If not, what sampling methodologies exist that would provide a better proxy of abundance at a locale?
	If the previous two questions cannot be adequately answered without additional study, what would be an appropriate study to answer the questions?
Biological monitoring, Refugia collections, and Applied Research collections	What changes are recommended for the Biological monitoring sampling program? What are the stated goals behind those changes?
	What changes are recommended for Refugia removal efforts? What are the stated goals behind those changes?
	Are the current and proposed levels of physical activity in the CSRB habitat protective of the species? If not, what level of activity is appropriate?
Long-term biological goals	Are the current population and habitat LTBGs for the CSRB appropriate? What are the criteria for more appropriate goals?
	What is an appropriate means to monitor the habitat quality goal?
	How can Biological monitoring, Refugia efforts, and Applied Research studies be used to establish new LTBGs?

The CSRBWG was comprised of members from the EAHCP Science Committee as well as external experts: Butch Weckerly (Science Committee & Texas State University), Chad Norris (Science Committee & Texas Parks and Wildlife Department, Conrad Lamon (Science Committee), and Kenneth Ostrand (U.S. Fish and Wildlife Service). Additionally, Tom Arsuffi (Science Committee) participated in the work group. The CSRBWG was moderated by Chad Furl meeting six times over 2018-2019. Agendas, minutes, and presentations from each meeting are included in Appendix B.

Background: EAHCP Biological monitoring, refugia collections, and LTBGs for the CSR

Cotton lure methodology

The current capture technique for CSRBs was developed during the EAA variable flow study (present day EAHCP Biological monitoring program) and consists of placing a folded cotton cloth lure contained within a wire cage in a spring orifice for approximately thirty days. Over the course of thirty days, the lures become inoculated with local organic matter and invertebrates. Since 2004, several entities not associated with the Biological monitoring program have completed CSR collections. In 2016, a CSR standard operating procedure was developed through the EAHCP to provide consistencies in construction of cotton lures, their placement, and standardized metadata requested during placement and retrieval.

Biological monitoring procedures

CSR sampling conducted as part of the Biological monitoring program occurs during the Spring and Fall as well as during critical flow periods described in the EAHCP. For the routine biannual sampling, 10 cotton lures are placed into spring openings/upwellings in the Comal system at three reaches (30 lures total): Spring Run 3, Western Shoreline of Landa Lake, and Spring Island (Figure 1). After 30 days, the lures are retrieved and all invertebrates collected on the cotton lures are identified, counted, and returned to the spring of capture. Environmental variables (i.e., depth and current velocity) are measured at the time of lure placement and retrieval. Since 2016, additional environmental variables have been recorded, including spring type, substrate composition, and general water quality parameters (i.e., DO, temperature, specific conductance, TDS, and pH). Presence of biofilm, percent coverage, and color has also been recorded since 2016.

Refugia collection procedures

The Refugia program for the CSR requires that wild-caught adults are kept in captivity during all times to serve as standing stock. The program started collecting CSRBs in January 2017 to achieve the required standing stock numbers. USFWS utilizes the cotton lure method to collect CSR as described in the CSR SOP. Wooden dowel lures are also co-located with the cotton lures as a secondary attractant for the CSR. Cotton lures and wooden dowels are placed into springs at several locations: Spring Runs 1 – 3, Western Shoreline of Landa Lake, and Spring Island. Cotton lures and dowels are retrieved after 30 days and no more than 25% of CSR collected per lure are retained for refugia stock. The remaining 75% of the catch is returned to the spring orifice. Environmental variables (i.e., depth and current velocity) are measured at the

time of lure placement and retrieval including spring type, substrate composition, and general water quality parameters (i.e., DO, temperature, specific conductance, TDS, and pH). Individuals kept for the refugia stock are placed in quarantine for approximately 30 days before being incorporated into the refugia stock population. Census and mortality of the CSRБ refugia population is completed every other month.

Beginning in Fall 2018, the Refugia staff collected CSRБ from cotton lures set for the Biological monitoring program. The coordination between the Biological monitoring sampling and Refugia collections was developed to reduce the level of physical disturbance within the CSRБ habitat.



Figure 1. Comal Spring riffle beetle study reaches for EAHCP Biological monitoring program.

CSRБ Long-term Biological Goals

The CSRБ LTBGs include a population measurement and a qualitative habitat component that are shown in Table 2 (Table 4-7 of the EAHCP). Three representative reaches (i.e., Spring Run 3, Western Shoreline, and Spring Island, refer to Figure 1) were selected to monitor the CSRБ

for LTBGs. The Biological monitoring program is the means to assess whether median densities are maintained.

CSRB have been found highly associated with coarse substrates that remain silt-free. The qualitative habitat goal component states that $\geq 90\%$ of the LTBG reaches should contain silt-free gravel and cobble.

Table 2. EAHCP CSRB long-term biological goals (Table 4-7 of EAHCP).

COMAL SPRINGS RIFFLE BEETLE LONG-TERM BIOLOGICAL GOALS

	Spring Run 3	Western Shoreline	Spring Island Area
Habitat	Silt-free gravel and cobble substrate $\geq 90\%$ of each study area		
Density (# of CSRB/ Lure)	≥ 20	≥ 15	≥ 15

Charge question 1. Cotton Lure Sampling Methodology

There is a fundamental information gap that limits the interpretation of the cotton lure data. Currently, it's unclear whether the number of beetles on a lure is representative of the number of beetles in the immediate area available for sampling. Put another way, does the cotton lure data tell us anything about the number of beetles living in a specific area. NAS had a similar criticism in their review of the program citing a "lack of quantitative monitoring" for the beetle.

The initial charge question posed to the committee seeks to understand 1) whether the cotton lure is appropriate to determine abundance at a location, 2) whether there are better sampling alternatives available, and 3) what would be an appropriate study design to aid interpretation of the lure data.

The first two sub-questions in charge question number one were addressed simultaneously by the committee. The committee reviewed summary analyses on the cotton lure dataset provided by Dr. Furl. The data set consists of routine and critical period biological monitoring from 2004 – 2019 and refugia cotton lure collections from 2017 – Feb 2019.

Summary statistics were presented on the distribution of raw beetle counts at each location by adults and larva, time series plots by reach, and time series plots by individual orifices that had been determined to have been repeatedly sampled over the course of the biological monitoring program.

Additionally, the committee heard from Dr. Arsuffi on topics related to stream ecology and the hyporheic zone, disturbance and recolonization patterns, sampling methodologies of the CSR B and other riffle beetle species, and life history aspects of the CSR B.

Outcome of CSR BWG for Charge 1: Experimental Design: CSR B preference/Cotton lure efficiency study

The CSR BWG decided to move forward with the cotton lure sampling method at the present time. However, the need was recognized to create a better understanding of what the lure data indicates about CSR B occupancy and their population. A basic laboratory study was proposed to satisfy this need. The study design described below is the outcome of multiple discussions by the work group and is the recommendation for addressing Charge 1 of the CSR BWG. The proposed study is scheduled to occur in 2020. At the conclusion of the study, results will be presented to the EAHCP Science Committee and the annual meeting of the CSR BWG.

In order to conduct the experiment, 5 circular tanks approximately 24 inches in diameter will be plumbed such that water emanates from the bottom of the tank approximately 3 inches from the tank sides. This will be achieved by drilling out PVC (or other tubing material) and fashioning into a circle. Water will exit a standpipe located in the center of the circular tank in an attempt to distribute flow evenly across the tank. Flow circulating in the tank will be approximately 0.5 L/min.

The experiment will be housed in an area with low-light conditions. Tank material will consist of wood, leaves, limestone gravel, and a cotton lure. Well-conditioned woody material will be harvested from Landa Lake, split into 5 equal proportions, and kept in flowing well water until tank construction is complete. Leaves will be collected from the bank along western shoreline and conditioned in flowing well water for approximately 4-12 weeks. It is anticipated that Anaqua or Sycamore leaves will be used for the experiment. Both the woody material and leaves will be placed into a wire-frame cage like the cotton lure. This will be done to standardize the approximate amount of each material and allow for easier collection when the experiment is finished. Gravel in the tank will consist of store-bought limestone primarily in the size range of ~ 1.5 inch. Larger pieces of cobble (3-5 inches) will also be interspersed throughout the tank. The limestone will be thoroughly washed and conditioned for _ weeks prior to tank set up. The cotton lure placed in the tank will follow protocol described by BIO-WEST.

The depth of the gravel in the tank will be as thin as possible and still allow for covering up the drilled out piping in the bottom of the tanks and allow for burial of the 3 wire cages on top of the

piping. Once the hydraulics are functioning correctly and the gravel is in place, the woody debris and leaf packets will be gently buried on the perimeter of the tanks just inside of the drilled-out piping. These two packets will be left in place for approximately one week before gently burring the cotton lure packet. To avoid potential toxicity issues, the cotton lure will be thoroughly flushed with Deionized water before placing into the tank. When all three packets are in place, they will be located equidistant from each other. Once all three packets are in place, 20 adult beetles will be randomly selected and added near the center of the tank with a baster or other piping device. Beetles will be held for a period of 3-7 days at the research facility prior to being placed in a tank.

The cotton lure will be checked at 10, 20, and 30 day intervals, and the number of beetles in the lure will be recorded. An attempt will be made to check the lure with as little disturbance as possible and return to the microcosm as quickly as possible. Standard water quality parameters will be monitored throughout the 30 day study period. On day 30, beetles will be enumerated in the cotton lure, leaf packets, woody debris packets, and substrate within the tank. The sex of the beetles will be determined at this point and the number of individuals that did not survive the experiment will be noted. Notes will be taken on the condition of the packets within the tanks.

Charge 2: Biological monitoring, Refugia collections, and Applied Research collections

Biological monitoring

The first objective of Charge 2 of the CSRBWG was to examine the current Biological monitoring program and develop any changes recommended for the sampling program. The goals of the EAHCP biological monitoring program are two-fold: 1) monitor changes to habitat availability and the population abundance of the covered species and 2) provide information to assess whether the conservation measures are achieving the biological goals and objectives. Specific topics presented to the CSRBWG for consideration included:

- Maintaining repeated sampling of individual orifices at the three LTBG reaches.
- Randomizing spring orifice selection.
- Expanding outside of the historical LTBG sampling areas.
- Adjusting the number of lures set for each survey.
- Adjusting the frequency of surveys.

Outcome of CSRBWG for Charge 2: Biological monitoring program

The CSRBWG ultimately decided to leave the current Biological monitoring program in place. The program will continue to sample 10 orifices at each of the three historical LTBG locations during Spring and Fall. No changes were made to the current sampling frequency and number of cotton lures set for the biological monitoring program.

The CSRBWG agreed that additional sampling should occur beyond the LTBG reaches in order to better understand system-wide abundance and near-surface populations. Currently (2019), an occupancy and abundance study is underway through a Texas State University master's student in Dr. Weston Nowlin's lab (not funded by EAHCP). The study methodology includes using the cotton lure approach for four repeated sampling events of 85 orifices that were selected randomly. This study will provide occupancy and population estimates and serve as the basis for additional system-wide studies.

The CSRBWG recommends two additional occupancy and population studies be conducted through the EAHCP program before the end of the current permit. It is anticipated these studies will be conducted in 2022 and 2025. The EAA anticipates contracting for study design after results from the Texas State University occupancy and site abundance study are made available.

Refugia collections

The Refugia program is required to maintain wild individuals for standing, refugia, and salvage stock populations. Since the inception of the Refugia program in 2017, maintaining a standing stock of 500 adults has proven difficult. To date, approximately 100-300 adult beetles have been maintained in Refugia standing stocks (as of Sept 2019). Unknown ages of the beetles during collections and the relatively short life span are contributing factors to the low survivability rates in captivity (~ 50% survival rate at 4-5 months). On-going research through the Refugia program is being conducted to improve CSRB husbandry and propagation techniques that will hopefully lead to increased CSRB survival rates in captivity.

Based on the high number of beetles collected to date, low survival rates in captivity, and active research on the topic the committee considered the following topics:

- Appropriate standing stock size.
- Refugia removal locations and total amount of beetles removed from system each year.
- System-wide and individual orifice disturbance from Refugia removals.
- Overlap and effect upon Biological Monitoring program.

- The percentage of beetles from a cotton lure that should be kept or returned to an orifice during collection events.

Outcome of CSRBWG for Charge 2: Refugia collections

The CSRBWG decided to adjust the standing stock number to 150 CSRBs, holding 75 at each of the Refugia facilities. To achieve this average standing stock number, it is estimated that 300-400 CSRBs will need to be collected annually. The CSRBWG will revisit standing stock numbers annually as population and husbandry studies become available and Refugia staff refines the level of effort needed to achieve this standing stock number.

Presently, the Refugia standard operating procedures state that only 25% of CSRB found on one lure will be retained while returning the other 75% to the site of collection. With the uncertainty on the survival rates of the 75% of individuals returned after lure collection and to further reduce disturbance, the CSRBWG approved the 100% collection of CSRB captured on a cotton lure. It is anticipated this will reduce overall system disturbance and lessen the level of effort. The increased collection percent was suggested with additional guidelines: alternating the use of spring orifices between sampling events, and all Refugia collections will occur separate from the Biological monitoring program (no longer collect beetles from the Biological monitoring lures). The decision to separate Refugia and Biological monitoring collection events was based on preventing any potential influence that 100% take might have on monitoring collection sites.

Charge 3: CSRB Long-term biological goals

The established EAHCP LTBGs for the CSRB are strongly tied to the cotton lure collection methodology (Table 2). Section 4.1.1.1 of the EAHCP states “The population measurement is to maintain greater than or equal to the median densities observed over the past six years of the EAA Variable Flow Study monitoring.” The Biological monitoring program samples twice a year to evaluate if median densities goals are being met.

The CSRBWG was charged with determining if the population LTBG for the CSRB was appropriate. In their discussions, the work group members agreed that changes should be made to LTBG population goals, but the absence of CSRB population and abundance data prevent the development of new ones. Their recommendation was to revisit the LTBGs after CSRB population studies have been completed. A comparison analysis can then be performed between the population survey and biological monitoring data to evaluate the appropriateness of the CSRB population LTBGs.

The other component of the LTBGs for the CSRБ relates to maintaining adequate habitat for the species. The LTBG habitat stated in the EAHCP for the CSRБ is to maintain silt-free gravel and cobble in $\geq 90\%$ of each study area (Spring Run 3, Western Shoreline, and Spring Island).

Discussion by the workgroup members on the CSRБ habitat LTBG resulted in no alternations to the current goal.

Outcome of CSRБWG for Charge 3: Long-term biological goals

The consensus of the work group members was to postpone any changes to the LTBGs until more data becomes available with the completion of the CSRБ system wide population and abundance study. At this time, the work group did not feel they had sufficient information available to evaluate the current population goal or develop a more appropriate population goal.

Conclusions and Work Group Recommendations

This report outlines the discussions by the CSRБ work group members and other interested members of the community that resulted in recommendations to improve CSRБ sampling techniques associated with the Biological monitoring and Refugia programs of the EAHCP. The work group provided several adjustments to current sampling efforts of the EAHCP programs. To summarize, the recommendations include the following:

- 1) An in-situ experiment will be conducted to assess the sampling efficiency of the cotton lure.
- 2) The Biological Monitoring program will continue using the cotton lure method to monitor CSRБ at the long-term biological goal reaches twice a year but will add two system wide population surveys before 2028.
- 3) The Refugia program will reduce standing stock numbers to 150 individuals (75 per refugia facility).
- 4) Refugia collections will retain 100% of CSRБ captured on a cotton lure; however, sampling collection locations must be alternated between collection events and occur separate from Biological monitoring events.
- 5) An annual meeting will be held to discuss CSRБ topics



***COMAL SPRINGS RIFFLE BEETLE WORK GROUP
REPORT – Appendix A Work Group Charge***

Charge of the Comal Springs Riffle Beetle (CSRB) Work Group

Overview

As part of regular execution of the Edwards Aquifer Habitat Conservation Plan (EAHCP), multiple activities require physical sampling or removal of the CSRB in its habitat. A Work Group is being formed to provide input on a specific set of questions concerning management of the CSRB as part of implementation of the EAHCP.

Background

The EAHCP mandates Applied Research, Biological monitoring, and Refugia programs; all of which require in situ sampling or removal of the CSRB from the Comal system (cite). The Biological Monitoring program (Biomonitoring) has sampled the CSRB at least twice annually at three locations since 2004. The Applied Research program has required some removal of the CSRB since 2013 to conduct ex situ experiments. The Refugia program has required regular removal of the beetle since 2016.

Historically, the CSRB have been captured (for sampling or removal) using a passive cotton lure methodology. Results from cotton lure samples as part of Biomonitoring are used in part to examine the CSRB LTBGs provided in the HCP (cite). These LTBGs are written as number of CSRB per lure at three Comal locations, and to obtain silt-free gravel and cobble substrate (90%) at the locations. During the review of the EAHCP, the National Academies of Science expressed concern over the use of the cotton lure approach for monitoring the beetle. Additionally, members of the EAHCP Adaptive Management Science Committee have raised concern over the appropriateness of the cotton lure methodology and CSRB LTBGs.

Creation

The HCP Program Manager and the Science Committee jointly determined to create a Comal Springs Riffle Beetle Work Group comprised of members from the Science Committee as well as external experts to examine questions regarding the EAHCP handling of the CSRB.

Charge

The Work Group's charge consists of examining questions related to three primary areas 1). sampling methodology, 2). field activities, and 3). EAHCP LTBGs.

1. Cotton lure sampling methodology

Is the current cotton lure sampling methodology an appropriate means to monitor abundance at a locale?

If not, what sampling methodologies exist that would provide a better proxy of abundance at a locale?

If the previous two questions cannot be adequately answered without additional study, what would be an appropriate study to answer the questions?

2. Biological monitoring, Refugia collections, and Applied Research collections

What changes are recommended for the Biological monitoring sampling program? What are the stated goals behind those changes?

What changes are recommended for Refugia removal efforts? What are the stated goals behind those changes?

Are the current and proposed levels of physical activity in the CSRB habitat protective of the species? If not, what level of activity is appropriate?

3. Long-term biological goals

Are the current population and habitat LTBGs for the CSRB appropriate? What are the criteria for more appropriate goals?

What is an appropriate means to monitor the habitat quality goal?

How can Biological monitoring, Refugia efforts, and Applied Research studies be used to establish new LTBGs?

Administration

The Work Group will meet on an as needed basis. The recommendations of the Work Group will be reported in the form of a written report and communicated to the full Science Committee. The Work Group will consist of the following members:

- Conrad Lamon (SC)
- Chad Norris (SC & TPWD)
- Floyd Weckerly (SC & TXSTATE)
- Ken Ostrand (USFWS)
- Eric Benbow (Michigan State University)*

*Dr. Benbow will begin serving on the Work Group following the conclusion of the NAS *Report 3*.



***COMAL SPRINGS RIFFLE BEETLE WORK GROUP
REPORT – Appendix B Agendas and Meeting
Minutes***



NOTICE OF OPEN MEETING
Available at eahcp.org

As approved by the Edwards Aquifer Habitat Conservation Plan (EAHCP) Science Committee, the Comal Springs riffle beetle (CSRB) Work Group has been formed to provide input on a specific set of questions concerning management of the CSRB as part of implementation of the EAHCP. A meeting of this Work Group for the EAHCP is scheduled for **Thursday, May 24, 2018, at 9 a.m. at the City of San Marcos Activity Center – Multipurpose Room, 501 E. Hopkins, San Marcos, Texas 78666**. Lunch will be provided. Please RSVP to spayne@edwardsaquifer.org

1. Call to order--Establish that all Work Group members are present - 9:00 am.
2. Public Comment.
3. Review of the CSRB Work Group Charge:
 - i. Cotton lure sampling methodology
 - ii. Biological monitoring, Refugia collections, and Applied Research collections
 - iii. Long-term biological goals
4. Presentation and discussion proposed CSRB Work Group meeting schedule.
5. Presentation of background regarding the current cotton lure sampling methodology and discussion on the major areas of literature review focus.
6. Presentation and discussion on the information needs pertaining to Refugia collections and Biological monitoring.
7. Questions from the public.
8. Adjourn.



CSRB WORK GROUP MEETING MINUTES
May 24, 2018

1. **Call to order--Establish that all Work Group members are present - 9:00 am.**

Chad Furl began by introducing the Work Group members and communicating Dr. Arsuffi will be acting as an aid to the literature review process and not an active member on the work group. Work Group members present included Ken Ostrand, Chad Norris, Butch Weckerly, and Conrad Lamon.
2. **Public Comment.**

There was no comment from the public.
3. **Introductions and communication of individual roles and Work Group process.**

Dr. Furl provided the work group members a summary of the process, and presented work group operating rules.

Dr. Arsuffi commented that “data” driven may not be solely the appropriate means of making decisions. Rather, it would be important to include other scientific means to make effective decisions that is determined by the literature.
4. **Review of the CSRB Work Group Charge as approved by the Science Committee on May 9th, 2018.**

Dr. Furl provided a list of past and present efforts put forth to inform the EAHCP staff and committees about the CSRB.

The first charge of the work group consists of analysis of whether the cotton lure sampling methodology is an appropriate means of sampling abundance at a specific location in the systems.

The second charge of the work group consists of discussion about the collections done for all EAHCP programs (Biological Monitoring, Refugia, and Applied Research), and how to better coordinate these collections in order to be more protective of the current CSRB population.

The third charge of the work group consists of how appropriate the current long-term biological goals, established in the EAHCP, are.
5. **Presentation and discussion proposed CSRB Work Group meeting schedule.**

Dr. Furl quickly discussed the proposed schedule of the work group and how the work done over the next several months will provide direction in final recommendations to improve activity surrounding the CSRB in the EAHCP process.

6. Presentation of background regarding the current cotton lure sampling methodology and discussion on the major areas of literature review focus.

Dr. Furl provided a comprehensive summary of the EAHCP requirements in regard to the CSRB. There was discussion about the median numbers, that are intended to be maintained, where they come from, and the rationale regarding the set values.

Additionally, Dr. Weckerly asked about how sample locations are identified (ie. GPS). Chad Norris mentioned that establishing an accurate GPS location at the springs is difficult. Mr. Norris has been part of an effort to identify and tag major upwellings where CSRB have been sampled overtime. This effort was undertaken in order to provide more accurate determination of lure location.

Conrad Lamon discussed the importance of establishing what the information coming from the lure is telling us (ie. Density, abundance, population, distribution, etc). There was a discussion about distance between lures. Dr. Arsuffi communicated how knowing location of the lures, and their distance from other lures, helps understand if certain springs are “oversampled” over any period of time.

Dr. Furl communicated the difference in goal between the biological monitoring and refugia programs. Monitoring focuses on developing consistent running numbers from a specific location across time, and the refugia program looks to collect as many beetles as possible with each collection event.

Dr. Arsuffi asked the rationale for using median instead of mean. Dr. Weckerly responded that it would make sense to use median due to the relatively small sample size. Dr. Lamon mentioned median provides a more statistically useful figure to determine probability of collection. Mr. Norris also mentioned a possible reason could have been because the median was a slightly lower figure than the mean and could have been chosen to provide a more conservative figure.

Dr. Furl presented a series of analyses done with the current CSRB data. He stated to the work group that much of these effort is designed to look at the data in a variety of ways and determine if this type of analysis is appropriate given our current collection methodology and whether there needs to be a change in that collection or a change in how the data is analyzed.

Dr. Furl mentioned that the data collected thus far is not appropriate to run analyses. Dr. Weckerly suggested using estimation methods to account for imperfect detection due to these methodologies. He mentioned there are tools that can be used if a specific location is determined accurately. Dr. Furl mentioned one goal would be to be able to determine trends in the data over time. Dr. Lamon described that knowing the physical location of the lure for knowing distance between other sampling locations and distance from other physical features would be useful.

Dr. Lamon proposed the question of whether keeping track of locations used would be a difficult effort. Dr. Furl communicated much of that information should be able to be collected

accurately this fall. Dr. Ostrand mentioned that location should not be difficult to record and that variability in the collection data is what is the issue at hand. Dr. Weckerly asked what other options are out there in order to make less variable data. Dr. Arsuffi mentioned that variability is the name of the game in this type of work.

Dr. Furl presented feedback from NAS regarding the difficulty of sampling for organisms like the CSRB and possible alternatives as well as the issues that could be produced out of an inappropriate methodology to estimate population. There was discussion of the variety of methods attempted and how the lure has been the most effective. Dr. Arsuffi mentioned there was not a stream ecologist on the NAS committee when the previous two reports were produced. He identified there is now an ecologist who may be able to provide a different opinion. Dr. Ostrand commented that any organism that receives a population estimate, there is a huge amount of variation. Dr. Lamon commented that the current methodology provides a fairly reasonable detection probability and another variable of analysis can be useful (ie. Accurate location of lure). He stated that the first step is to quantify what we have now from the data. Dr. Arsuffi proposed the question whether or not a lure is appropriate. Dr. Lamon stated that he would not be the one to determine the appropriateness of a method but give one method of collection there is a way to make the data useful.

Dr. Furl proposed the question: What are some of the problems with the cotton lure sampling methodology?

Dr. Arsuffi commented that aquatic fungi is the primary driver in colonization of the cotton lure. Mr. Norris communicated that the period of time found best for collection rates were examined. A four-week colonization time was found to be the most effective.

Dr. Furl asked the work group what their specific issues are regarding the current sampling methodology?

- Dr. Arsuffi mentioned that a collection method that was “non-lure” in order to estimate abundance. The issue with a lure is that it begs the question of whether the organisms that our found on the lure actually are found at that location or have traveled to the food source. Dr. Furl mentioned that the heterogeneity of the system is a major limiting factor in choosing a location specific methodology. He stated there would be variability in any method.
- Dr. Ostrand described a variety of methods that have been attempted.
- Dr. Arsuffi mentioned collecting a list of attempted methods in order to perform a literature review and construct a list of pros and cons.
- Dr. Lamon commented that whether or not we change the collection methods we should analyze the current data to get a better idea of what we have.
- Mr. Norris mentioned Joe Fry was an individual who has tried a variety of different methods of collection.
- Nathan Pence commented that looking into what other agencies in the country/world on collection of riffle beetles located elsewhere. Dr. Arsuffi commented that there is an abundance of sampling techniques out there to look at.
- Arsuffi proposed a question on whether the CSRB is specifically a hyporheic organisms.

- Dr. Ostrand mentioned possibly looking at genetics as a driving factor in sampling. Dr. Weckerly agreed that exploring genetic techniques could be a viable option.
- Dr. Ostrand mentioned sampling methodologies cannot be too intrusive.
- What are some of the recolonization studies done comparing various methods. This could be helpful in knowing the disturbance of various methods, as well as our current method.
- Dr. Weckerly commented that if you had special location data for each lure, and relative location to physical characteristics, you would be able to run an analysis on possible influences. Dr. Furl was tasked to look at sampling data from continual locations over time and measuring the space between the lures. If you have two nearby springs and are the same quality of habitat, and there are differences in the numbers you will be able to determine how far apart you should separate the lures in order to not influence the other lure.
- Dr. Ostrand commented that there is a section of the system that can be used for experimentation and not disturb current sampling locations.
- Dr. Arsuffi asked to look at the data regarding number of lures set in a location in a given month and compare it to the number of lures set in a period of time (1,2, or 3 samples in a given month compared to 4,5, or 6 in more intense sampling periods).
- Dr. Weckerly commented that flow could also be a helpful variable in understanding the data.

Mr. Norris discussed what the real purpose is of estimating population is and if the Biomonitoring program is intended to pursue those questions. He described the sampling currently compares data from locations in order to find trends from over time and not necessarily to know the population in the species.

The work group took an informal break and continued to discuss some of the possibilities of the current methods and any possible alternatives. The work group discussed presence/absence as a use of the data. There was a brief discussion regarding long-term biological goals. Mr. Norris commented that the spring runs are expected to go dry in a repeat of the drought of record (DOR) and how do the data that are being collected in the spring runs for CSRБ matter.

- There was mention of reviewing Lucas et al. for an examination of genetic distribution from the DOR.
- Dr. Lamon mentioned it may be important to analyze larvae and adult data separately.
- Dr. Arsuffi asked the question on how long the CSRБ can live outside of water. Mr. Norris mentioned there has been an informal examination of that but nothing official.
- Dr. Lamon commented that the usefulness of the current methodology may not be the right question right now without putting effort into a real analysis of the current data. For example, he discussed the use of non-detects or zero numbers in data being used in analysis rather than ignoring those figures.

7. Presentation and discussion on the information needs pertaining to two critical aspects of CSRБ activity.

Dr. Furl began discussing the primary issues regarding the biological monitoring and refugia programs. These issues include: returning beetles, CSRБ cage setup, water quality measures,

discharge measurements, sample location identification, sample a portion of range, no habitat quality component, data interpretation, cotton lure methodology. Topics discussed included:

- Issue of overlap of sampling, recolonization issues
- Need for coordination with other sampling efforts (inside and outside the EAHCP)
- Two studies are necessary for the lure; effectiveness, and beetle mortality
- Mr. Norris communicated that the handling issues regarding mortality would be more of an issue for refugia. If there were issues about handling and returning beetles to the system you would see that issue more when removing beetles for refugia. Dr. Furl commented that if coordination was increased we could minimize demand on the species if monitoring lures and refugia lures can be used interchangeably.
- There is a need to avoid sampling the same springs repeatedly in both programs.
- Mr. Norris says there may be data with water depth at specific spring locations.
- We want to be sure to sample the same spring location across time will be the best way to sample. If our goal is to identify where the beetles are in the system, a more random distribution of lures can be helpful. Mr. Norris and Dr. Weckerly communicated that it depends upon our goals.
- Before we go to data interpretation, we need to know what questions we are asking that are appropriate for data interpretation.

Dr. Furl asked about the “maintain silt-free environment component.” There was a discussion about optimal habitat/suitable habitat and using it to extrapolate out much like the SAV for fountain darter densities.

- If you’re after abundance than you will need a methodology with constraints (N mixture model – repeated sampling). Random selection of springs (even when habitat is not suitable and beetles will unlikely be collected) would be helpful for distribution.
- A model would help keep all variables or springs characteristics in order to make assumptions of suitable habitat.
- Presence/absence sampling can be used to assess distribution, environmental correlates. Does not tell you anything about abundance. Occupancy estimates require repeated samples for each spring in able to be used. Mark and recapture is the best method, but reality causes this to not be possible, or effective. N-mixture model can be used when mark and recapture cannot be used.

Dr. Furl will pull together all the current location data with characteristics as well as repeated sampling locations. He introduced the second topic of system disturbance with the work group.

- Dr. Weckerly mentioned that harm to the species seems unlikely considering refugia transport success.
- Dr. Arsuffi commented that the level of disturbance as a possible variable affecting the collection rate. There was a concern with the level of refugia collection and removal and the recent decline of beetles found through the monitoring program.
- There is a possibility that life history and/or genetic cohort distribution could affect sampling rate.
- We could look at intensity of sampling effort versus what was happening in the system.

- We need to look at who sampled what, where and when.
- Reducing the unaccounted-for variability is necessary to find a trend in the data.

Dr. Furl proposed the last topic to be discussed; whether the current long-term biological goals are appropriate, and do they need to be changed and what would be the criteria (not what will those goals be).

- Mr. Norris identified that median numbers as a goal separated by reaches does not seem to be the best method of establishing a metric.
- Dr. Arsuffi asked whether the number on the lure is representative of something that means anything.
- Dr. Weckerly proposed a variety of statistical methods that provide an analysis that can fit the current sampling methodology.
- LTBG seem to be constructed out of the data that had been collected (should be the other way around).

The work group had a brief discussion with Tanya Sommer (USFWS) about this issue in relation to other HCPs along presence/absence versus population studies.

8. Questions from the public.
No comments from the public

9. Adjourn.



NOTICE OF OPEN MEETING
Available at eahcp.org

As approved by the Edwards Aquifer Habitat Conservation Plan (EAHCP) Science Committee, the Comal Springs riffle beetle (CSRB) Work Group has been formed to provide input on a specific set of questions concerning management of the CSRB as part of implementation of the EAHCP. A meeting of this Work Group for the EAHCP is scheduled for **Tuesday, July 2, 2019, at 9 a.m. at the City of San Marcos Activity Center – Multipurpose Room, 501 E. Hopkins, San Marcos, Texas 78666.** Lunch will be provided. Please RSVP to kkollaus@edwardsaquifer.org

1. Call to order--Establish that all Work Group members are present - 9:00 am.
2. Public Comment.
3. Review of the CSRB Work Group Charge:
 - i. Cotton lure sampling methodology
 - ii. Biological monitoring, Refugia collections, and Applied Research collections
 - iii. Long-term biological goals
4. Presentation and discussion of CSRB literature review and data analyses.
5. Questions from the public.
6. Adjourn.



Comal Springs riffle beetle Work Group
07/02/2019 Meeting Minutes
Available at eahcp.org

Members of this committee include: Conrad Lamon, Chad Norris, Butch Weckerly, Ken Ostrand, and Eric Benbow

1. Call to order.

All members were present except for Ken Ostrand and Eric Benbow.

2. Public comment.

There were no comments from the public.

3. Review of the Comal Springs riffle beetle (CSRB) Work Group Charge

- a.) CSRB Sampling Methodology: currently use cotton lures in spring orifices. The group will consider alternatives to the current sampling methodology and develop potential research projects that may inform and improve the sampling methodology.*
- b.) Biological Monitoring, Refugia and Applied Research collections: assess and develop recommendations to improve how and why we collect CSRB. Are we oversampling the CSRB?*
- c.) Long-term Biological Goals: how do we evaluate our long-term biological goals? NAS was unable to determine, how do we improve this or establish new long-term biological goals?*

4. Presentation and discussion of the CSRB literature review and data analysis.

Dr. Tom Arsuffi presented an overview of his CSRB literature review (the presentation is available on the CSRB Work Group website). His research focused on stream ecology related to the hyporheic zone, disturbance and recolonization patterns, sampling methodologies of the CSRB and other riffle beetle species, and life history aspects of the CSRB. A summary on the topics discussed during the presentation are listed below.

Inductive and Deductive Enhancement

- 1.) Stream ecology and the hyporheic zone: Consider CSRB and Comal Springs at a broader-scale to inform our understanding of the empirical data and theories (Boulton et.al., 2010).*
- 2.) Life-history research on other benthic macroinvertebrates: Used to inform how life-history information is applied through a species-trait approach (Resh and Rosenberg, 2010)*

3.) **Patch Dynamics:** *How spatial patterns are created and are linked to the ecology of a species (Winemiller et. al., 2010). Why do we see different CSRFB densities between spring orifices? Example: Is there a link between the riparian vegetation and CSRFB densities (tree roots=food?).*

a.) *Landscape ecology perspective: how spatial patterns are created and effect ecological processes over different spatial and temporal scales.*

b.) *Metacommunity: The influence of periodic disturbances, refugia, and dispersal in maintaining nonequilibrium communities in patch mosaics. (Findlay, 2010)*

4.) **Disturbance:** *The role of disturbance and recolonization in stream ecology (Resh et. al., 1988; Stanley et. al., 2010). How do we define disturbance and apply it to the CSRFB? Are we looking at CSRFB disturbance at the right spatial and temporal scales?*

**direct and indirect disturbances: flood, drought, habitat characteristics, and resource availability.*

**additional disturbances may include aquatic recreation, ducks and vultures, and even sampling. Sediment deposition from flooding and overland runoff could affect their respiration rates.*

**Dr. Lamon asked: Do we know if there is a lower count after a disturbance? Need to consider population estimates and identify trends within the data we currently have*

**Need to account for all disturbances (even time between sampling by all/different entities) when assessing the number of CSRFB on lures.*

Currently, sampling can be triggered by extreme drought and flood conditions to assess their numbers; however, sampling may exacerbate the disturbance from drought or flood. Members discussed postponing the sampling after an event to let the species recover; but, for how long? We need to document and consider the disturbances when sampling and improve our understanding of their resilience and recovery rates (life history studies).

5.) **Resistance, resilience, and recolonization**

**recolonization patterns: could establish in new areas (logs, adjacent springs, etc.). Need to combine data from other entities to assess spatial variation.*

**pathways: aerial, hyporheic, upstream migration, and downstream drift*

**what is the recovery time, how do we analyze that? Consider their fecundity and duration of life stages.*

Members discussed adding more variables to the data collected during sampling events to help ascertain habitat conditions that influence the population and potentially develop population models to analyze and predict densities. CSRFB population estimates would be ideal for analyzing observed trends, but the subterranean hyporheic zone is complex. Members then discussed what we do know about the CSRFB –

CSRFB life history

The CSRFB live in the hyporheic zone, the conduits, spring orifices, and on logs and woody debris near springs. More info on slide 17 and 18. CSRFB are K-strategist, which means they have slow

growth rates and low fecundity (“elephant” inverts), most of their biomass (females) is dedicated to egg production. As a K-strategist, they recover slowly from disturbances.

CSRB and other riffle beetle sampling methodologies

**Bore hole samples help assess the hyporheic zone, but they disturb the habitat significantly more than the cotton lure.*

**Drift nets have been used in the past, but the cotton lures have proved more effective at capturing CSRB*

**Cotton strip is used in other systems. Some argued that the lack of folds (cotton lure is folded square) causes the strip to turn anoxic. Members discussed changing the duration from 30 days to 3 days to avoid the strip turning anoxic and reducing the travel time to assess those that are close to the strip.*

**Currently sample the same three reaches (i.e., Spring Run 3, Westernshore at Landa Lake, and Spring Island) each sampling event (typically Spring and Fall). Members suggested collecting more information during sampling events and potentially adding new sampling areas.*

**How far do they travel to the lure? Anecdotal evidence suggests that CSRB can travel 3 meters within 30 days and can be found up to 1 meter from a spring.*

**What attracts them to the cotton lure? Does the conditioned lure, “lure” them or are they just stopping by, or are they just lost? US Fish and Wildlife refugia staff are working on a food preference study and Dr. Nowlin’s lab (Texas State) has analyzed the gut content of the CSRB. Dr. Kosnicki (Biowest) has found certain logs have a higher density of CSRB, these may offer some insight about their food preferences: shredder vs. scrapper.*

Dr. Weckerly emphasized the need for a hierarchical framework to tease-out the abundance predictions vs. estimates which Dr. Nowlin’s research findings may provide.

We must be careful about making assumptions about the CSRB population based on the lure counts. Statistics would be difficult because we don’t have dependent variables.

The group collectively agreed that the current methodology works and consistency is important, but there are still many unknown factors.

Questions:

**What portion of the population within the sampled spring orifice/area is attracted to the lure? Is it 90% or just a small portion of the population?*

**Why are the CSRB attracted to the lure? Is the microbial content on the conditioned lure and how does it compare to other food sources?*

**What does the number of CSRB captured on the lure represent?*

**How long does it take the CSRB to recover after a sampling event?*

**Should we sample in other spring orifices?*

Recommendations (pilot studies):

- 1.) Test cotton strip sampling method used by other riffle beetle researchers.*
- 2.) Determine microbial content of the conditioned cotton lures*
- 3.) Reduce sampling time from 30 days to 2-4 days*
- 4.) Evaluate the effectiveness of the lure in a controlled environment*

5. Questions from the public.

The public offered comments and questions during the discussion which were incorporated above.

6. Adjourn.

Meeting adjourned around noon.



NOTICE OF OPEN MEETING
Available at eahcp.org

As approved by the Edwards Aquifer Habitat Conservation Plan (EAHCP) Science Committee, the Comal Springs Riffle Beetle (CSRB) Work Group has been formed to provide input on a specific set of questions concerning management of the CSRB as part of implementation of the EAHCP. A meeting of this Work Group for the EAHCP is scheduled for **September 4, 2019, at 9:00 a.m. at the San Marcos Rec Hall (City Park), 170 Charles Austin Drive, San Marcos, Texas 78666**. Lunch will be provided. Please RSVP to kkollaus@edwardsaquifer.org.

1. Call to order--Establish that all Work Group members are present - 9:00 am.
2. Public Comment.
3. Approve minutes from July 2, 2019 Work Group meeting (Attachment 1).
4. Review of the CSRB Work Group Meeting 2 held on July 2, 2019 to discuss Charge 1: Cotton lure sampling methodology.
 - i. Presentation on proposed research project to address the question: What is the proportion of CSRB beetles found on the cotton lure compared to surrounding environment?
5. Discussion on CSRB Work Group Charge 2: Biological monitoring, Refugia collections and Applied Research collections associated with the CSRB.
6. Questions from the public.
7. Adjourn.



**Comal Springs Riffle Beetle Work Group
Meeting Minutes
Revised
San Marcos Rec Hall
September 4, 2019**

Members of this committee included: Conrad Lamon, Chad Norris, Tom Arsuffi, Butch Weckerly, and Ken Ostrand

Audience: Amelia Hunter, Lindsay Campbell, Ely Kosnicki, Ashley Jackson, Mark Enders, Phillip Quast, Rachel Sanborn, and Brandon Payne.

- 1. Call to Order:** 9:00 am – All members of the work group were present.
- 2. Public Comment:**
There were no comments from the public.
- 3. Approve minutes from July 2, 2019 Work Group meeting.**
The Work Group approved the meeting minutes from July 2, 2019. There were no objections.
- 4. Review of the CSRB Work Group Meeting 2 held on July 2, 2019 to discuss Charge 1: Cotton lure sampling methodology.**

Dr. Chad Furl discussed what was presented at the prior work group meeting and recapped the lessons learned. As a result of the last meeting, EAHCP staff developed a proposed research project aimed at understanding the efficiency of the cotton lure for sampling riffle beetles. The general concept of the proposed research project is to recreate cotton lure sampling in a controlled laboratory setting. Dr. Chad Furl presented the details of the proposed project, informing the Work Group of the ideas surrounding tank construction, materials in the tank and specific intervals to count the number of beetles on the lure.

Dr. Tom Arsuffi had concerns of replication with regards to the distances of the cotton lure and the position of the riffle beetle to other treatments (i.e. leaves, woody debris, etc.). Dr. Conrad Lamon expressed concerns of the utility of a laboratory sampling to help understand the natural environment. Dr. Ken Ostrand suggested simplifying the project by decreasing the number of treatments available to the riffle beetle in a lab setting. Dr. Butch Weckerly supported the proposed project and the overall evaluation of the cotton lure's efficiency.

Treatment Types: Amelia Hunter suggested using one leaf type, one wood type and the cotton lure. Dr. Arsuffi noted the issue on decomposition rates of leaves and recommended prefacing the experiment with three types of substrates. Dr. Kosnicki and Ms. Hunter recommended using Sycamore leaves.

Ms. Hunter questioned the method of observing the riffle beetle and the possibility of disturbance (looking vs grabbing). Dr. Arsuffi recommended that the surface area of the substrate should be taken into consideration.

Substrate: Dr. Furl noted that the project would include wood, gravel and leaves in the tank and possibly buried 4 inches deep into the substrate. Dr. Campbell suggested that 4 inches is too deep and recommended to reduce the thickness just below the surface. Ms. Hunter noted that riffle beetles do not typically use rocks but rather gravel. The Work Group recommended using store bought gravel and condition it prior to experiment.

Woody Debris: Ms. Hunter recommended using conditioned balsam wood and offered to provide some that she has already prepared. Chad Norris had concerns using something that hasn't been historically used and is not found in the field. Additionally, Mr. Norris commented that there are too many unknowns using woody debris, however, if there is a choice, natural wood would be the best option. Dr. Kosnicki noted that popular debris takes about three months to condition where as balsam may take only a month to condition. Dr. Campbell suggested using harvest natural log cut into segments.

Leaf Type: Ms. Hunter noted that there has not been an experiment to determine which types of leaves riffle beetles prefer. Sycamore, anacua, and pecan leaves are most common near riffle beetle habitat. Dr. Kosnicki recommended using a single leaf type, conditioned, and in a cage. Dr. Arsuffi recommended keeping the leaf types separate.

Number of Beetles: The work group agreed that 20 beetles was sufficient. Dr. Campbell suggested a 50:50 sex ratio.

Replication and frequency: The work group recommended sampling five tanks at one time at 10/20/30 day intervals. Dr. Arsuffi recommended introducing the beetles at equidistant locations from a treatment type.

Tank recommendation: The work group suggested using a 10-gallon round tank.

Dr. Ostrand questioned if gravel was necessary and if not, could it be replaced with tile for easier observation. Dr. Kosnicki noted that substrate is used for mobility. Tile would work for this experiment, plastic mesh is not a good substrate, and gravel could take up too much space. Dr. Campbell had concerns with tile being too different from the field.

Proposed measurements at the conclusion of experiment: Dr. Arsuffi suggested research into the microbial biomass associated with substrates. The work group discussed the color change of the cotton lure; however, it was noted that observations can be subjective.

5. CSRB Work Group Charge 2: Biological monitoring, Refugia collections and Applied Research collections associated with the CSRB.

Dr. Furl reminded the work group of the goals of the biological monitoring program.

- "...will provide a means of monitoring changes to habitat availability and the population abundance of the Covered Species..."

- “...will provide information to effectively determine whether the conservation measures are achieving the biological goals and objectives...”

In regard to sampling locations, the work group agreed that sampling should occur beyond the reaches that are typically measured. Sampling two times at three locations is insufficient. Dr. Furl proposed, due to duplications of efforts by multiple agencies, a population study every 3-5 years and to continue cotton lure sampling in the LTBG reach.

Dr. Weckerly suggested that the findings of the Texas State population study be duplicated in the future so that the results can be used for comparison with the concurrent biomonitoring studies.

Mr. Norris recommended adding more sampling locations at deeper spring depths in addition to the monitoring that is already occurring. Furthermore, Mr. Norris suggested studying migration and genetics.

Dr. Arsuffi recommended monitoring the fix sample sites every 2 years and random sampling of the 85 sites used in the Texas State population study using their methodology. Dr. Weckerly recommended using the same sampling times that are used in the Texas State study.

6. Questions from the public.

None.

7. Adjourn – 11:42 am



NOTICE OF OPEN MEETING
Available at eahcp.org

As approved by the Edwards Aquifer Habitat Conservation Plan (EAHCP) Science Committee, the Comal Springs Riffle Beetle (CSRB) Work Group has been formed to provide input on a specific set of questions concerning management of the CSRB as part of implementation of the EAHCP. A meeting of this Work Group for the EAHCP is scheduled for **October 9, 2019, at 9:00 a.m. at the San Marcos Activity Center (Multipurpose Room), 501 E. Hopkins St., San Marcos, Texas 78666**. Light refreshments will be provided. Please RSVP to kkollaus@edwardsaquifer.org.

1. Call to order--Establish that all Work Group members are present - 9:00 am.
2. Public Comment.
3. Approve minutes from September 4, 2019 Work Group meeting (Attachment 1).
4. Review the CSRB Work Group Meeting 3 held on September 4, 2019 and take any further comments on experimental design of the cotton lure efficiency tank study.
5. Review discussion from Meeting 3 on the Biological Monitoring Program and develop final recommendations.
6. Discuss Refugia CSRB collections to date and review of program goals.
7. Discuss habitat disturbance and develop recommendations regarding system disturbance.
8. Questions from the public.
9. Adjourn.



**Comal Springs Riffle Beetle Work Group
Meeting Minutes
San Marcos Activity Center
October 9, 2019**

Members of this committee included: Conrad Lamon, Chad Norris, Tom Arsuffi, Butch Weckerly, and Ken Ostrand

- 1. Call to order:** 9:02 am – All members of the work group were present.
- 2. Public Comment:**
There were no comments from the public.
- 3. Approve minutes from September 4, 2019 Work Group meeting.**
Chad Norris requested a revision to the prefix of his name in the meeting minutes from September 4, 2019. A motion was made by Butch Weckerly to approve the meeting minutes with revisions. Chad Norris seconded the motion. There were no objections.
- 4. Review the CSRB Work Group Meeting 3 held on September 4, 2019 and take any further comments on experimental design of the cotton lure efficiency tank study.**
Dr. Chad Furl presented an overview of the cotton lure efficiency tank study. Five circular tanks will be used with wood, leaves, limestone gravel, and cotton lure material. Wood and leaves will be collected from the wild, conditioned appropriately, and kept in wireframe. Cotton lures will be washed thoroughly with DI water to remove any potential toxins before being placed in the tank. Twenty adult Comal Springs riffle beetles will be placed in the tank, and lures will be checked at 10, 20, and 30-day intervals. All food items will be inspected for beetles at 30 days and mortalities will be documented. Dr. Furl and EAHCP staff will be moving forward with this design. A report is expected to be presented at the 2020 Science Committee meeting.
- 5. Review discussion from Meeting 3 on the Biological Monitoring Program and develop final recommendations.**
Dr. Furl reminded the staff of the goals of the Biological Monitoring Program. As per the prior work group meeting, two recommendations were determined: 1) the continuation of monitoring in the three LTBG reaches and follow established sampling protocols and 2) the addition of system wide population surveys to be completed twice before the end of the incidental take permit.

The Work Group mentioned Weston Nowlin's research on the Comal Springs riffle beetle. Dr. Furl and Butch Weckerly agreed that a review of Nowlin's research should be

considered prior to commitment to his study designs. Chad Norris mentioned population genetic studies. Dr. Furl noted that genetic studies can be conducted through the refugia program, outside of the biological monitoring program.

The Work Group concluded that the final recommendations suggested at the September meeting would suffice.

6. Discuss Refugia CSRB collections to date and review of program goals.

Dr. Furl presented to the Work Group a background on Refugia collections. Currently, the U.S. Fish and Wildlife Service is tasked with collecting 500 Comal Springs riffle beetles for a standing stock and 500 for salvage stock.

Mr. Norris asked how many Comal Springs riffle beetles are actively kept at Refugia. Dr. Furl answered about 200-300 at both the San Marcos and Uvalde Refugia locations. Ken Ostrand recommended using a model, based on aquifer conditions and genetic diversity, to determine effective refugia populations in 2020. Mr. Norris commented that 500 individuals seems too high and 200 is too low. Tom Arsuffi recommended 250 individuals collected for standing stock, Butch Weckerly seconded that recommendation. Mr. Norris commented that 250 individuals may not be sufficient for standing stock.

7. Discuss habitat disturbance and develop recommendations regarding system disturbance.

Ken Ostrand recommended a survivorship study to develop recommendations on habitat disturbance. Butch Weckerly noted, based on Dr. Furl's data, any time the population falls below 25%, there is a problem with disturbance. Tom Arsuffi requested a disturbance table to better develop appropriate recommendations.

8. Questions from the public.

None.

9. Adjourn.

11:37 am



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Available at eahcp.org

As approved by the Edwards Aquifer Habitat Conservation Plan (EAHCP) Science Committee, the Comal Springs Riffle Beetle (CSRB) Work Group has been formed to provide input on a specific set of questions concerning management of the CSRB as part of implementation of the EAHCP. A meeting of this Work Group for the EAHCP is scheduled for **October 30th, 2019, at 9:00 a.m. at the Meadows Center for Water and the Environment, SLH 107 Conference Room, 201 San Marcos Springs Dr., San Marcos, TX 78666**. Breakfast items and lunch will be provided. Please RSVP to kkollaus@edwardsaquifer.org.

1. Call to order--Establish that all Work Group members are present - 9:00 am.
2. Public Comment.
3. Approve minutes from October 9th, 2019 CSRB Work Group Meeting 4 (Attachment 1).
4. Review discussion from Meeting 4 on Refugia CSRB collections and system disturbance and develop final recommendations.
5. Review and discuss CSRB Long-Term Biological Goals and develop recommendations.
6. Questions from the public.
7. Adjourn.



**Comal Springs Riffle Beetle Work Group
Meeting Minutes
Meadows Center for Water and the Environment
October 30, 2019**

Members of this committee included: Conrad Lamon, Chad Norris, Tom Arsuffi, Butch Weckerly, and Ken Ostrand

1. **Call to order:** 9:05 a.m. – All members of the work group were present.
2. **Public Comment:**
There were no comments from the public.
3. **Approve minutes from October 9, 2019 Work Group meeting.**
A motion was made by Ken Ostrand to approve the meeting minutes. Dr. Butch Weckerly seconded the motion. There were no objections.
4. **Review discussion from Meeting 4 on Refugia CSRFB collections and system disturbance and develop final recommendations.**

Chad Furl summarized the outcome of Meeting 4 of the CSRFB Work Group. Tom Arsuffi clarified that the standing stock amount is subject to change as husbandry techniques improve. Ken Ostrand recommended, based on collection and husbandry studies, a standing stock of 50 would be adequate. Dr. Arsuffi recommended 75 individuals at each Refugia facility, 150 individuals total. Chad Norris recommended a genetic study to develop appropriate standing stock numbers. The Work Group determined that 150 CSRFB individuals is sufficient for standing stock at Refugia and will revisit standing stock numbers in 2022 after population studies have concluded. Dr. Furl added, in 2021 a CSRFB pupation study will continue and in 2022 the population study will be complete. To monitor the development of these studies, the Work Group agreed to convene on an annual basis. Dr. Furl informed the group that the next CSRFB Work Group meeting will be in conjunction with the Research Work Group on December 11, 2019.

Regarding CSRFB collection sites, Lindsey Campbell clarified that collection for Refugia occurs beyond Spring Run 3. The Work Group determined that CSRFB collection for Refugia purposes should be separate from collection for Biological Monitoring.

Additionally, the Work Group determined that alternating collections should occur between spring orifices and that 100% of the CSRFB captured on the cotton lure can be collected.

5. Review and discuss CSRB Long-Term Biological Goals and develop recommendations.

Dr. Furl presented the CSRB Long-Term Biological Goals as stated in Section 4.1.1.1 of the EAHCP. Mr. Norris noted that the Long-Term Biological Goals for the CSRB were intended to be similar to the goals for the fountain darter and Texas wild-rice. That said, there should be major changes to the overall goals. Dr. Furl recommended to meet again to discuss the issues and concerns with the CSRB Long-Term Biological Goals after studies have been concluded and more information is made available. The Work Group agreed with Dr. Furl's recommendation to suspend the discussion.

6. Questions from the public.

None.

7. Adjourn.

11:42 a.m.



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Available at eahcp.org

As approved by the Edwards Aquifer Habitat Conservation Plan (EAHCP) Science Committee, the Comal Springs Riffle Beetle (CSRB) Work Group has been formed to provide input on a specific set of questions concerning management of the CSRB as part of implementation of the EAHCP. A meeting of this Work Group for the EAHCP is scheduled for **December 11th, 2019, at 9:00 a.m. at the Meadows Center for Water and the Environment, SLH 107 Conference Room, 201 San Marcos Springs Dr., San Marcos, TX 78666**. Breakfast items will be provided. Please RSVP to kkollaus@edwardsaquifer.org.

1. Call to order--Establish that all Work Group members are present - 9:00 am.
2. Public Comment.
3. Approve minutes from October 30th, 2019 CSRB Work Group Meeting 5 (Attachment 1).
4. Review and receive comments on the CSRB Work Group Report (Attachment 2).
5. Receive any final recommendations from the Work Group members.
6. Questions from the public.
7. Adjourn.



**Comal Springs Riffle Beetle (CSRB) Work Group
Meeting Minutes
Meadows Center for Water and the Environment
December 11, 2019**

Members of this committee included: Conrad Lamon, Chad Norris, Tom Arsuffi, Butch Weckerly, and Ken Ostrand.

1. **Call to order:** 9:07 a.m. – All members of the work group were present.
2. **Public Comment:**
There were no comments from the public.
3. **Approve minutes from October 30, 2019 Work Group meeting.**
A motion was made by Butch Weckerly to approve the meeting minutes. Ken Ostrand seconded the motion. There were no objections.
4. **Review and receive comments on the CSRB Work Group Report.**
Dr. Chad Furl recapped the overall discussion and recommendations provided at the previous CSRB Work Group meetings. Recommendations included continued use of the cotton lure for CSRB collection, a cotton lure mesocosm experiment to be completed in 2020, and to finalize CSRB population surveys expected to be completed in 2022 and 2025. Ken Ostrand asked if the mesocosm experiment was going to go through the RFP process. Dr. Furl responded that it will go through the formal Edwards Aquifer Authority's bid process and that the experiment should start in Spring-Summer 2020. Nathan Pence asked what the mesocosm experiment entailed. Dr. Furl summarized the details of the experiment. Dr. Furl recapped the biological monitoring, Refugia, and Applied Research collection recommendations that were provided during the previous CSRB Work Group meetings. The work group member recommended refugia standing stocks be lowered to 150, 75 in each facility. The CSRBWG will revisit standing stock numbers annually as population and husbandry studies become available and Refugia staff refines the level of effort needed to achieve this standing stock number. Lastly, Dr. Furl reminded the Work Group the recommendation to maintain the present Long-Term Biological Goals and revisit the goals after population studies have been completed.
5. **Receive any final recommendations from the Work Group members.**
There were no final recommendations from the Work Group members.
6. **Questions from the public.**
None.
7. **Adjourn.**
9:35 a.m.



***COMAL SPRINGS RIFFLE BEETLE WORK GROUP
REPORT – Appendix C Presentations of the
Work Group***

COMAL SPRINGS RIFFLE BEETLE WORK GROUP



May 9th Science Committee

CSRB WORK GROUP CHARGE

- The Work Group's charge consists of questions related to three areas:
 - 1). Cotton lure sampling methodology
 - 2). Biological Monitoring, Refugia Collections and Applied Research
 - 3). CSRB Long-term Biological Goals



CSRB WORK GROUP MEMBERS

- ▶ The Work Group will consist of the following members:
 - ▶ Conrad Lamon
 - ▶ Chad Norris
 - ▶ Ken Ostrand
 - ▶ Eric Benbow*

*Dr. Benbow will join the Work Group following the completion of *NAS Report 3*.



CSRB WORK GROUP: RATIONALE

- Due to expressed concerns from the Science Committee as well as the National Academy of Sciences, EAHCP staff have determined a comprehensive look at these three areas charged to the Work Group.



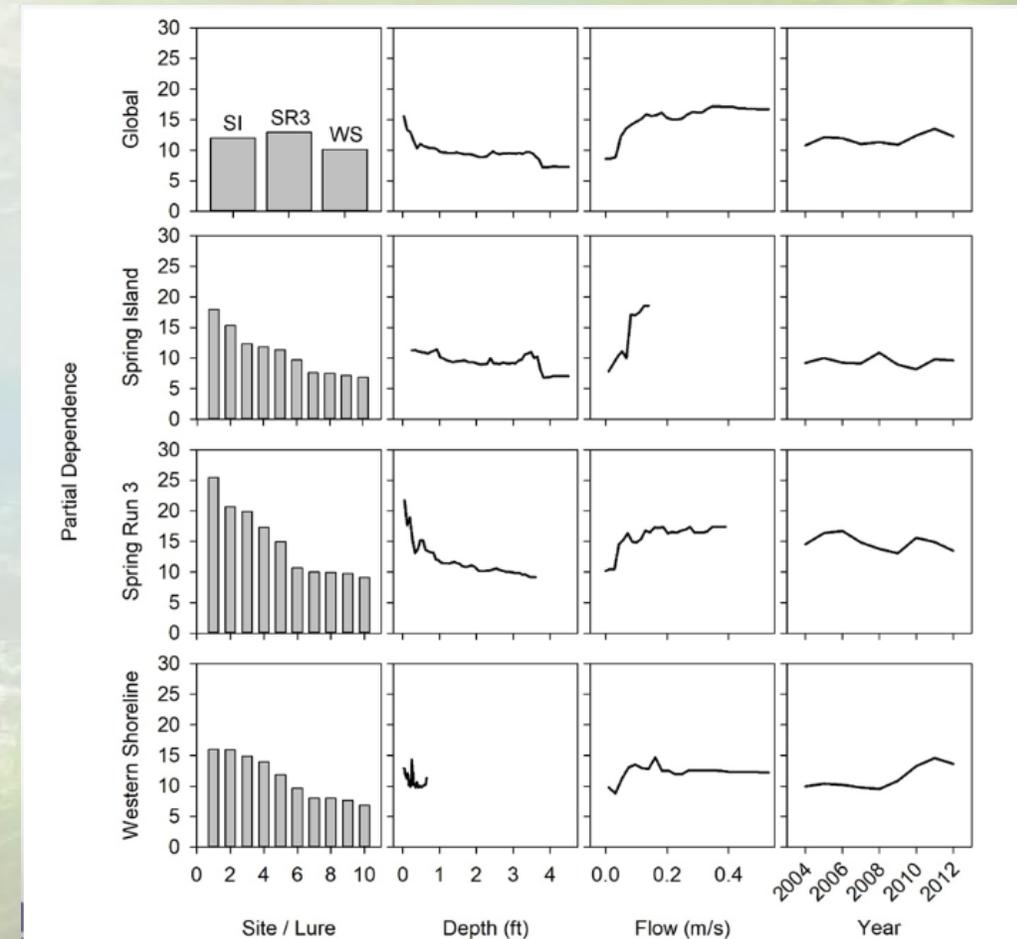


CSRB WORK GROUP: KICK-OFF MEETING

- ▶ The Work Group will meet on an as needed basis and will produce a written report documenting their recommendations.
 - ▶ Solicit data requests
 - ▶ Discuss literature review
- ▶ Kick-off meeting will be held on **May 24th at the San Marcos Activity Center**

CSRB WORK GROUP: DATA REQUESTS

- ➔ In order to better facilitate a productive conversation, Work Group members are asked to provide information requests regarding past data collection and analysis.



CSRB WORK GROUP: LITERATURE REVIEW

- Dr. Arsuffi will be tasked with compiling a robust literature review in order to provide the Work Group a better understanding of possible sampling methodologies used on similar species.



CSRB WG TIMELINE

Solicit Science Committee input on charge questions	March 8 - March 23
Finalize and approve work group charge through Science Committee	May 9 meeting
Hold initial work group meeting	May 24
Conduct literature and other work group requests	May - August
Perform data analyses and other work group requests	May - August
NAS Report 3 complete, literature review complete, and initial data analyses complete	September 1
Work group begins regular meetings	September - October
Finalize and document work group results	November - December
Implement work group suggestions	2019

COMAL SPRINGS RIFFLE BEETLE WORK GROUP

May 24, 2018





AGENDA

1. Introductions
2. Work Group Charge Review
3. Meeting Schedule Review
4. Cotton lure methodology
 - a. Current issues
 - b. Major areas of literature review focus
5. Biological Monitoring and Refugia Collections
 - a. Information needs



Individuals' Process Roles

- ▶ Work Group members:
 - ▶ Conrad Lamon
 - ▶ Chad Norris
 - ▶ Ken Ostrand
 - ▶ Eric Benbow
- ▶ Staff
 - ▶ Chad Furl
 - ▶ Shaun Payne
- ▶ Field Experts

CSRB WORK GROUP CHARGE

- ▶ The Work Group's charge consists of questions related to three areas:
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 - 3). CSRB Long-term Biological Goals





CSRB COTTON LURE SAMPLING METHODOLOGY

- ▶ Is the current cotton lure sampling methodology an appropriate means to monitor abundance at a locale?
- ▶ If not, what sampling methodologies exist that would provide a better proxy of abundance at a locale?
- ▶ If the previous two questions cannot be adequately answered without additional study, what would be an appropriate study to answer the questions?

BIOLOGICAL MONITORING, REFUGIA COLLECTIONS, AND APPLIED RESEARCH COLLECTIONS

- ▶ What changes are recommended for the Biological monitoring sampling program? What are the stated goals behind those changes?
- ▶ What changes are recommended for Refugia removal efforts? What are the stated goals behind those changes?
- ▶ Are the current and proposed levels of physical activity in the CSRB habitat protective of the species? If not, what level of activity is appropriate?



CSRB LONG-TERM BIOLOGICAL GOALS

- ▶ Are the current population and habitat LTBGs for the CSRB appropriate? What are the criteria for more appropriate goals?
- ▶ What is an appropriate means to monitor the habitat quality goal?
- ▶ How can Biological monitoring, Refugia efforts, and Applied Research studies be used to establish new LTBGs if needed?

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Background and purpose of CSRБ monitoring

- ▶ LTBGs for CSRБ involve a qualitative habitat component and quantitative population measurement.
- ▶ Population measurement goal is to maintain greater than or equal to the median densities observed

	Spring Run 3	Western Shoreline	Spring Island Area
Density (# of CSRБ/Lure)	≥ 20	≥ 15	≥ 15

Background of the CSRB cotton lure methodology

- A major unknown is the CSRB's use of subsurface habitat, thus a lure approach has been utilized to collect population data.
- Population measurements are based on long-term trends in specific locations.

COMAL SPRINGS RIFFLE BEETLE DENSITY (#/LURE)

	Spring Run 3	Western Shoreline	Spring Island Area
Minimum	7	9	7
25 th	12	13	11
Median	17	14	13
75 th	21	20	16
Maximum	32	26	23





First discussion question

What are the issues with the current Cotton lure methodology?



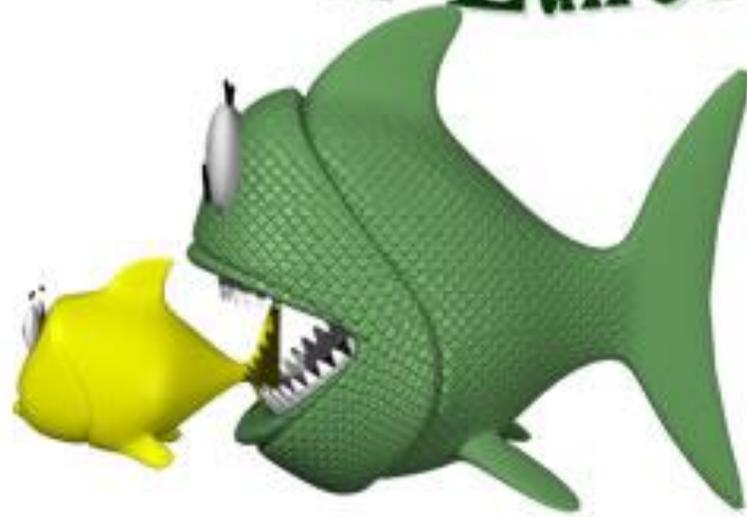


Second discussion question

What are the major areas of focus needed to inform fundamental changes to the current monitoring process?



Join Me For Lunch





Biological Monitoring, Refugia Collection and Applied Research

- ▶ Biological Monitoring is a comprehensive monitoring program was established by the EAA in 2000.
 - ▶ The program accumulates data to refine estimates of “average” conditions as well as producing monitoring data during low-flow periods (and after floods).
- ▶ Refugia collection is an essential aspect of building a “standing stock” of covered species at the USFWS SMARC refugia facility.
- ▶ Applied Research has been a major area of focus of over the past 5-years.
 - ▶ Research on the CSRB has required collection in the past and is expected to continue.



Biological Monitoring, Refugia Collection and Applied Research

- ▶ Preliminary data questions from Chad.
- 



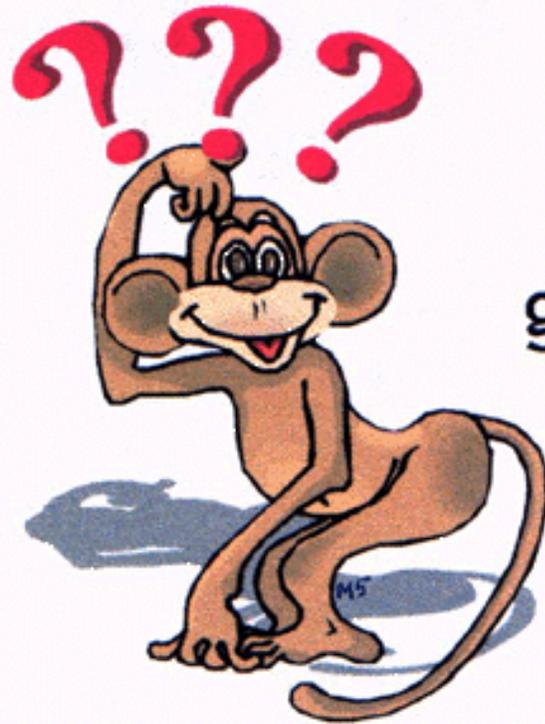
First discussion question

What are some information/data needs regarding Biological Monitoring that can help inform the Work Groups decision on how best to redefine CSRB sampling regime?



Second discussion question

What are some information/data needs regarding Refugia collection that can help inform the Work Groups decision on how best to redefine CSRB collection strategy?



Questions
are
guaranteed in
life;
Answers
aren't.



Schedule of next steps



Comal Springs Riffle Beetle Work Group

Meeting 2 – July 2, 2019

Meeting 2

- Review Work Group goals
- Presentation by Dr. Arsuffi
- Discuss updated dataset and repeated sampling
- Discuss next steps

Work Group Goals

- Cotton lure sampling methodology
 - Is the current cotton lure sampling methodology an appropriate means to monitor abundance at a locale?
 - If not, what sampling methodologies exist that would provide a better proxy of abundance at a locale?
 - If the previous two questions cannot be adequately answered without additional study, what would be an appropriate study to answer the questions?

Work Group Goals

- Biological monitoring, Refugia collections, and Applied Research collections
- Long-term biological goals

CSRB lure dataset

- Individual field sheets were reviewed against BioWest's Access database.
- Riffle beetle counts were almost identical to the previous version.
 - lures that were lost or disturbed were identified to confirm they were not recorded as zeros.
 - Values in the db for each lure were filled in when they were evidently not filled in, or simply averaged for the whole reach.
 - Slight changes were made to some of indexed values to better reflect the chronological order that the samples were taken.
- USFWS refugia collection events were added to the database.

CSRB lure dataset

- Orifices targeted for repeated sampling were identified in both BW and USFWS data.

Table 1. Biowest CSRB survey results 2004-2018.

<u>location</u>	<u>surveys</u>	<u>lures_set</u>	<u>adults_caught</u>	<u>larva_caught</u>
:-----:	-----:	-----:	-----:	-----:
Spring Island	42	425	2704	1407
Spring Run 3	42	451	4452	573
Western Shoreline	42	464	4321	1228

Table 2. USFWS CSRB survey results 2017 - 2/2019.

<u>location</u>	<u>surveys</u>	<u>lures_set</u>	<u>adults_caught</u>	<u>larva_caught</u>
:-----:	-----:	-----:	-----:	-----:
Spring Island	14	51	498	432
Spring Run <u>1</u>	14	54	634	50
Spring Run <u>2</u>	2	4	60	9
Spring Run <u>3</u>	21	162	2700	675

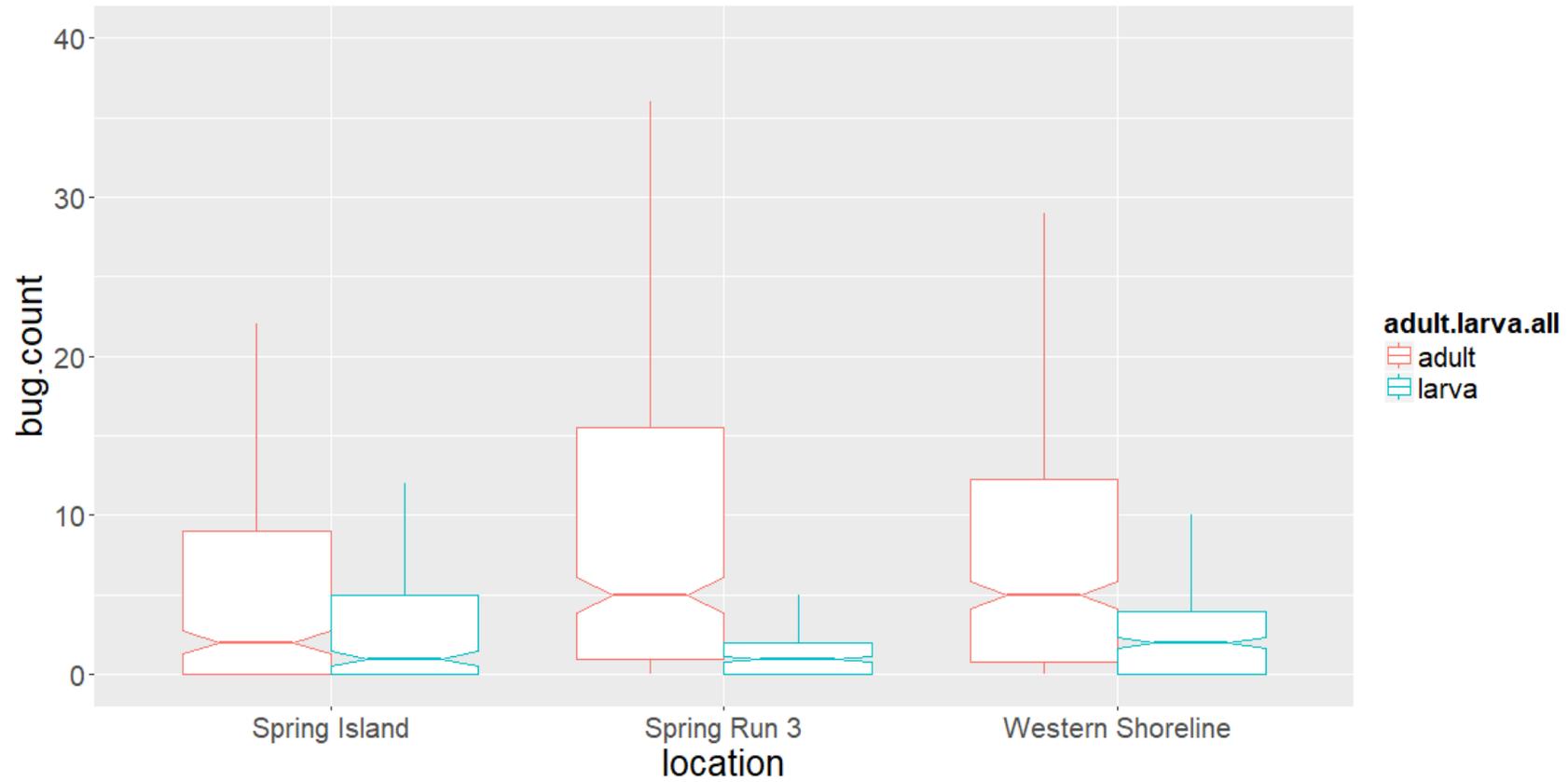
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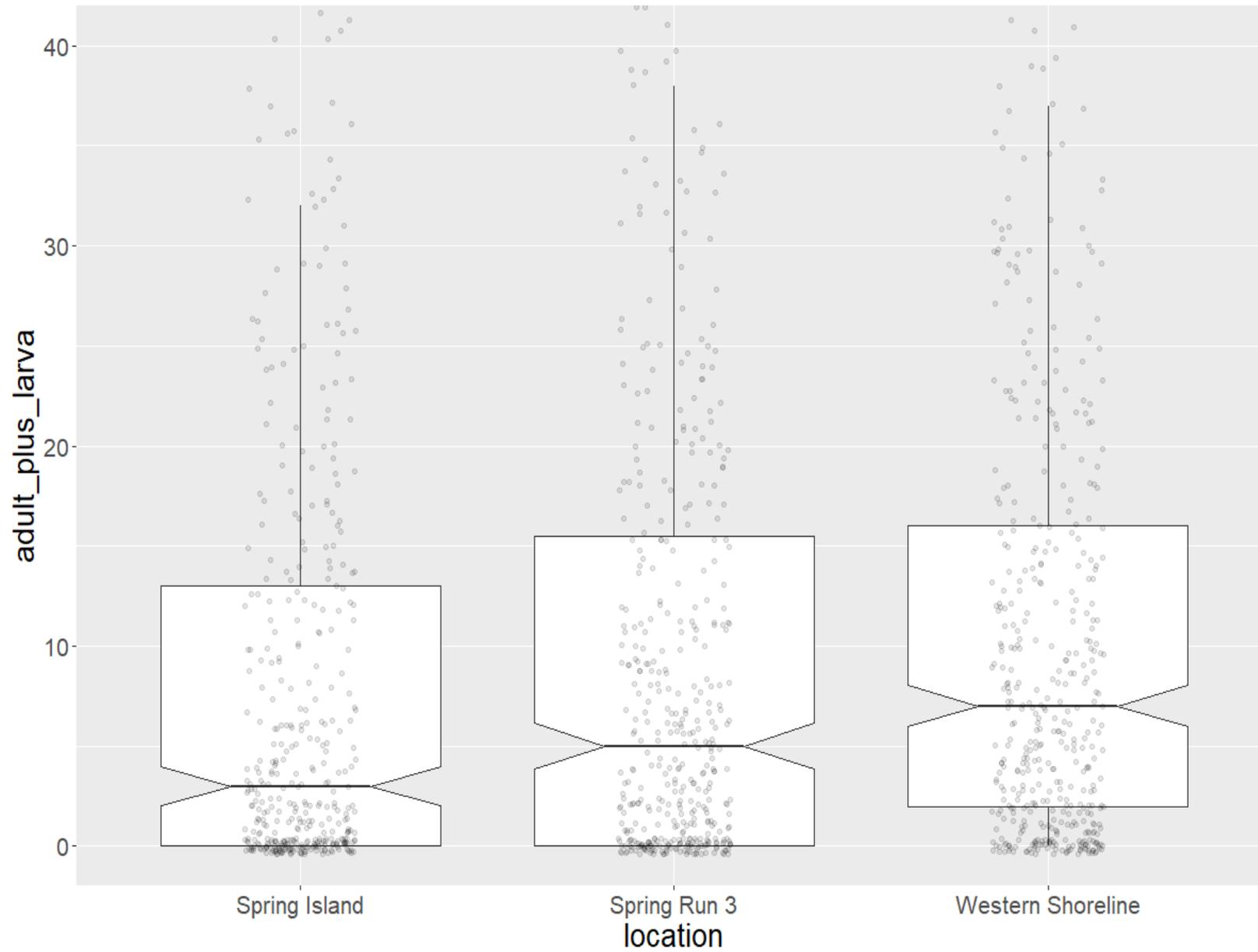
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Spring Run <u>3</u>	21	162	2700	675

- 1611 lures set and 19,743 beetles handled since 2004



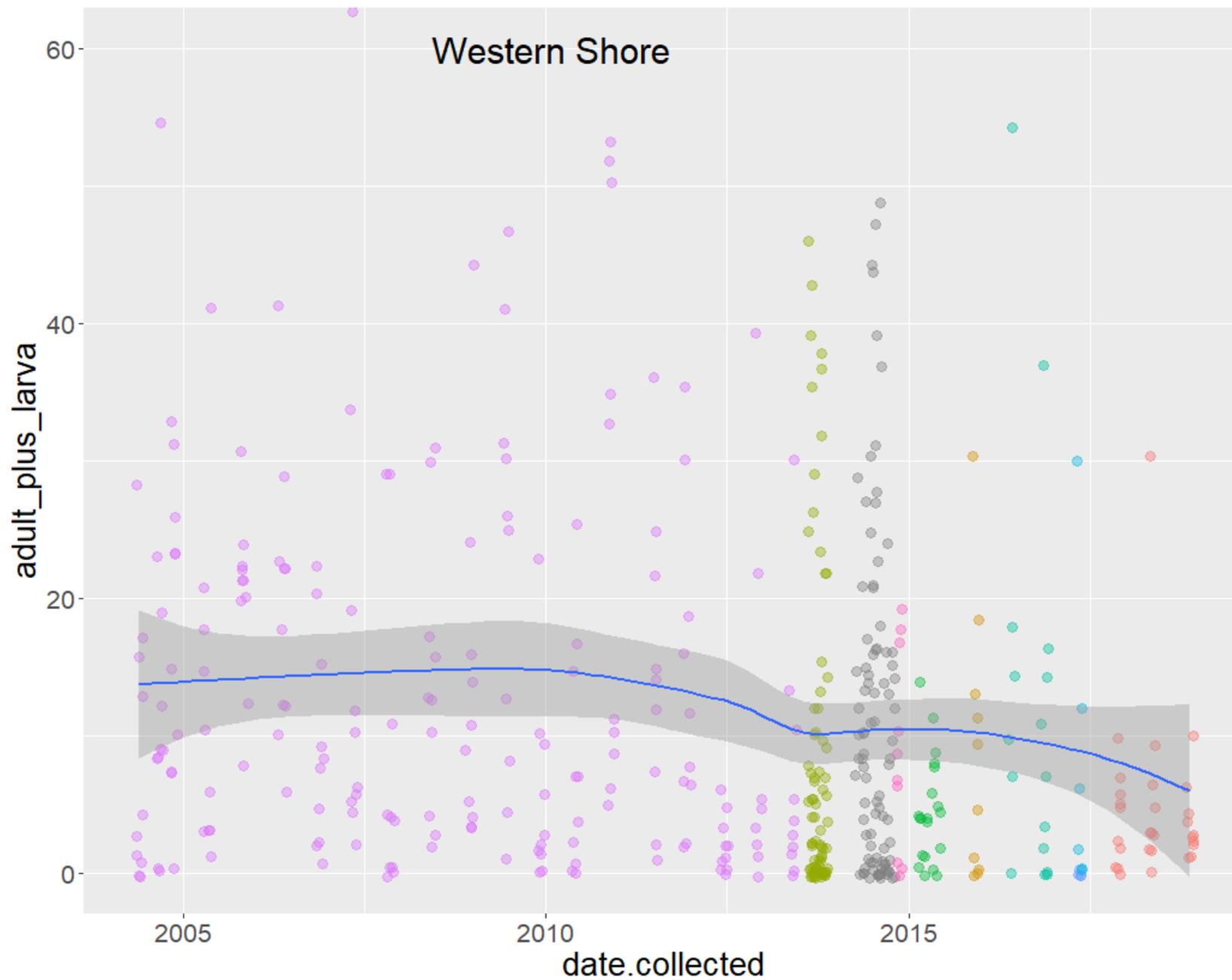
location	lures	zero counts adults	zero counts larva
Spring Island	425	125	147
Spring Run 3	451	97	173
Western Shoreline	464	110	111

Distribution of adult and larva bug counts by location for Biowest surveys 2004-2018.



Same as previous figure with adult and larva counts combined.

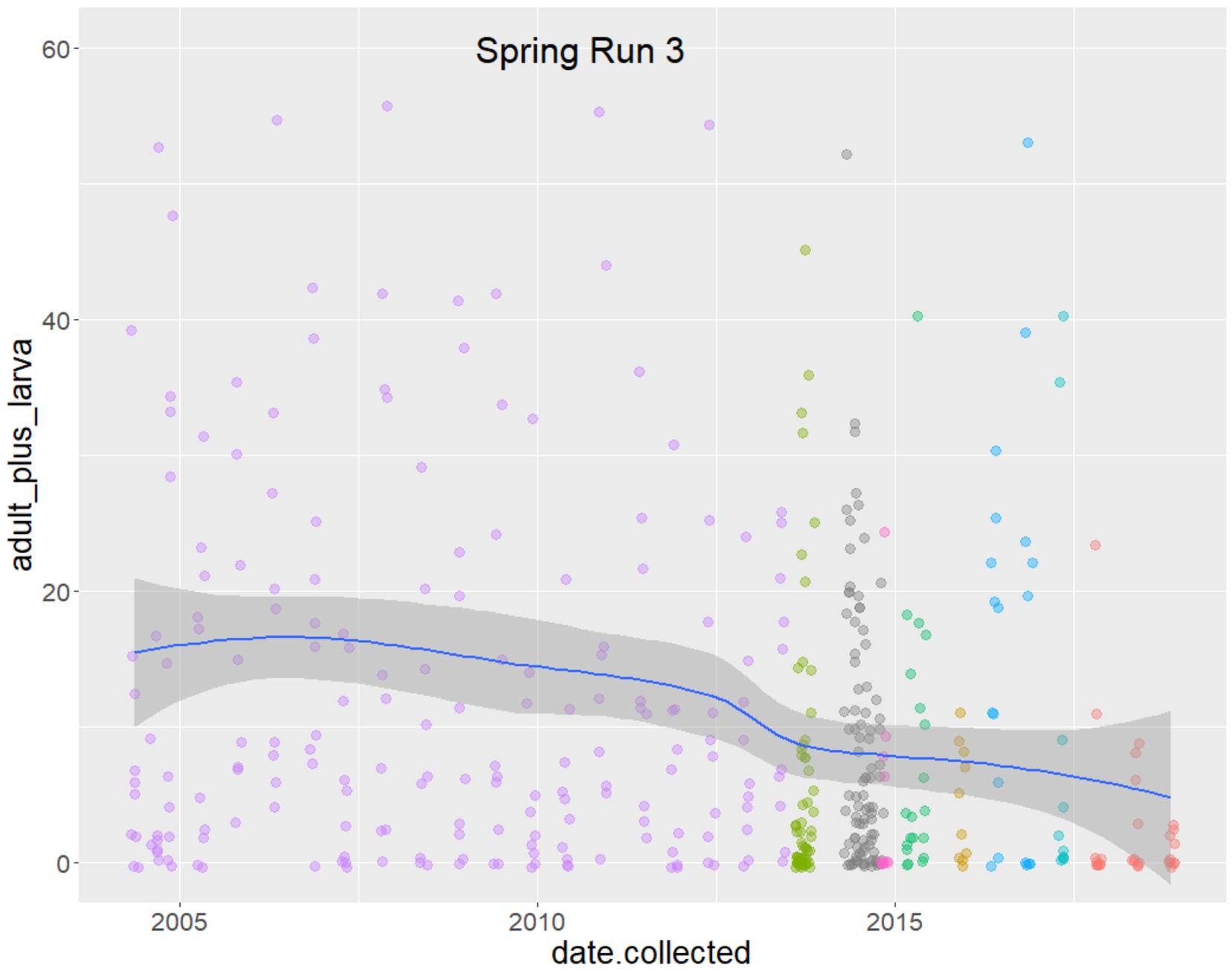
Western Shore



Combined adult plus larva counts for Biowest 2004-2018. Points are jittered off of x position. Line represents local polynomial regression fitting. Base geom_smooth() determined by LOESS function.
https://ggplot2.tidyverse.org/reference/geom_smooth.html

data.collector

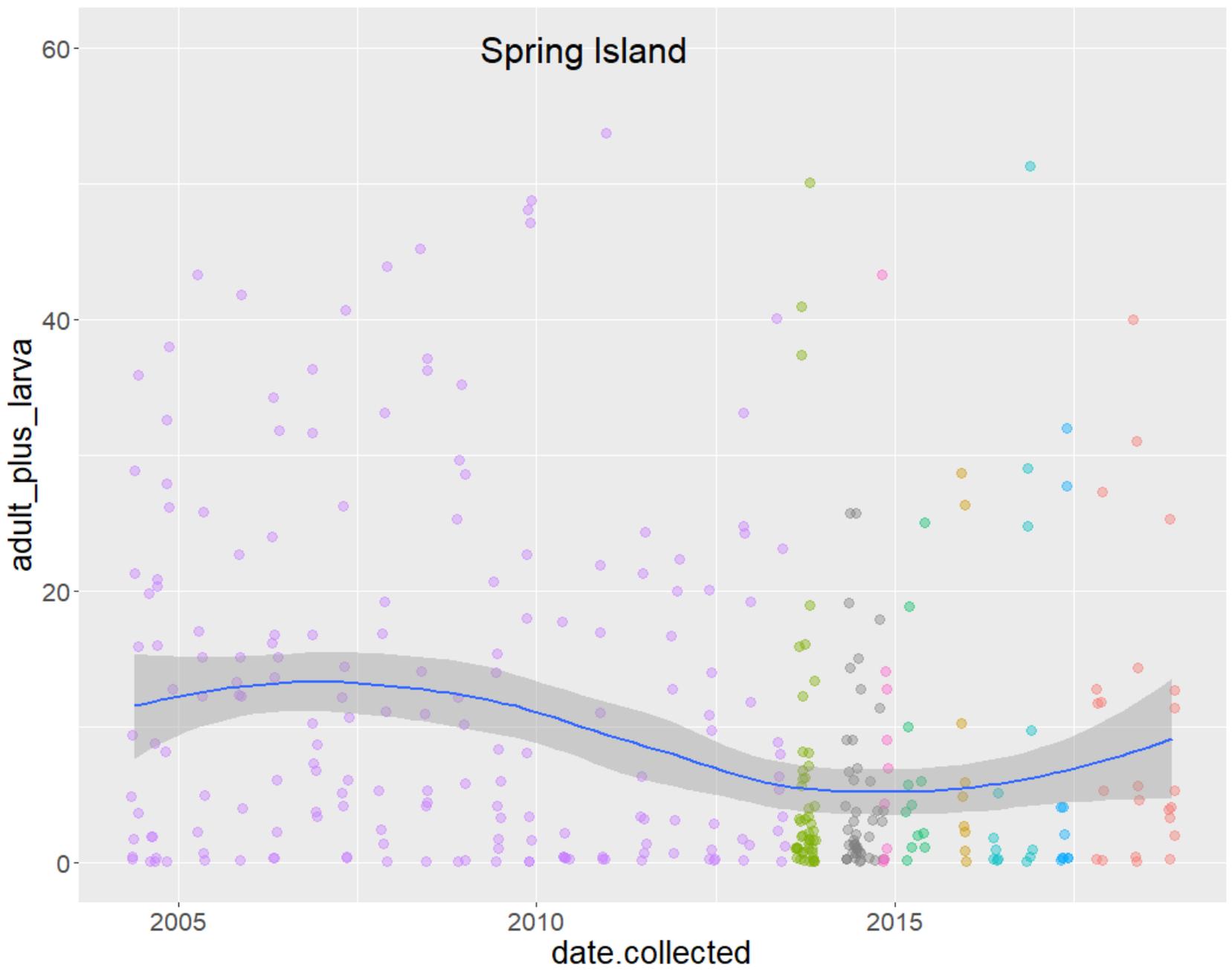
● EJ EK	● LL	● MW	● NK	● TJ ME KM RG
● JG ME	● ME TJ	● MW NK EJ	● RG	● NA



Spring Run 3

Combined adult plus larva counts for Biowest 2004-2018. Points are jittered off of x position. Line represents local polynomial regression fitting. Base geom_smooth() determined by LOESS function.
https://ggplot2.tidyverse.org/reference/geom_smooth.html

data.collector EJ EK LL MK NK RG NA
 JG ME ME TJ MW TJ ME KM RG

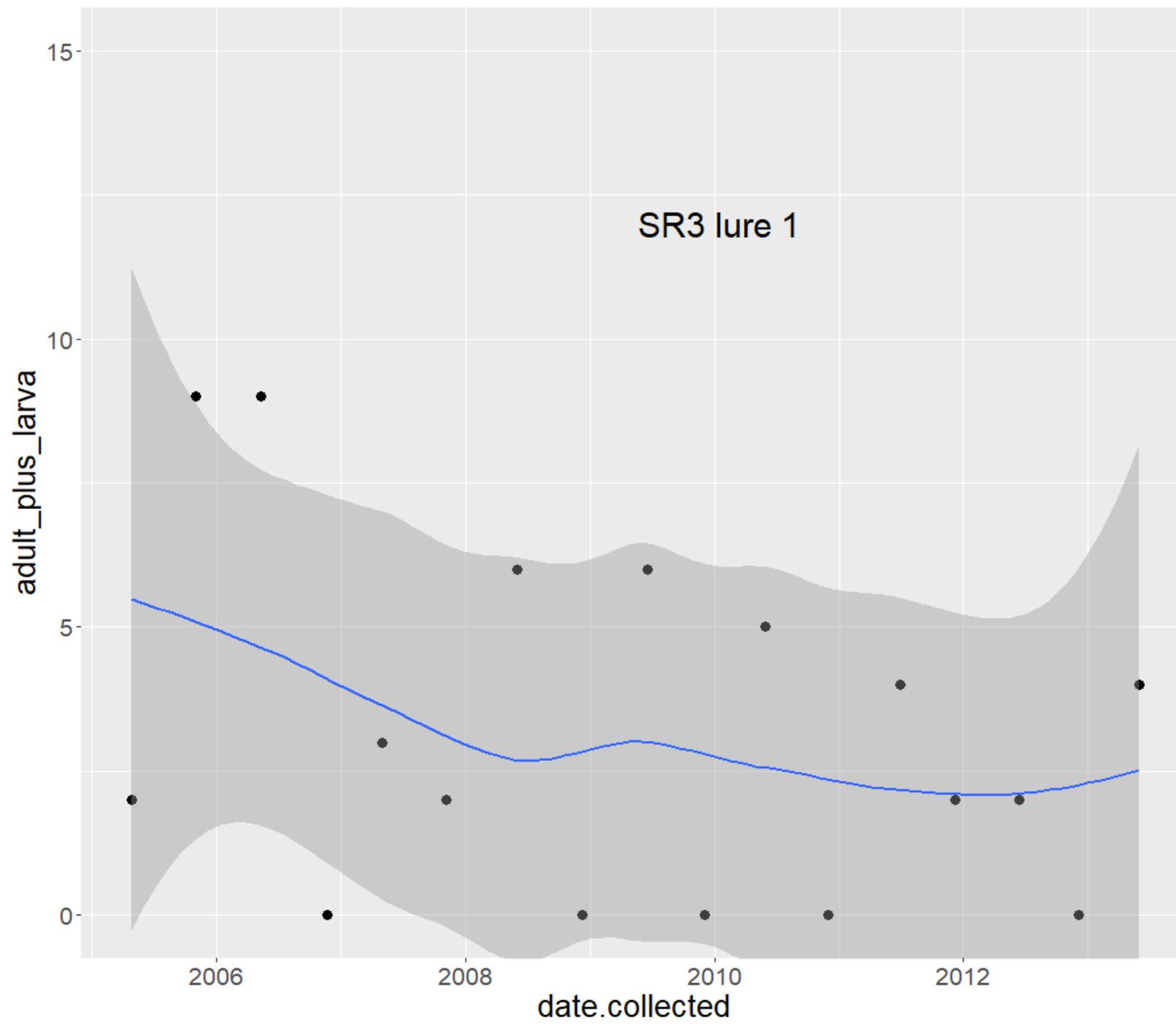


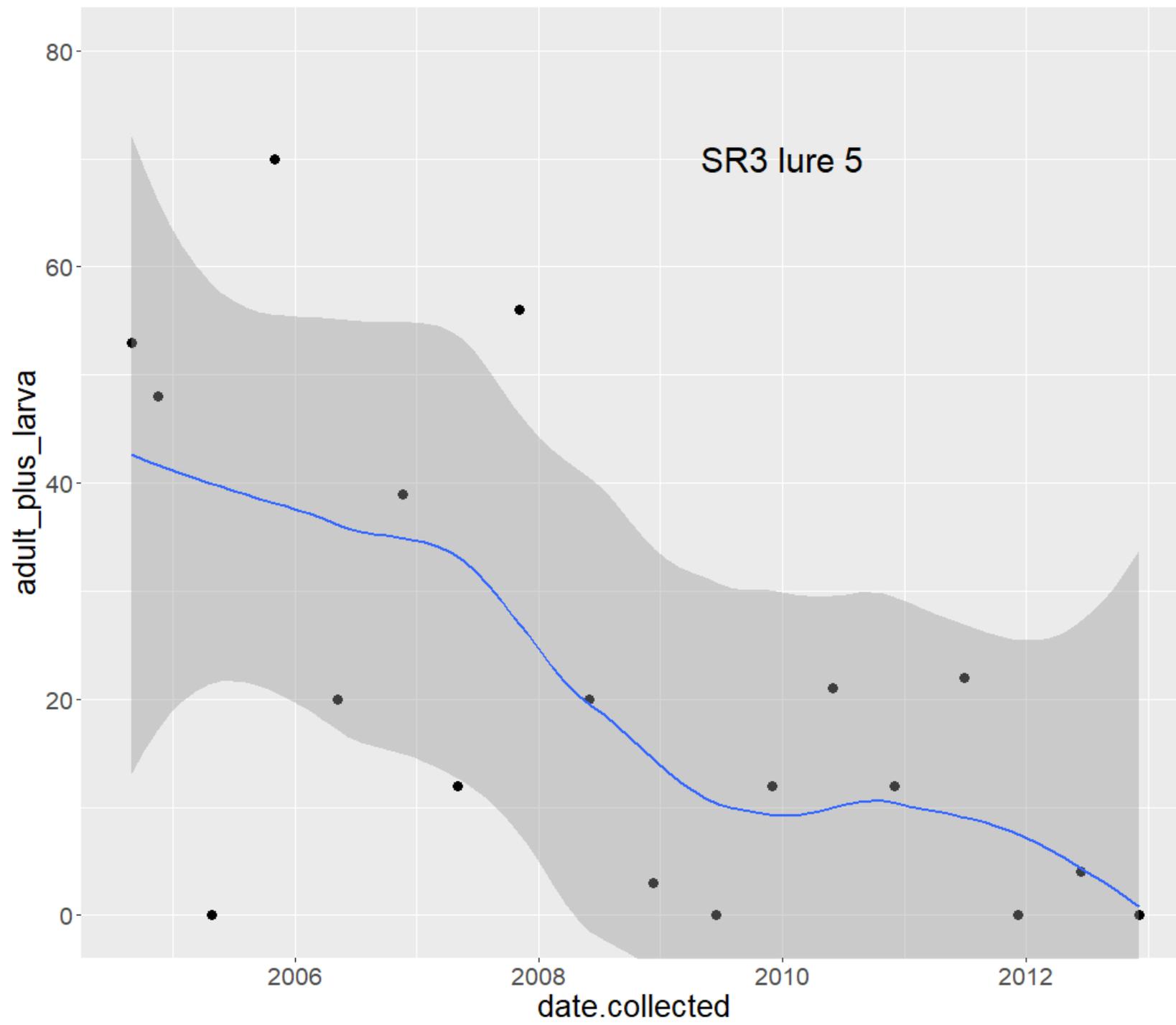
Combined adult plus larva counts for Biowest 2004-2018. Points are jittered off of x position. Line represents local polynomial regression fitting. Base geom_smooth() determined by LOESS function.
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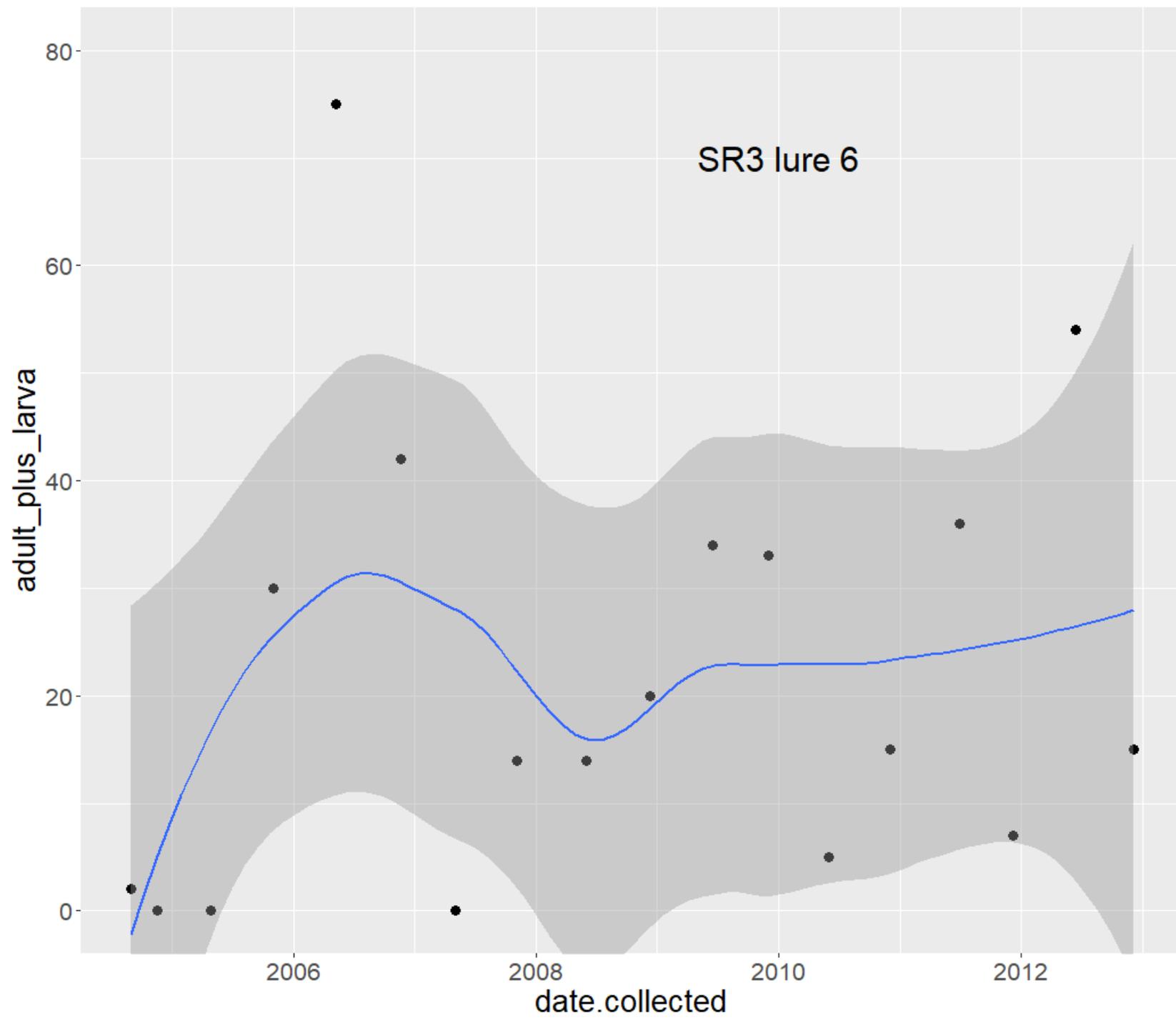
● EJ EK	● LL	● MW	● RG	● NA
● JG ME	● ME TJ	● MW NK EJ	● TJ ME KM RG	

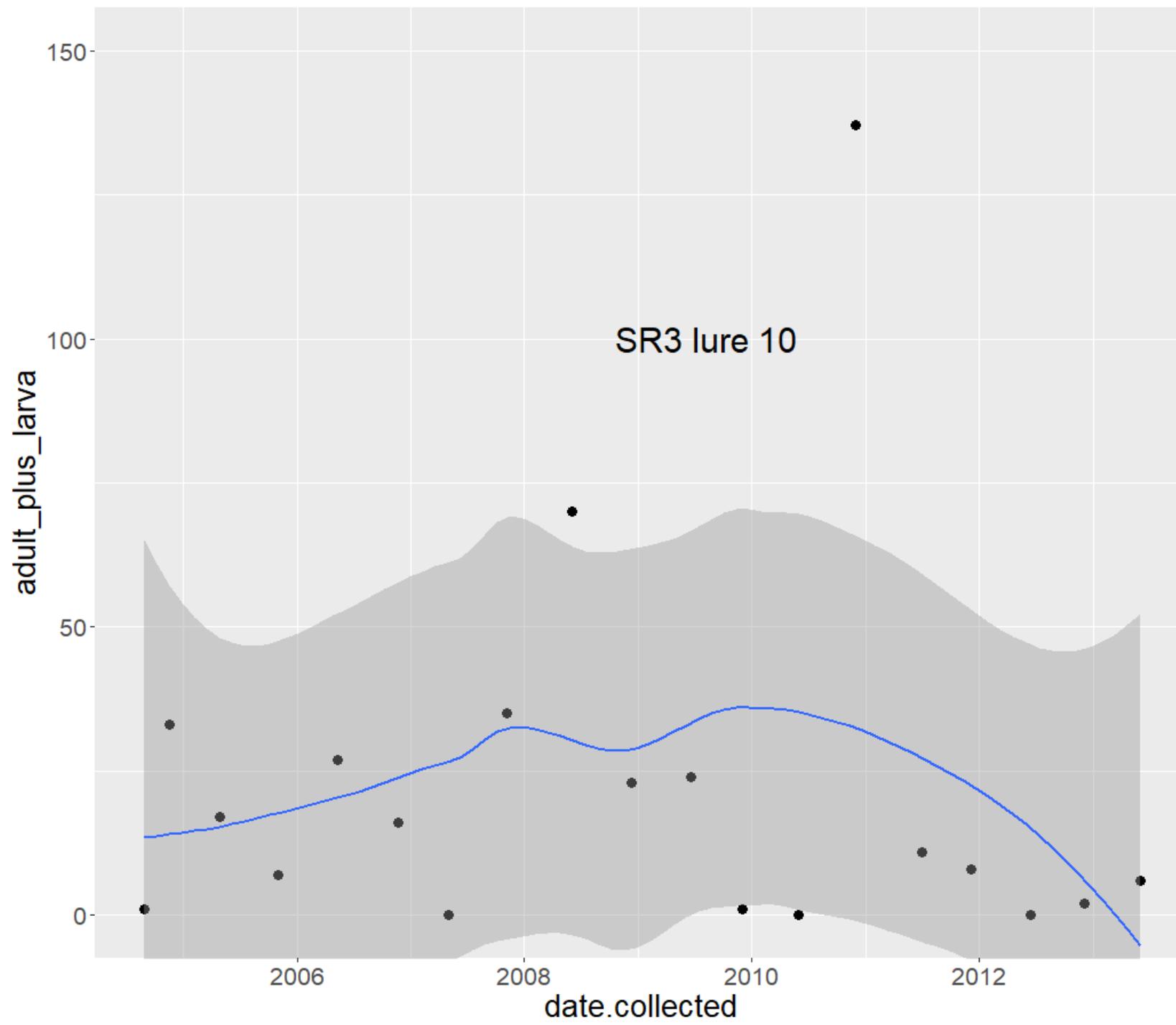
Sampling Reach	Lure Location	Dates/Period
Spring Run 3	1	April 2005 – May 2013
	5, 6	Aug 2004 – Dec 2012
	10	Aug 2004 – May 2013
Spring Island	1	Dec 2008 – May 2013
	2,3	Nov 2006 – Dec 2012
	4	June 2008 – May 2013
	8	May 2006 – June 2012, May 2013
	9,10	Nov 2006 – June 2012



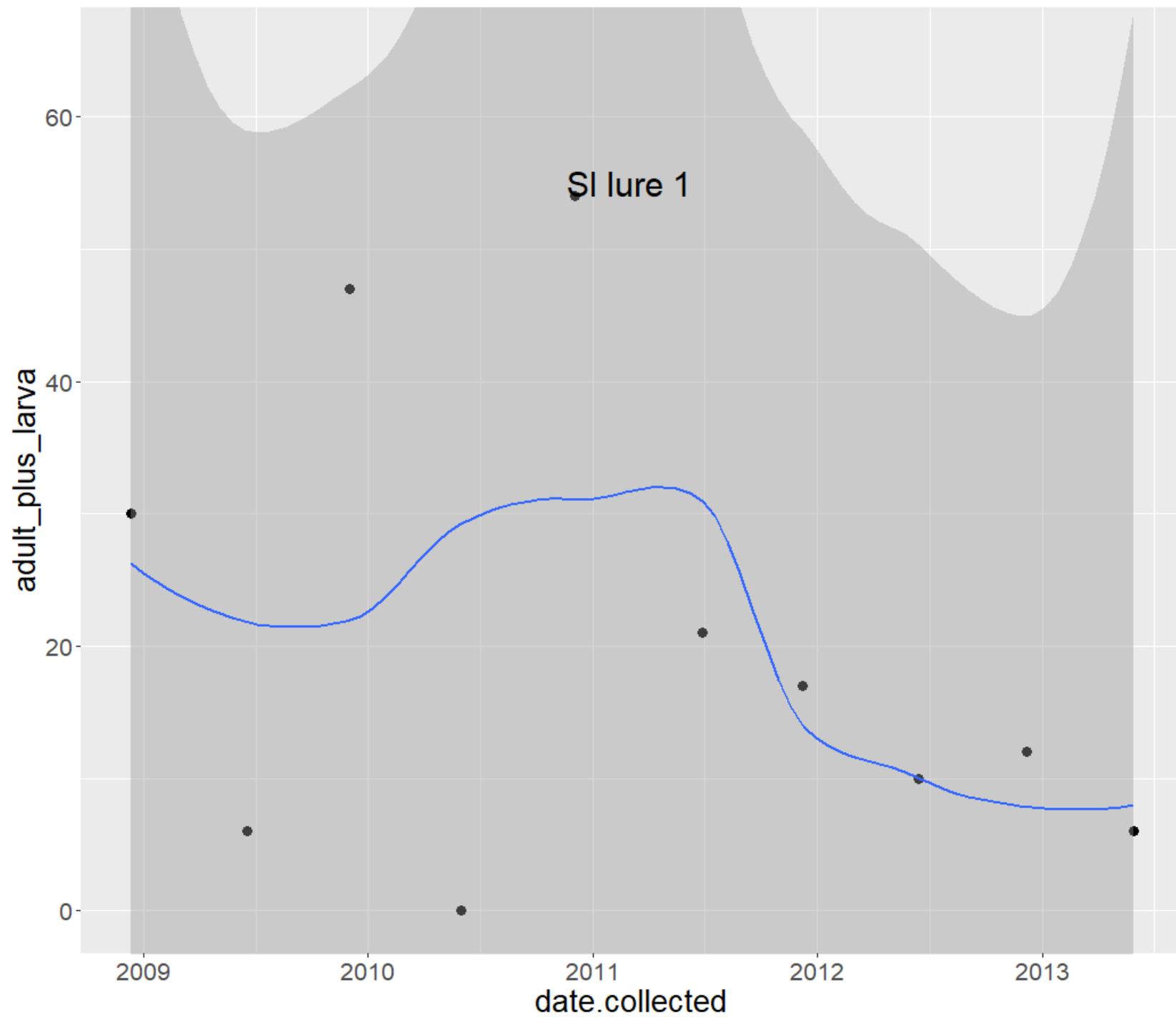


Repeated orifice
sampling - BioWest

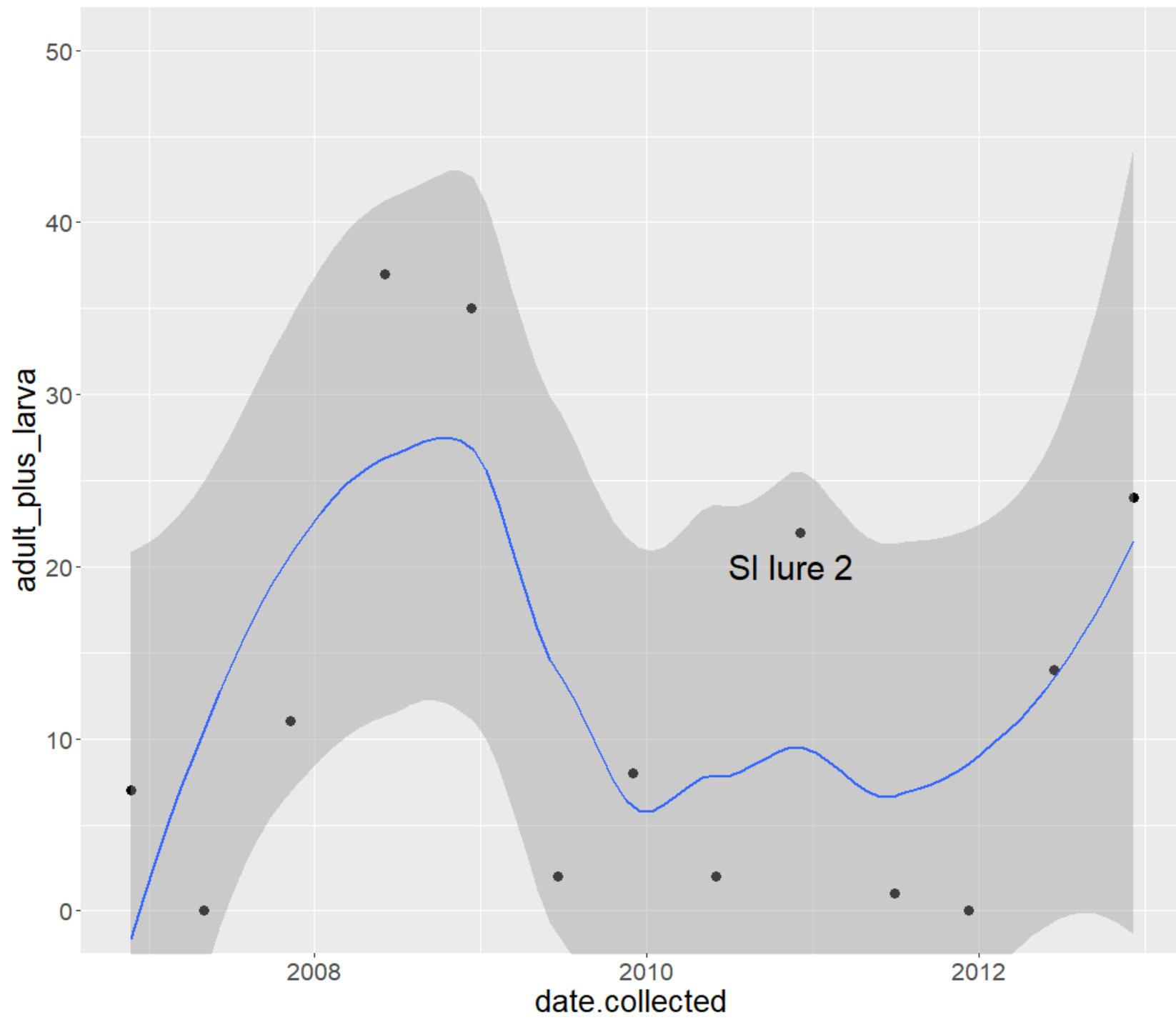




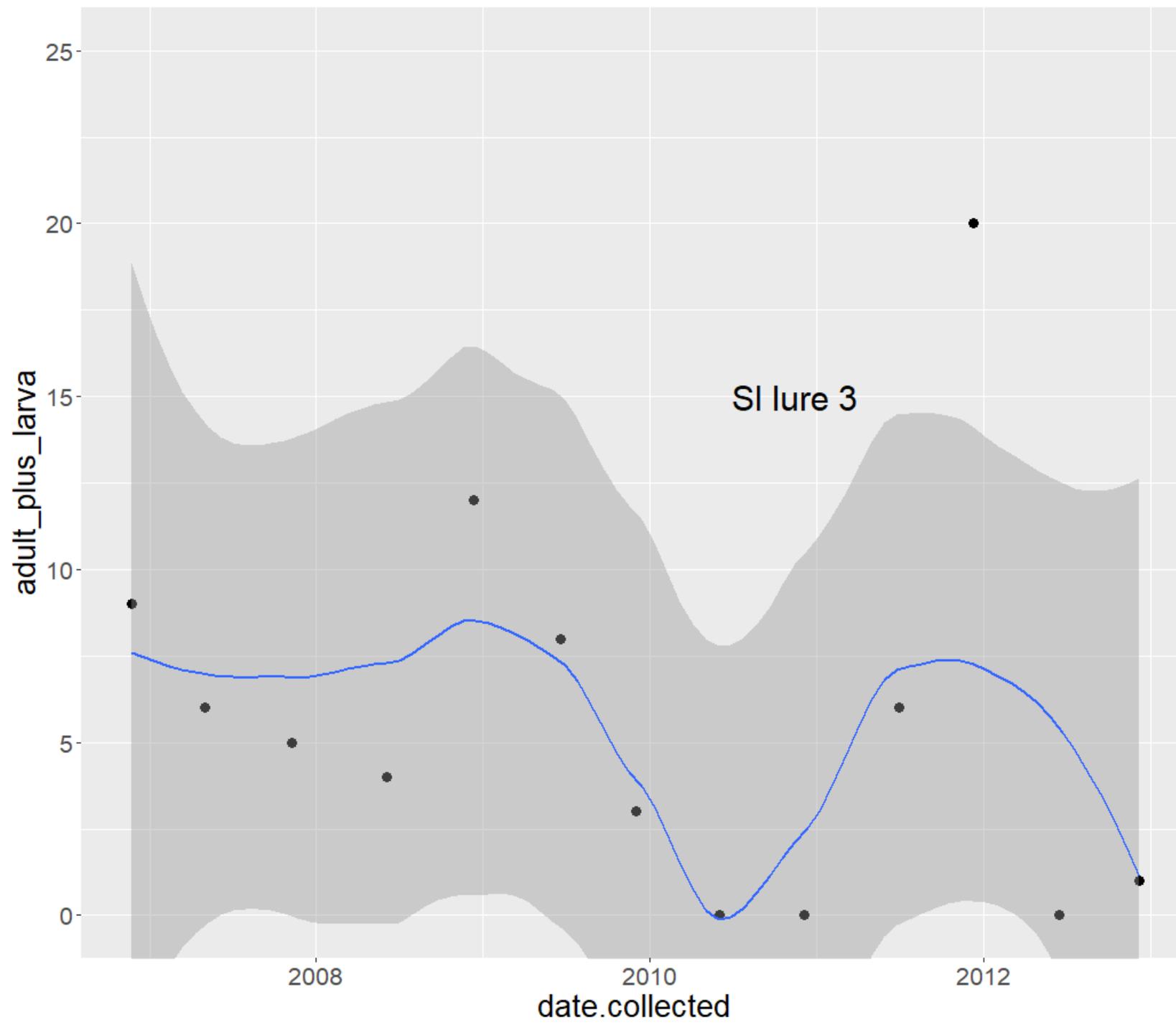
Repeated orifice
sampling - BioWest



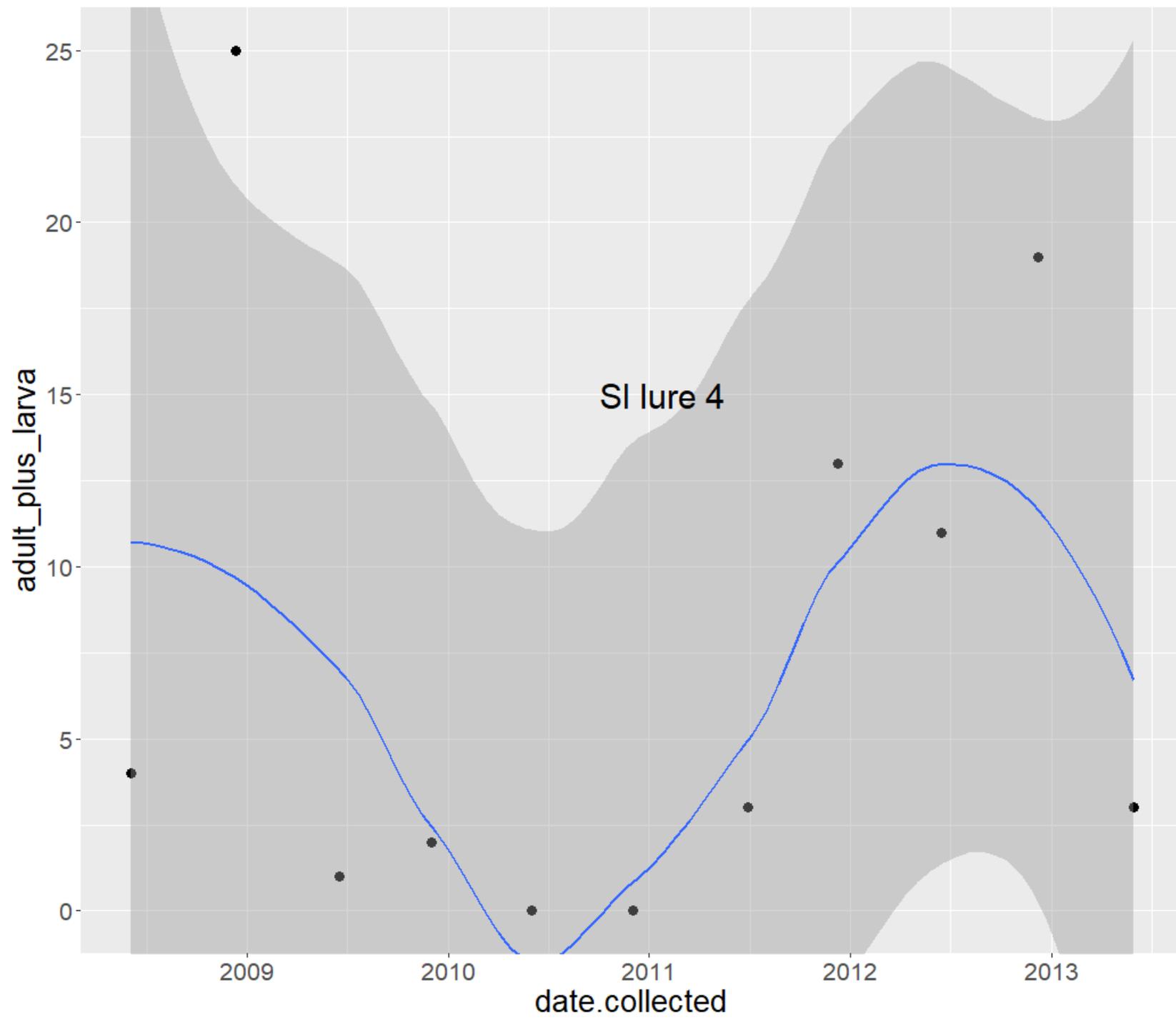
Repeated orifice
sampling - BioWest



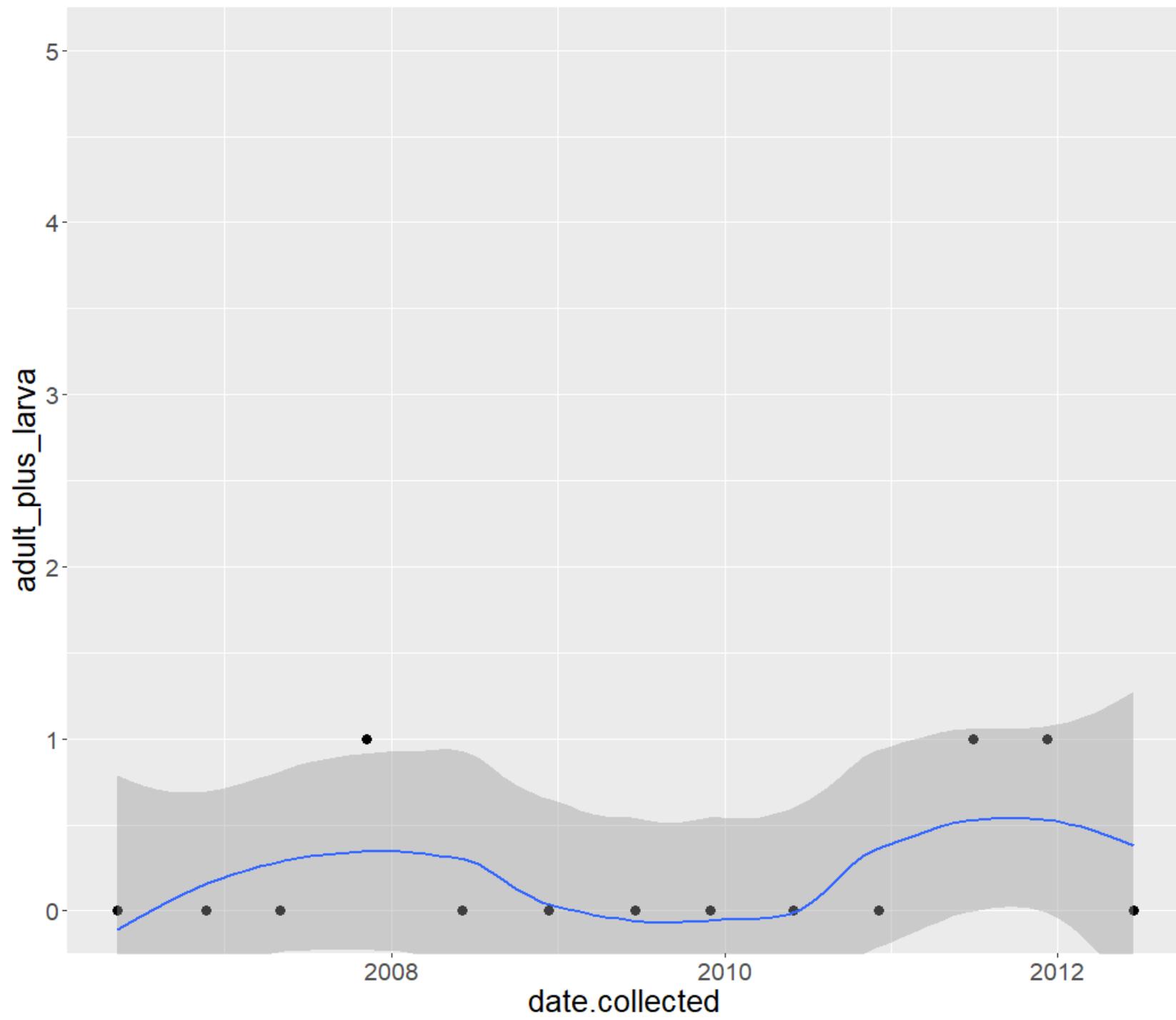
Repeated orifice
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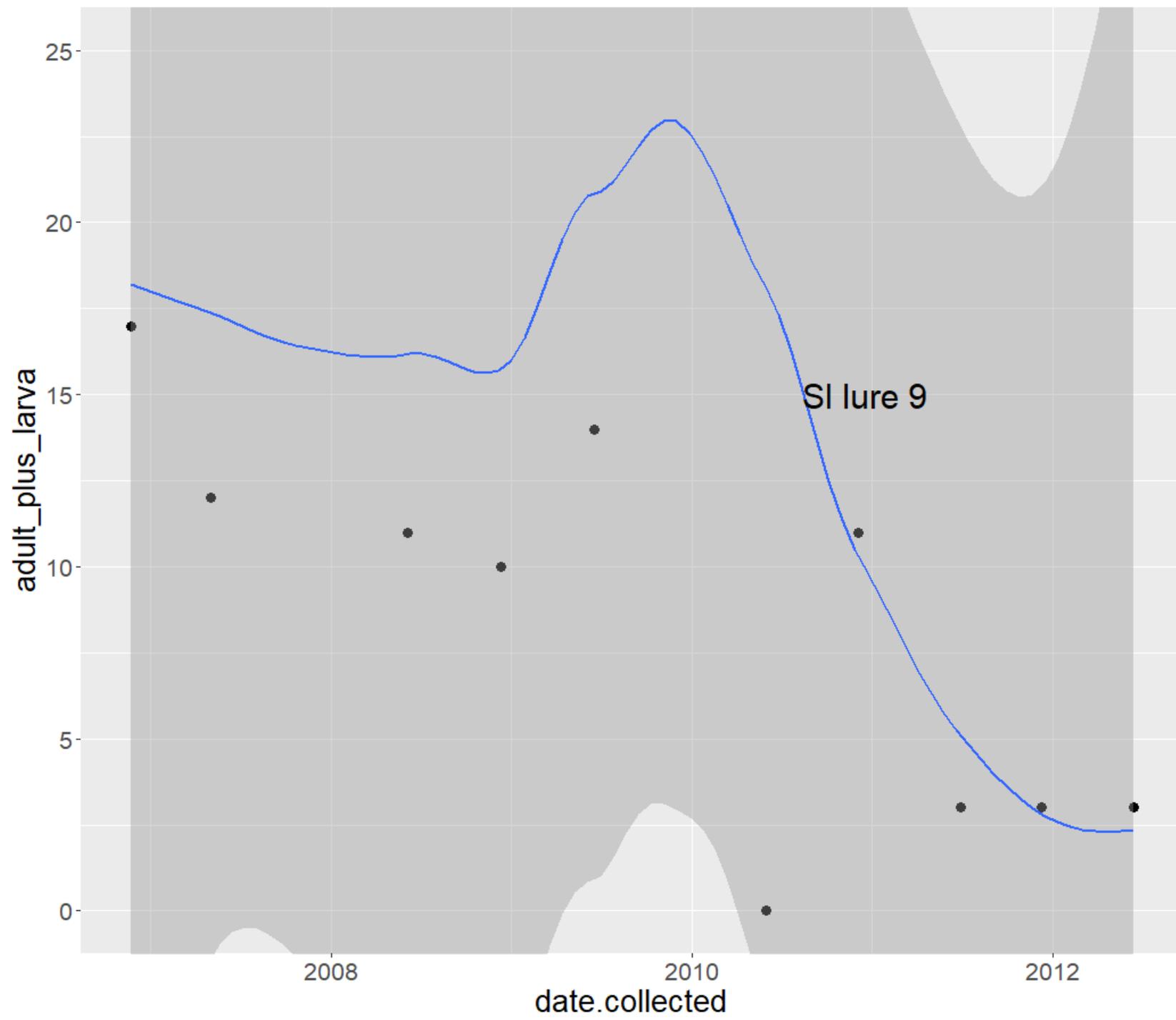
Repeated orifice
sampling - BioWest

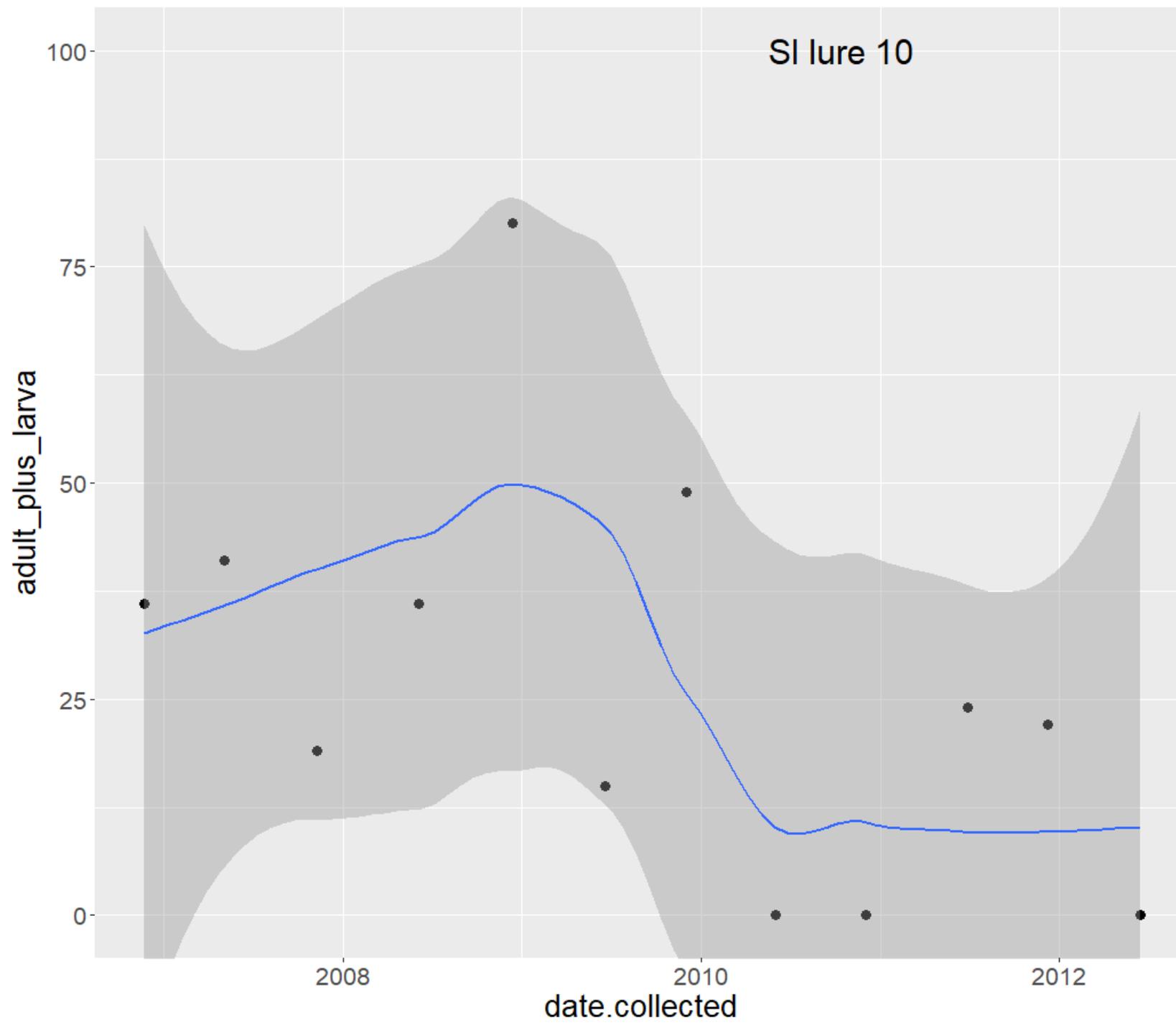


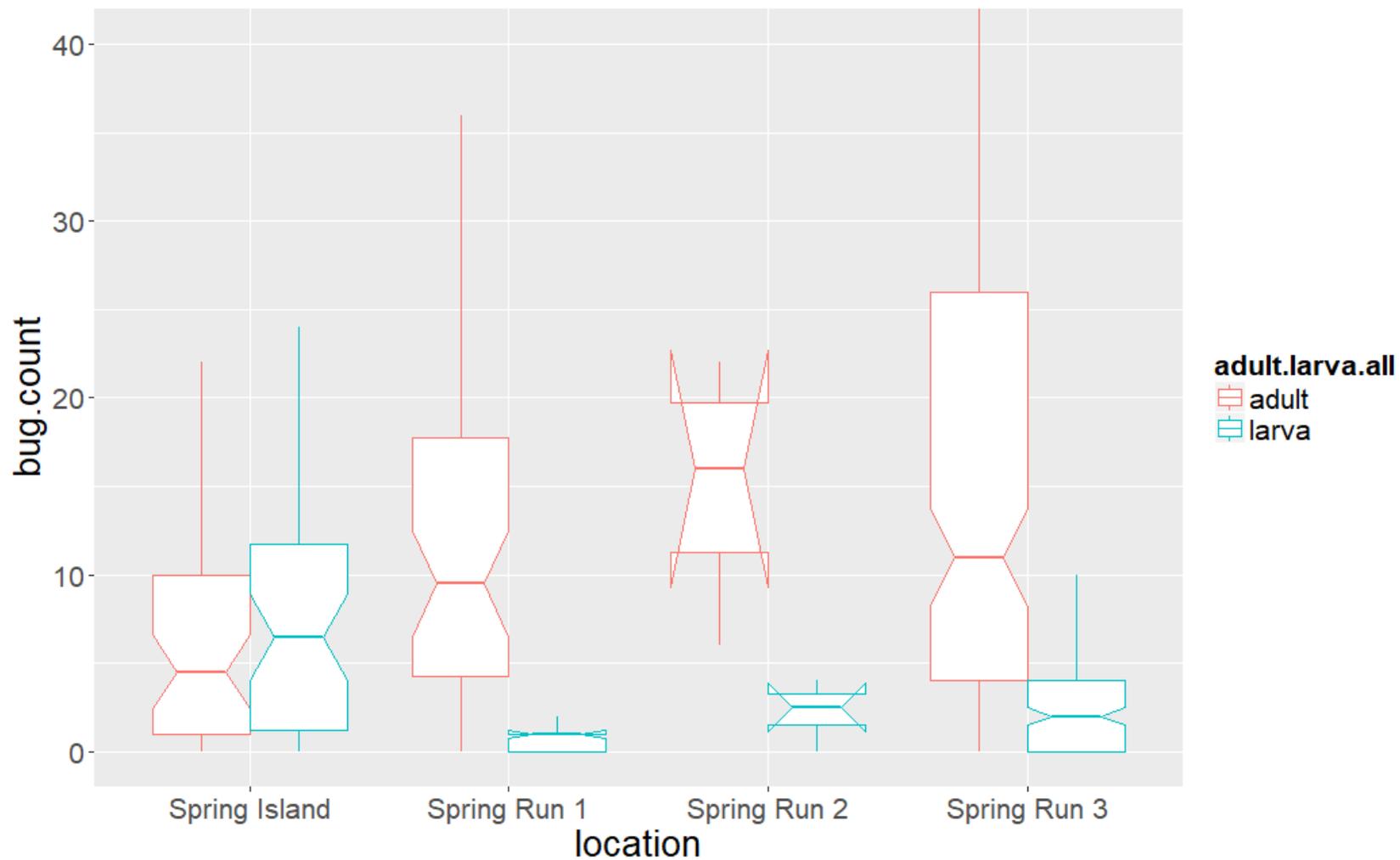
Repeated orifice
sampling - BioWest



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sampling - BioWest

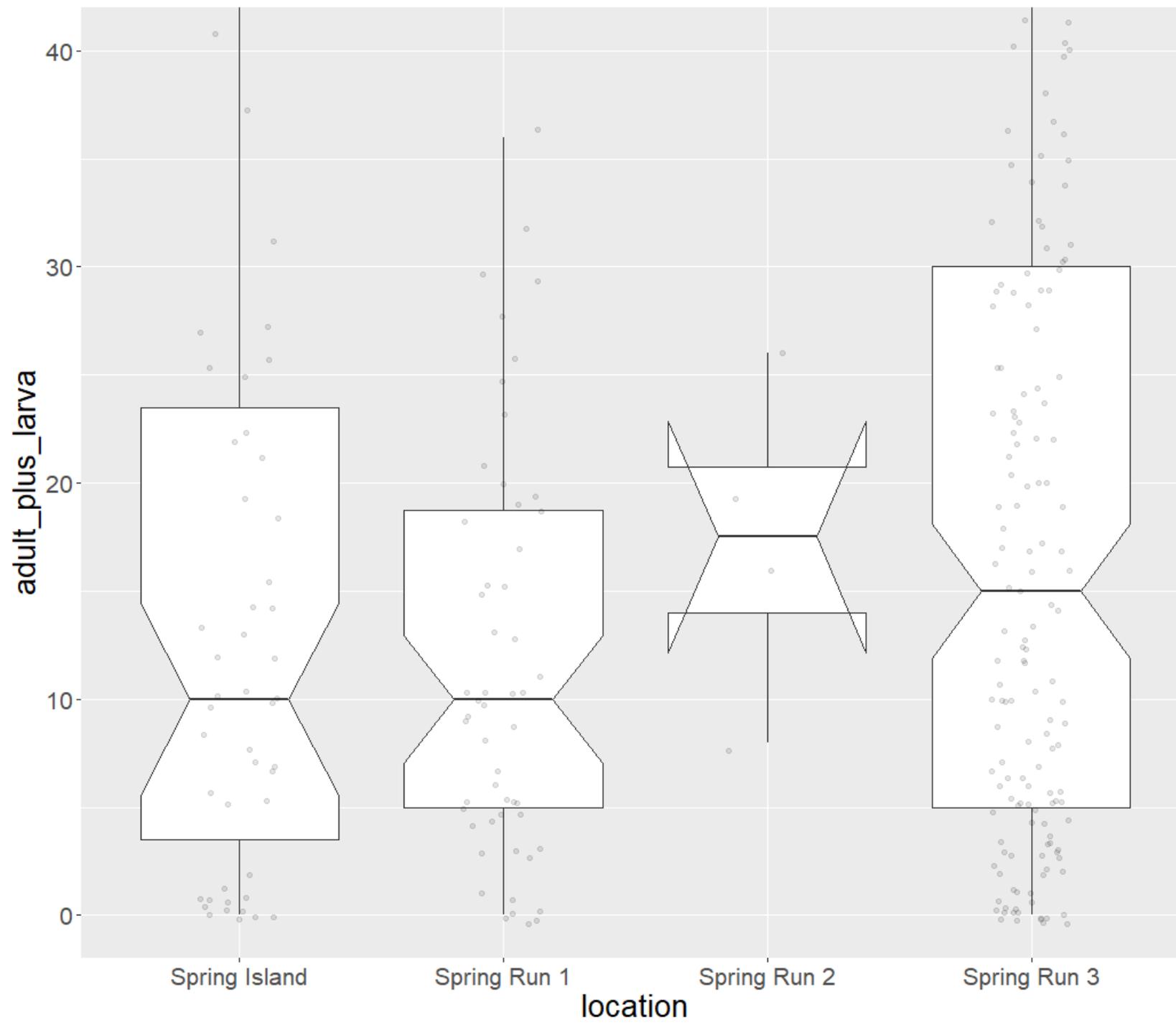




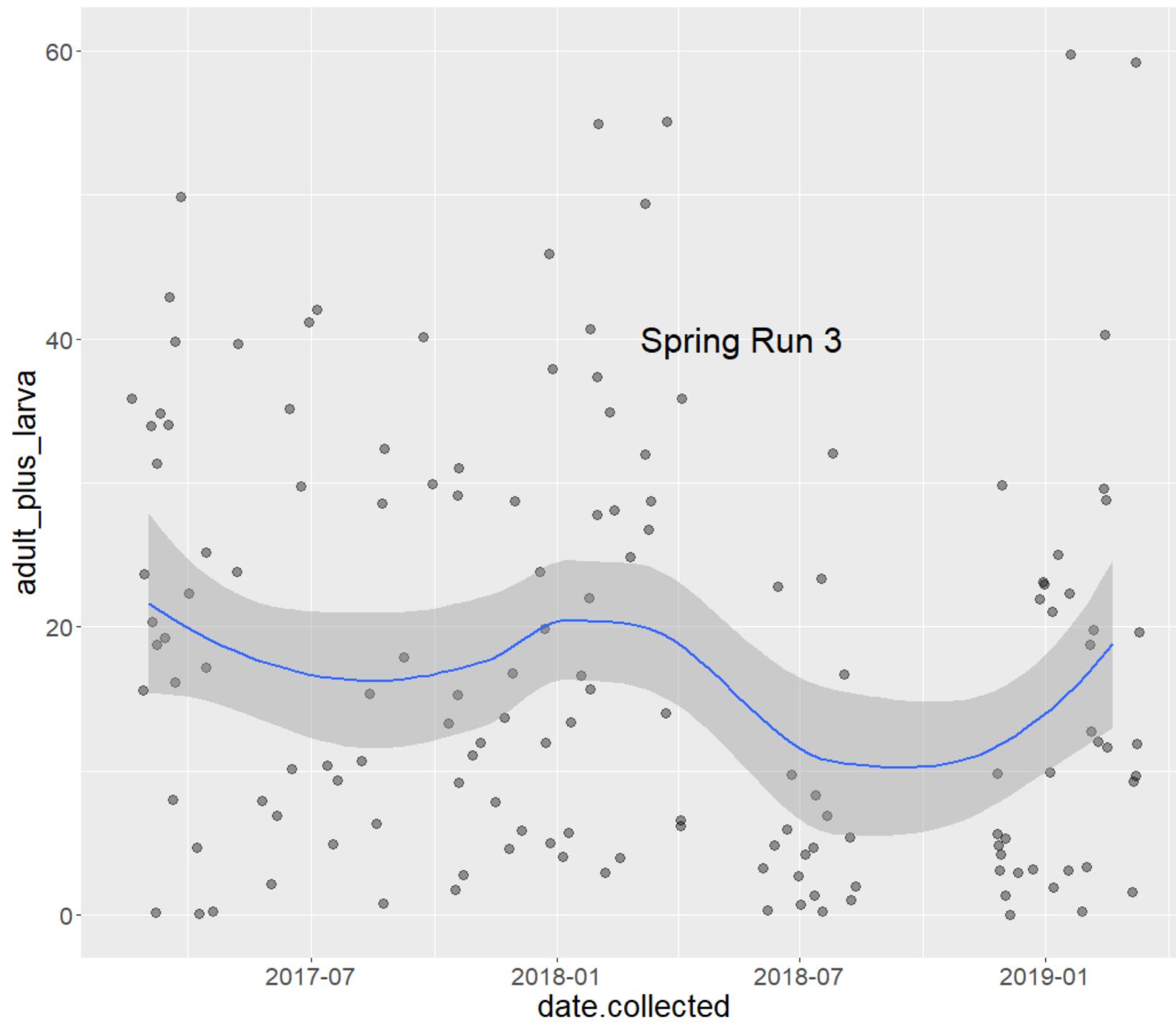


location	lures	zero counts adults	zero counts larva
Spring Island	51	11	5
Spring Run 3	162	13	47
Spring Run 1	54	1	24
Spring Run 2	4	0	1

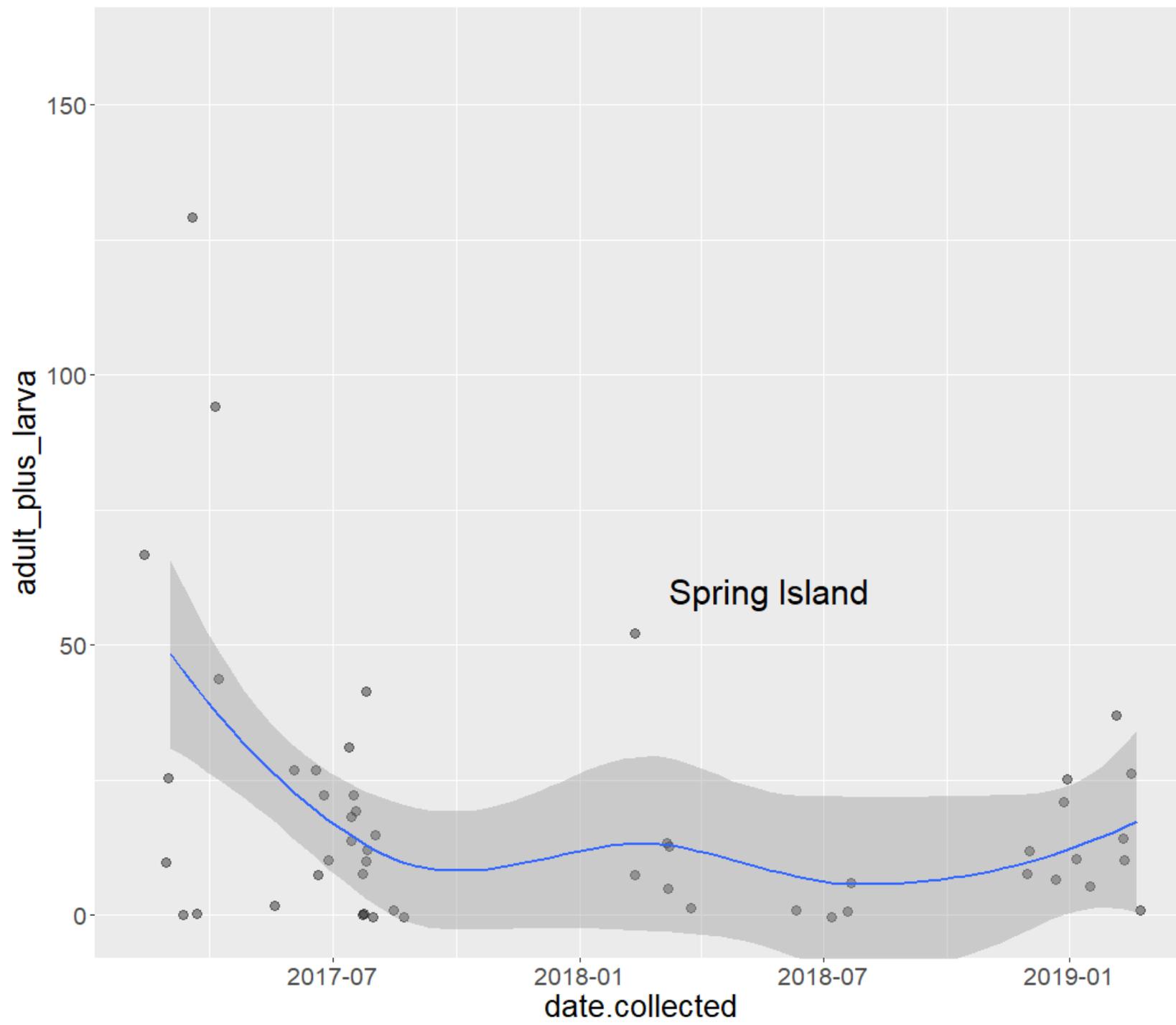
Distribution of adult and larva bug counts by location for Refugia collections 2017-2019.



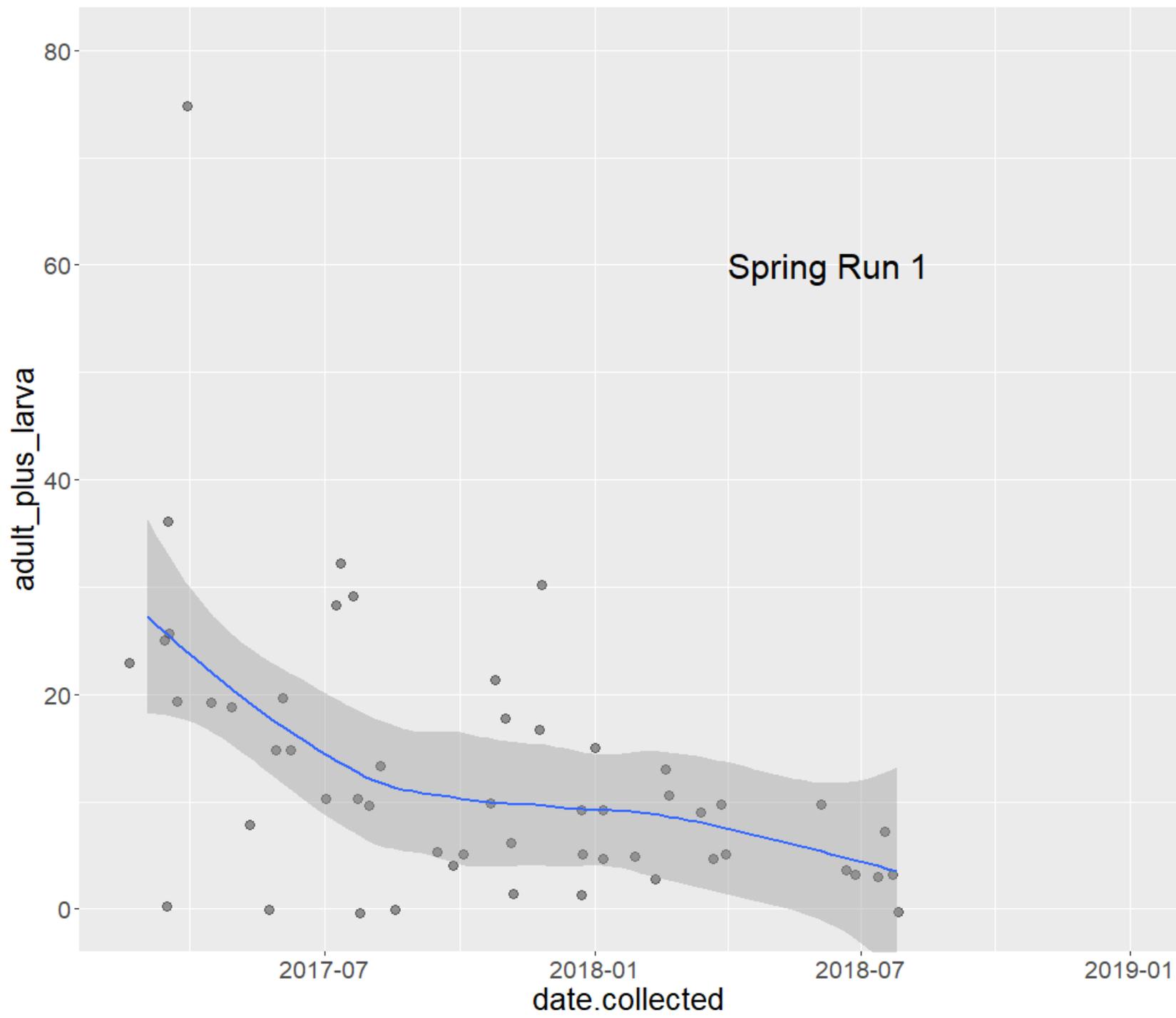
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Combined adult plus larva counts for USFWS 2017-2019. Points are jittered off of x position. Line represents local polynomial regression fitting. Base geom_smooth() determined by LOESS function. https://ggplot2.tidyverse.org/reference/geom_smooth.html

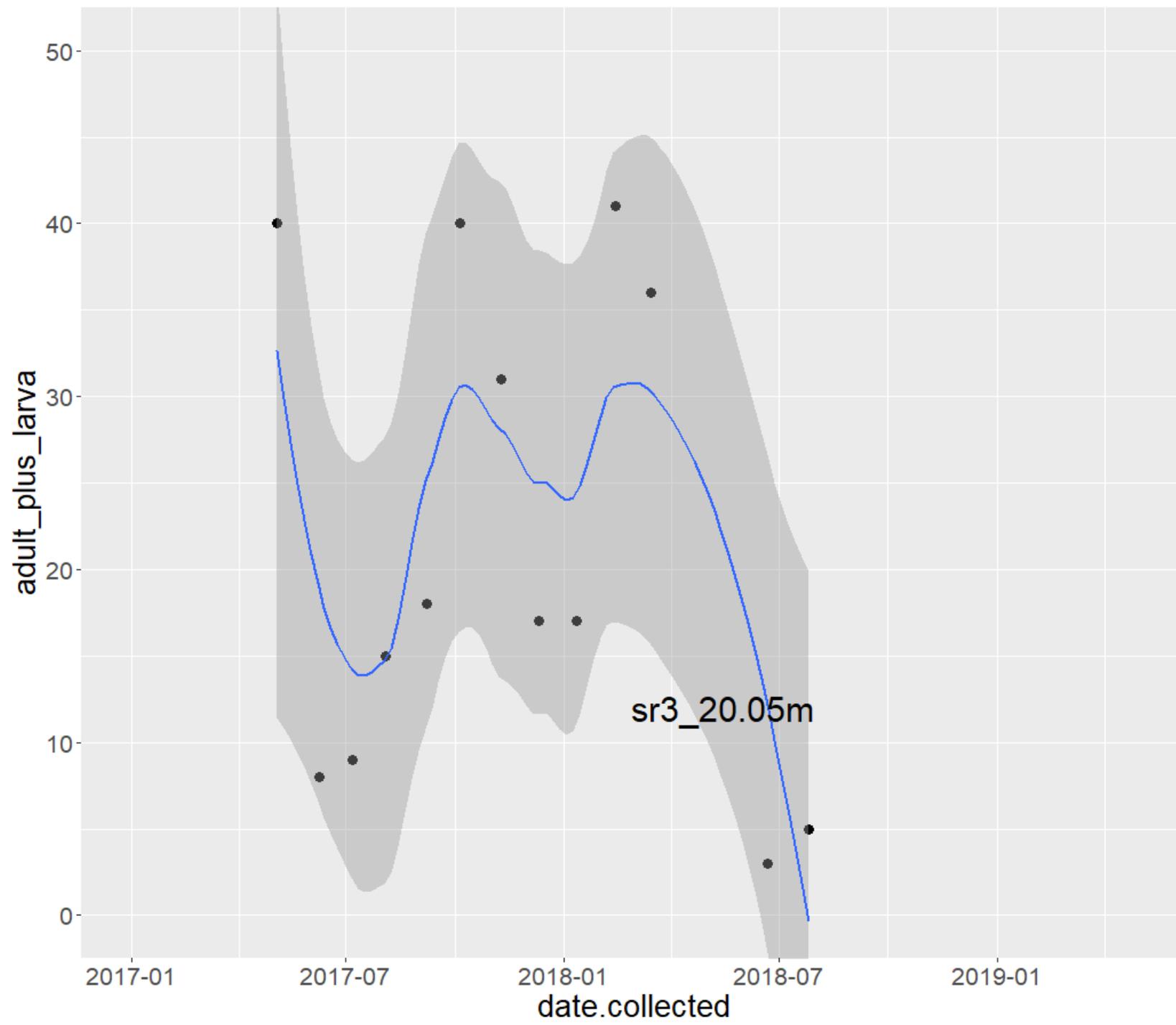


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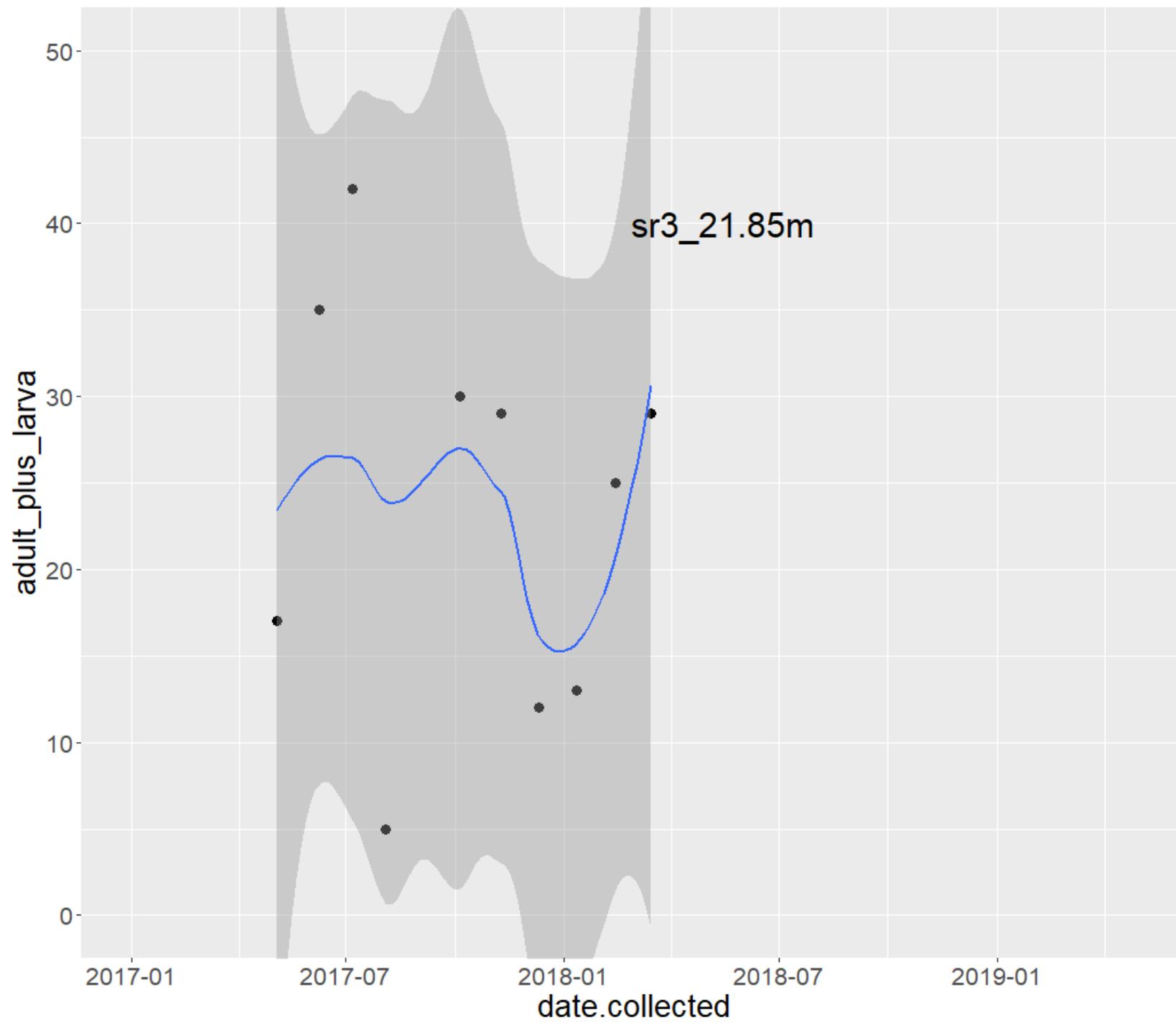


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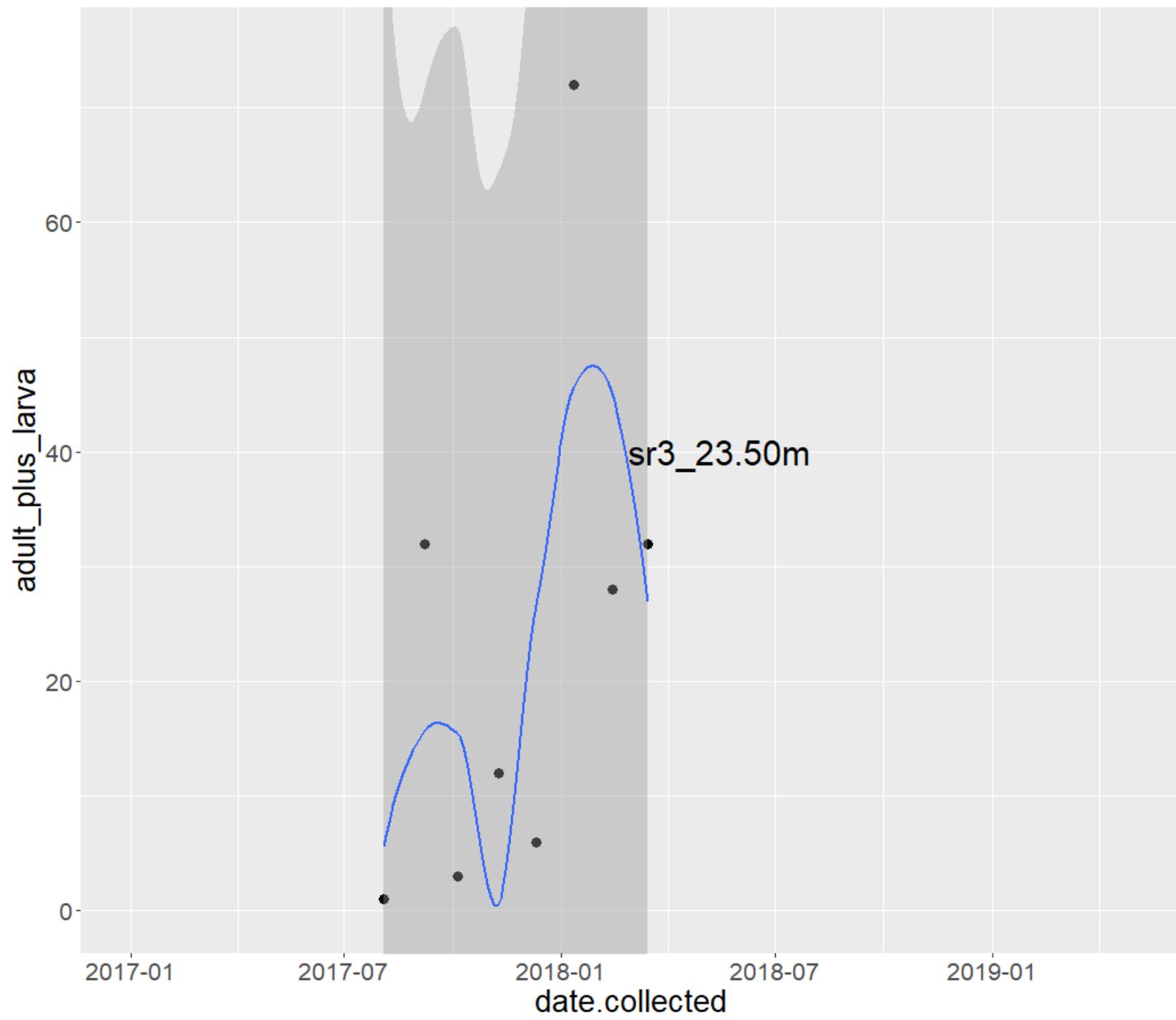
Repeated orifice
sampling - USFWS



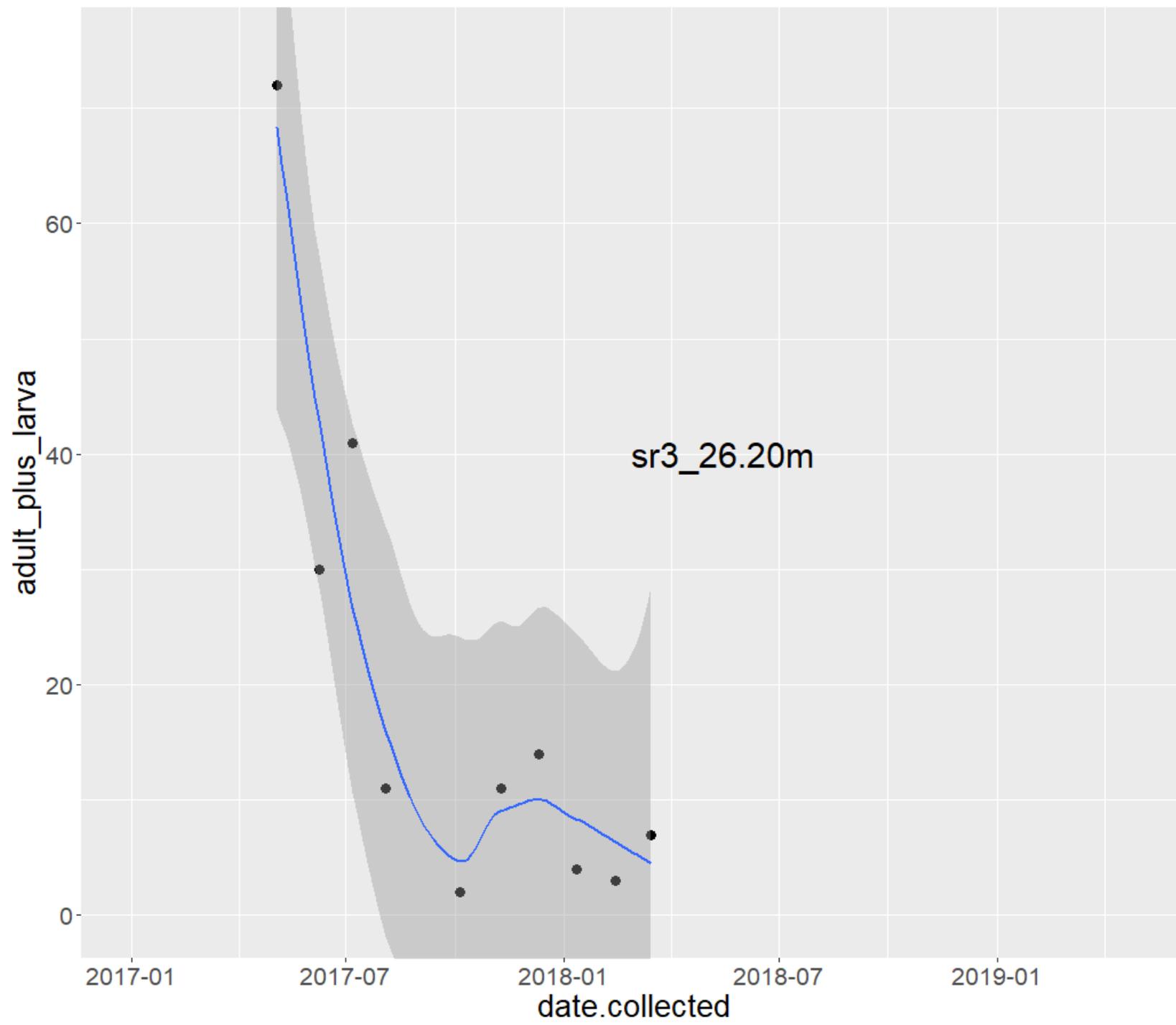
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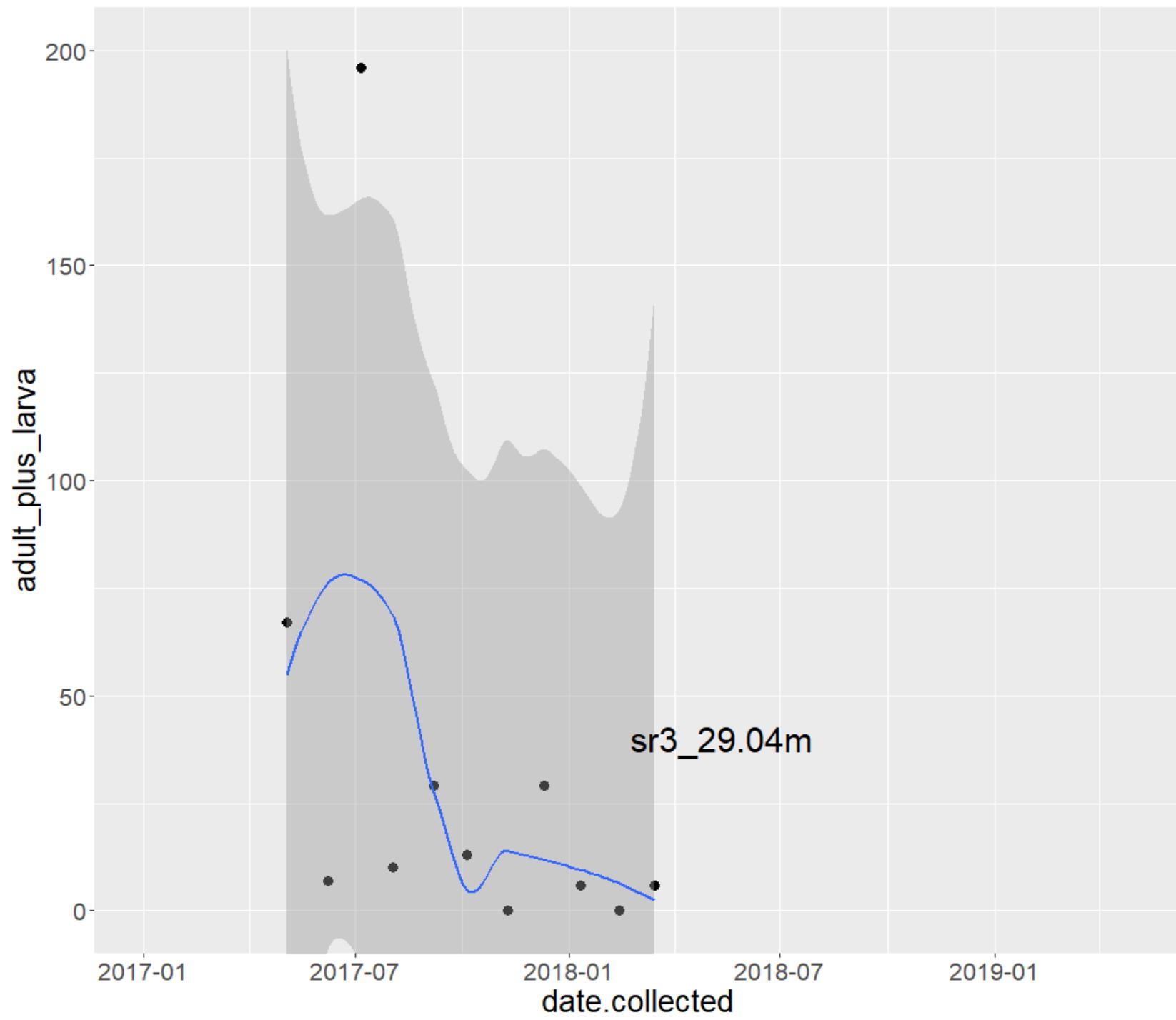
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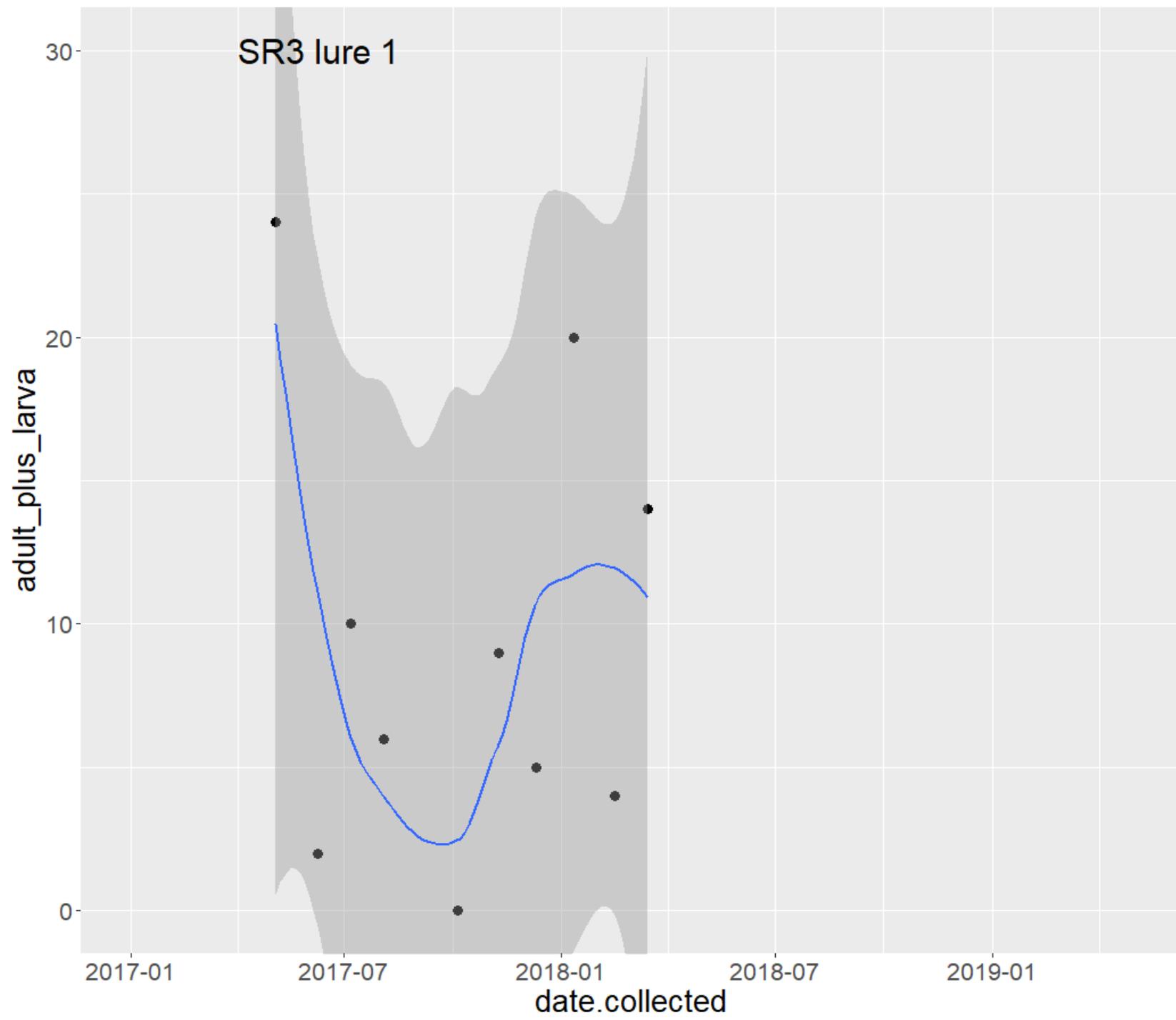
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sampling - USFWS



Repeated orifice
sampling - USFWS



Repeated orifice
sampling - USFWS





CSRB Research Assessment, Questions, Recommendations Next Steps

- Inductive/Deductive Enhancement
- Context of CSRB and Comal Springs in broader stream ecological empirical understanding and theoretical basis

Ecology and management of the hyporheic zone: stream– groundwater interactions of running waters and their floodplains

**Andrew J. Boulton^{1,6}, Thibault Datry^{2,7}, Tamao Kasahara^{3,8},
Michael Mutz^{4,9}, AND Jack A. Stanford^{5,10}**

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² *Aquatic Ecosystem Biology, CEMAGREF-Lyon 3 bis quai Chauveau, F-69336 Lyon cedex 09, France*

³ *Department of Watershed Sciences, Utah State University, 5210 Old Main Hill, Logan, Utah 84321 USA*

⁴ *Department of Freshwater Conservation, Brandenburg University of Technology Cottbus, Seestraße 45,
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⁵ *Flathead Lake Biological Station, The University of Montana, 32111 BioStation Lane, Polson, Montana,
59860 USA*

Recent trends in life-history research on benthic macroinvertebrates

Vincent H. Resh¹

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David M. Rosenberg²

Freshwater Institute, 501 University Crescent, Winnipeg, Manitoba R3T 2N6 Canada

4) the use of a species-trait approach to examine both basic and applied aspects of benthic biology, which began in the 1990s, is an expanding research area, and is a valuable application of life-history information.



J. N. Am. Benthol. Soc., 2010, 29(1):84–99
Patch dynamics and environmental
heterogeneity in lotic ecosystems

Conceptual models of patch dynamics can be traced to 2 basic approaches: 1) the landscape ecology perspective and 2) the metacommunity perspective. The former focuses on how spatial patterns are created and affect ecological processes over variable scales of space and time, whereas the latter emphasizes the important influence of periodic disturbances, refugia, and dispersal in maintaining nonequilibrium communities within patch mosaics.

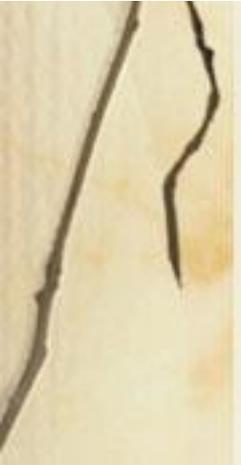


J. N. Am. Benthol. Soc., 2010, 29(1):170–181
© 2010 by The North American Benthological Society
DOI: 10.1899/09-023.1
Published online: 5 February 2010

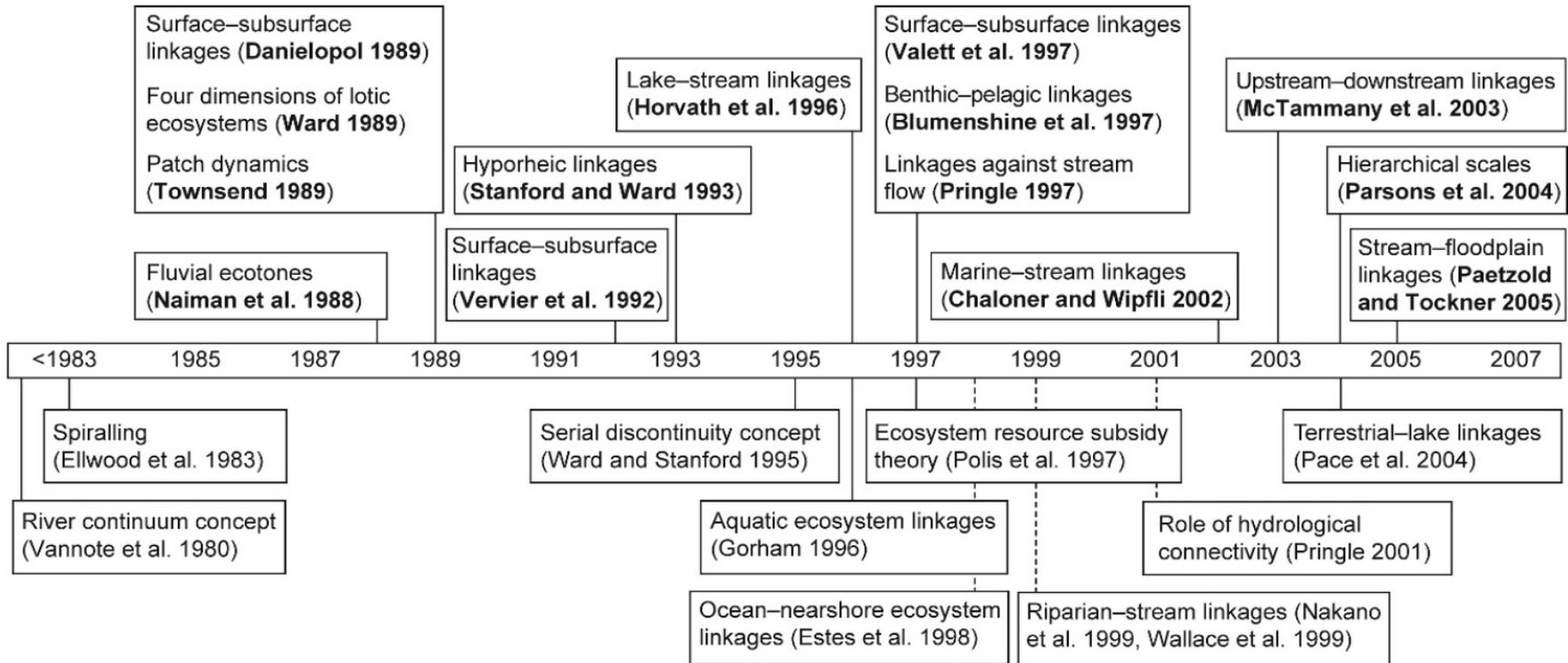
Stream microbial ecology

Stuart Findlay¹

Cary Institute of Ecosystem Studies, Box AB, Millbrook, New York 12545 USA



Linkages among aquatic ecosystems



The role of disturbance in stream ecology*

VINCENT H. RESH¹, ARTHUR V. BROWN², ALAN P. COVICH³,
MARTIN E. GURTZ⁴, HIRAM W. LI⁵, G. WAYNE MINSHALL⁶,
SETH R. REICE⁷, ANDREW L. SHELDON⁸, J. BRUCE WALLACE⁹,
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⁵ *U.S. Fish and Wildlife Cooperative Research Unit, Oregon State University,
Corvallis, Oregon 97331 USA*

⁶ *Department of Biological Sciences, Idaho State University, Pocatello, Idaho 83209 USA*

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Chapel Hill, North Carolina 27514 USA*

⁸ *Division of Biology, University of Montana, Missoula, Montana 59812 USA*

⁹ *Department of Entomology, University of Georgia, Athens, Georgia 30602 USA*

¹⁰ *Center for Streamside Studies, College of Forest Resources and Fisheries Research Institute,
University of Washington, Seattle, Washington 98195 USA*



J. N. Am. Benthol. Soc., 2010, 29(1):67–83
© 2010 by The North American Benthological Society
DOI: 10.1899/08-027.1
Published online: 5 February 2010

The evolving legacy of disturbance in stream ecology: concepts, contributions, and coming challenges

Emily H. Stanley¹, Stephen M. Powers², AND Noah R. Lottig³
Center for Limnology, University of Wisconsin, Madison, Wisconsin 53706 USA



Disturbance

 Definition: any process or condition external to the natural physiology of living organisms that results in the sudden mortality of biomass in a community on a time scale significantly shorter than the accumulation of the biomass.

Disturbance

5) Resource Availability: immediate mortality is the most dramatic effect, but most important usually are the longer-term consequences for resource availability (e.g., detritus, wood)

Drought

- Direct impacts - loss of water and flow, habitat reduction and reconfiguration
- Indirect impacts - interspecific interactions and the nature of food resources (Lake 2003)
- Reduced flow lower DO levels, harder for the fauna to persist; mortality <10 days

(Stanley et al. 1994)

Flood

- Macroinvertebrate density and diversity may decline following the flood (Scrimgeour and Winterbourne 1989)
- Substrate nature - Sandy or stable with algal mats
- Dislodgement, scouring and abrasion from high sediment loads and substrate mobilization (Collier and Quinn 2003)



Recolonization

- Organisms establish in new areas or disturbed habitats
- Occurs over broad and variable spatial and time scales (Sheldon 1984)
- Colonize on sediment surface, woody debris in streams (Thorp et al. 1985)

Recolonization continued

- Colonization of denuded substratum is a common phenomenon
- Response to sediment-scouring storms but also to other disturbances such as toxic pollutants and drying of the streambed during periods of drought

Recolonization pathways

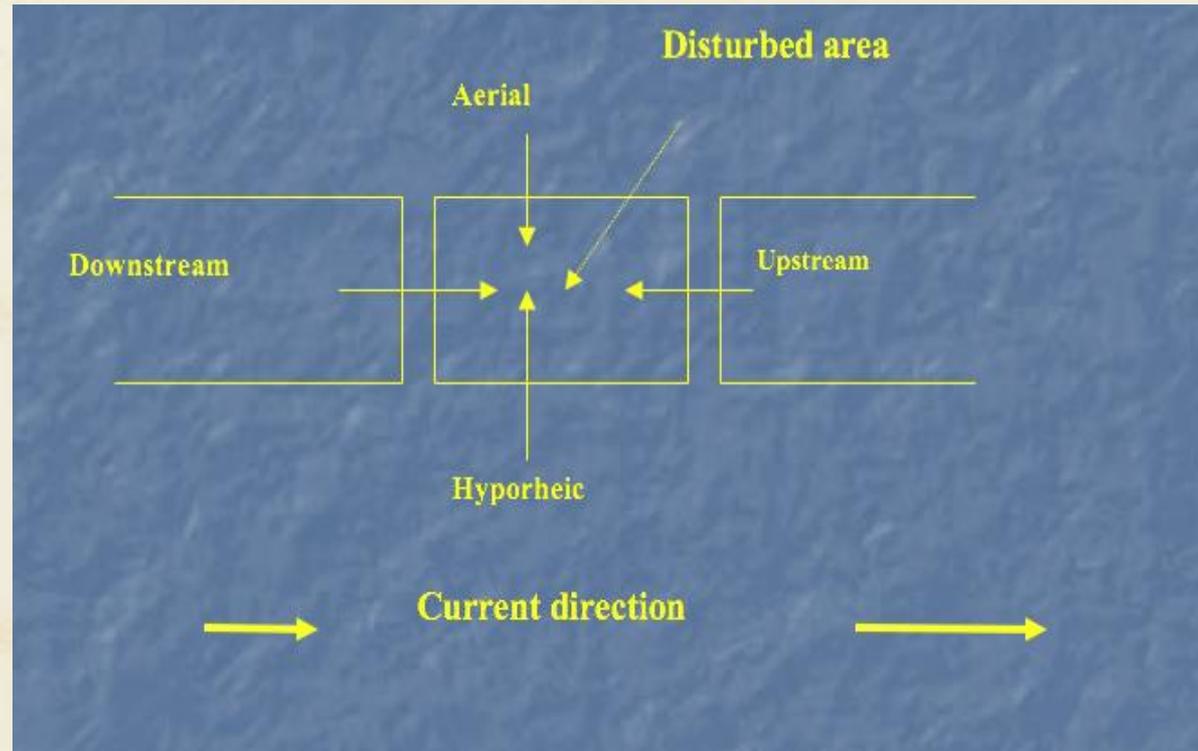
○ Aerial

○ Hyporheic

○ Upstream migration

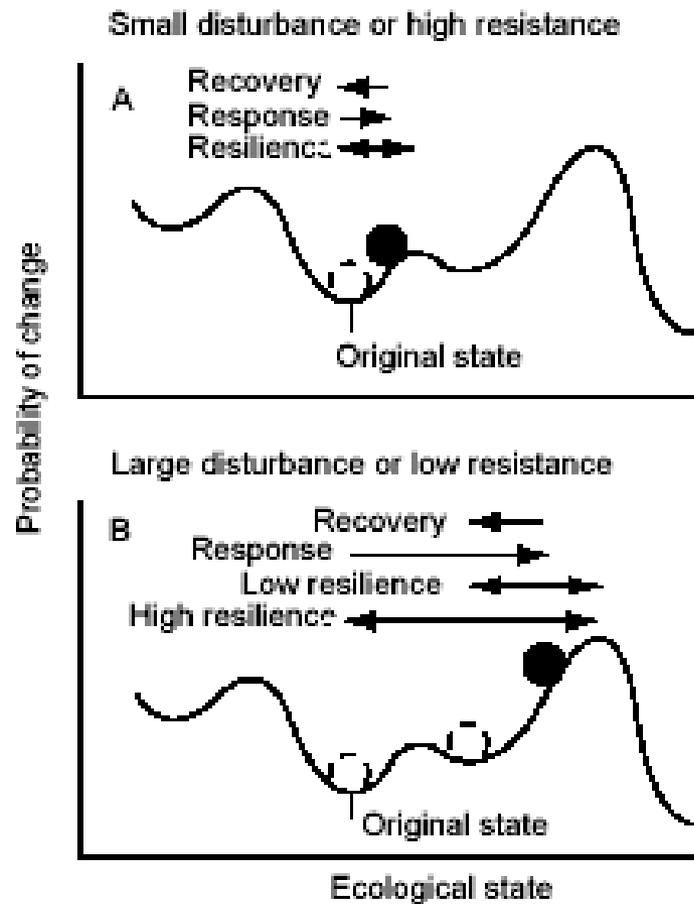
○ Downstream drift

(William and Hynes 1976)



What affects resistance and resilience?

Fig. 13.1



Comal Springs Riffle Beetle (CSRB), *Heterelmis comalensis*

- Species described from Comal Springs in 1988 (Bosse et al.)
- 1st reported in San Marcos Springs in 1993 (Barr)



Characteristics of *H. comalensis*

- Do not swim or fly
- Adults and larvae found living together
- Adults respire through plastron, larvae have gills
- Require the near-saturated oxygen levels associated with cool, fast-flowing shallow streams (Brown 1987)
- Presumably feed on fungus, algae, and bio-films
- Mostly found in substrate of the direct spring area (<80 cm), in gravel, on woody debris or roots, and under rocks (Gibson et al 2008, Cooke 2012)
- Subterranean habitat use not well-understood

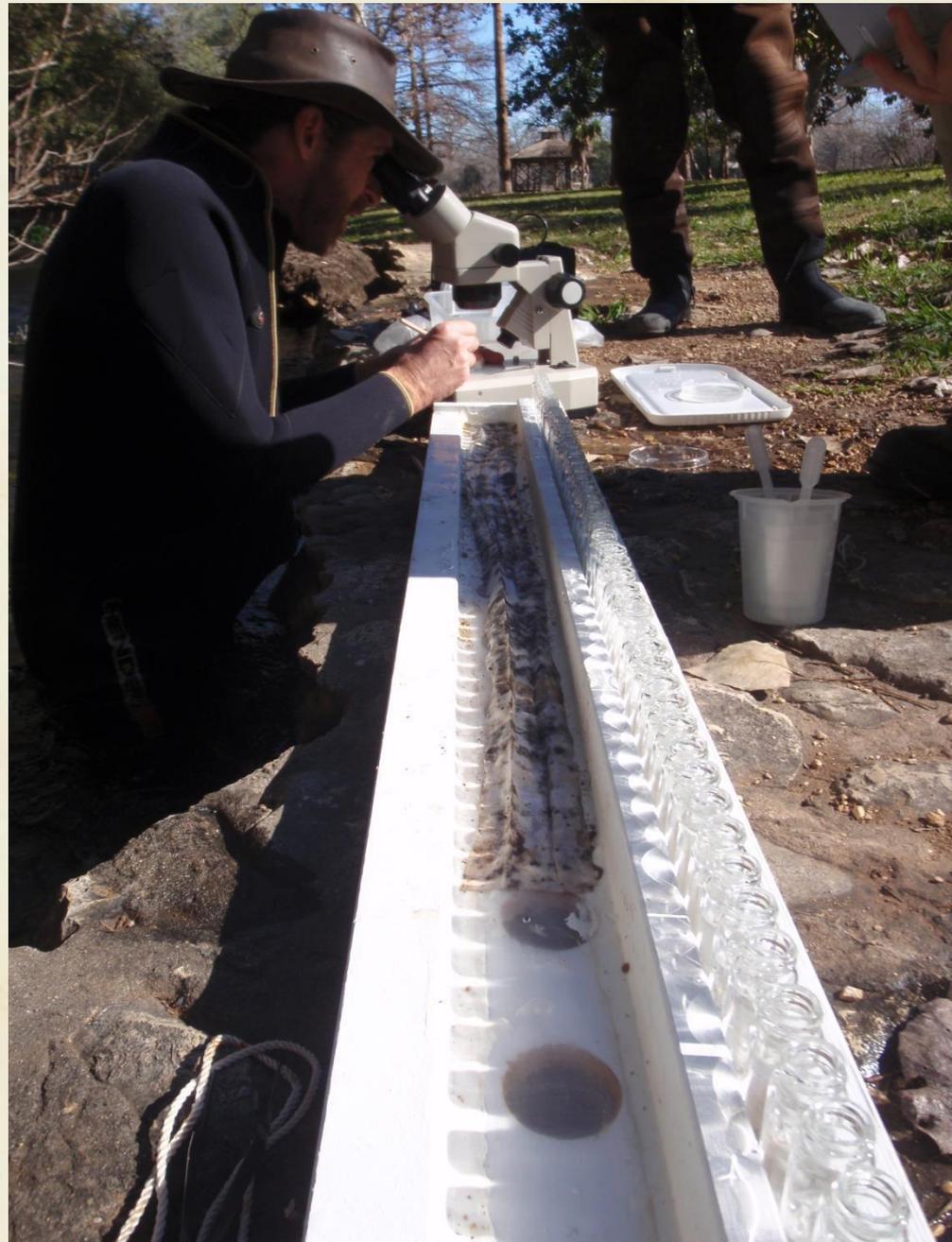


Historical Collections of CSRB

- **Various methods employed:**
 - Hand-picking
 - Meter Quadrat (Bowles)
 - Hess Sampler (Arsuffi)
 - Drift Nets (Arsuffi, Norris, Gibson)
 - Mop heads
 - Various cloth materials
 - Cotton cloth lures

Comal Springs Run 1 - Bore Hole Sampling

- CSRB “may have survived ...by retreating into the spring-heads, aquifer, or the hyporheos as spring-flows diminished” (Bowles et al. 2003).
- \approx 4 times more CSRB on lures than from surface sampling (BIO-WEST monitoring 2003-2004)
- CSRBs presumed interstitial habitat associated with spring sources (Cooke et al. 2012)

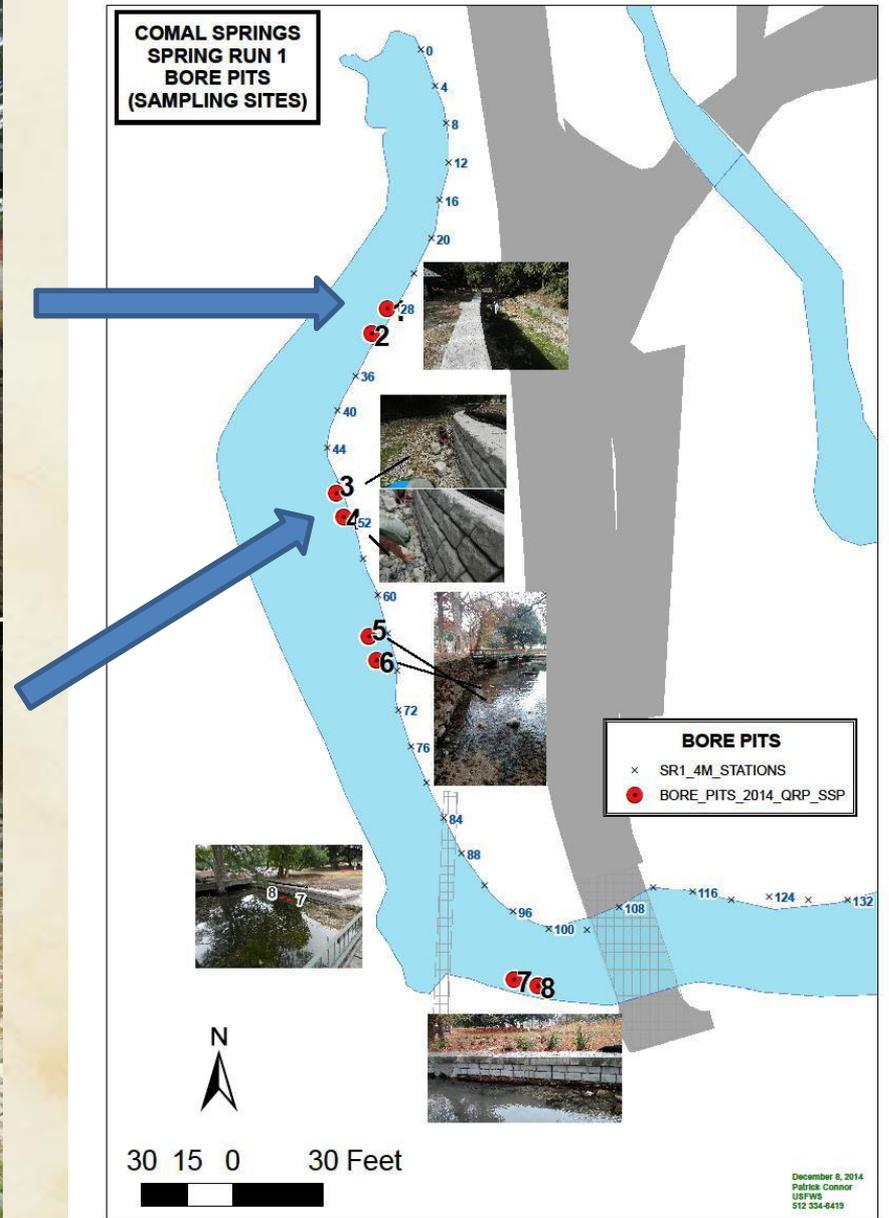


Comal Springs Run 1 - Bore Hole Sampling

- USGS/ES installed 4 pairs of sampling ports in Spring Run 1 from Sep to Oct 2014
- Stainless steel screen 4" diameter, ca. 2' deep, 5 mm pore size



Comal Springs Run 1 - Bore Hole Sampling



Comal Springs Run 1 - Bore Hole Sampling

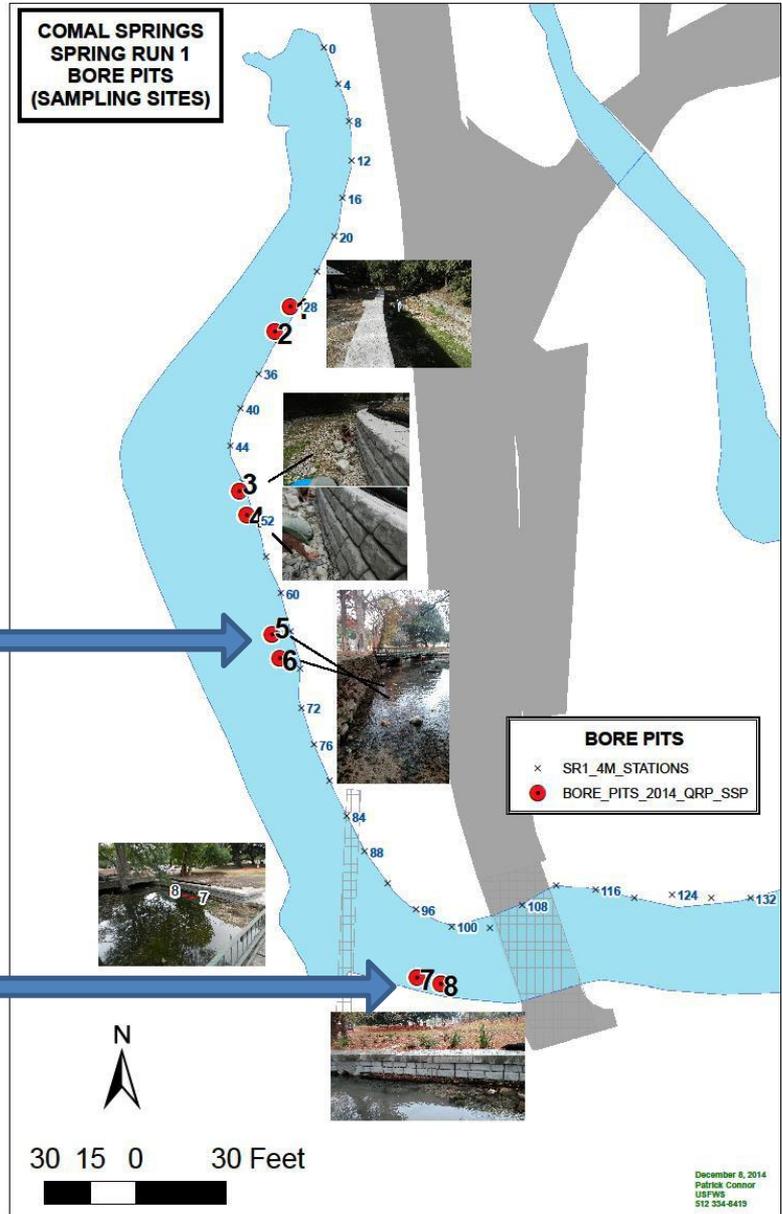
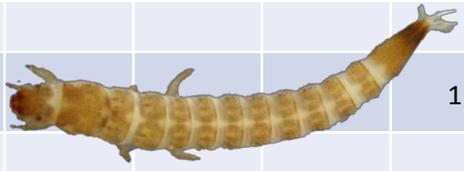


TABLE 2 — Vertical habitat parameters of each trap. Negative numbers are estimates of dry space inside a trap, estimated as vertical difference at stream surface equal to zero.

Trap #	Distance (m) from Primary Spring	Trap depth (cm)	Difference of water depth to trap top (cm)	Total depth from stream surface to trap bottom (cm)
1	29	71.12	-17.78	53.34
2	32.5	75.565	-16.51	59.055
3	49.3	71.12	-3.81	67.31
4	51.8	81.28	-2.54	78.74
5	78.8	66.04	15.24	81.28
6	80.9	62.23	7.62	69.85
7	83.3	92.71	0	92.71
8	84.6	83.82	31.75	115.57

TABLE 3 — Number and identification of fauna removed from traps during 26 Sept - 8 Oct 2014.

Taxa	Trap # 3			Trap # 4		
	Upper	Mid	Lower	Upper	Mid	Lower
Gastropoda						
Thiaridae						
<i>Tarebia</i> sp.	30			20	9	3
Insects						
Coleoptera						
Elmidae						
<i>Microcyloepus</i> sp. larval				1		
Psephenidae						
<i>Psephenus</i> sp.					2	1
Trichoptera						
Helicopsychidae						
<i>Helicopsyche</i> sp.						1
						

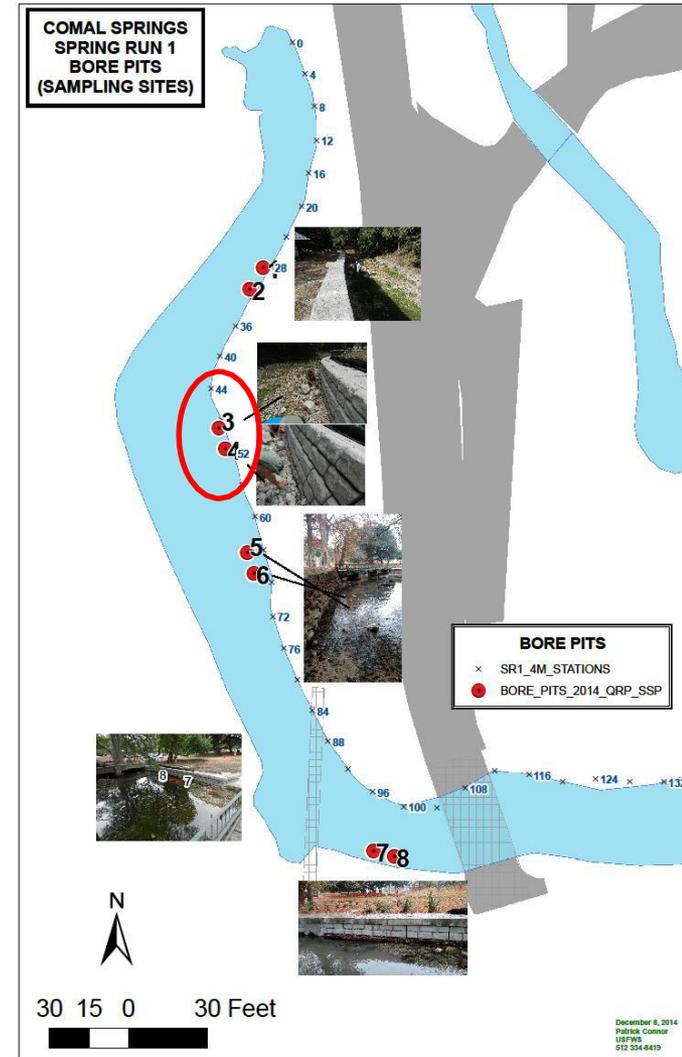
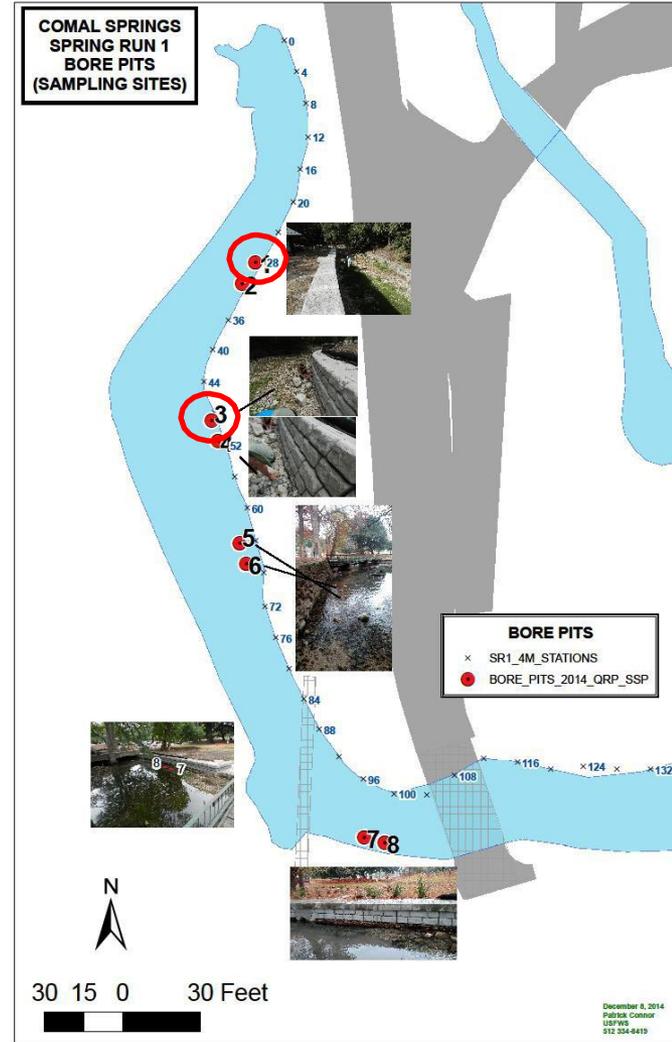


TABLE 3 — Number and identification of fauna removed from traps during phase one.

Taxa	Trap # 1		Trap # 3		Trap # 5		Trap # 7	
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
Gastropoda								
Physidae			12					
Thiaridae								
<i>Tarebia</i> sp.		1	4		2		2	
<i>Melanooides</i> sp.	2							
Decapoda								
Crayfish	1			1				
Insects								
Coleoptera								
Elmidae								
<i>Microcylloepus</i> sp.								
adult				1				
larva				1				
Diptera								
Chironomidae		2						



Conchal Springs Run 1 - Bore Hole Sampling

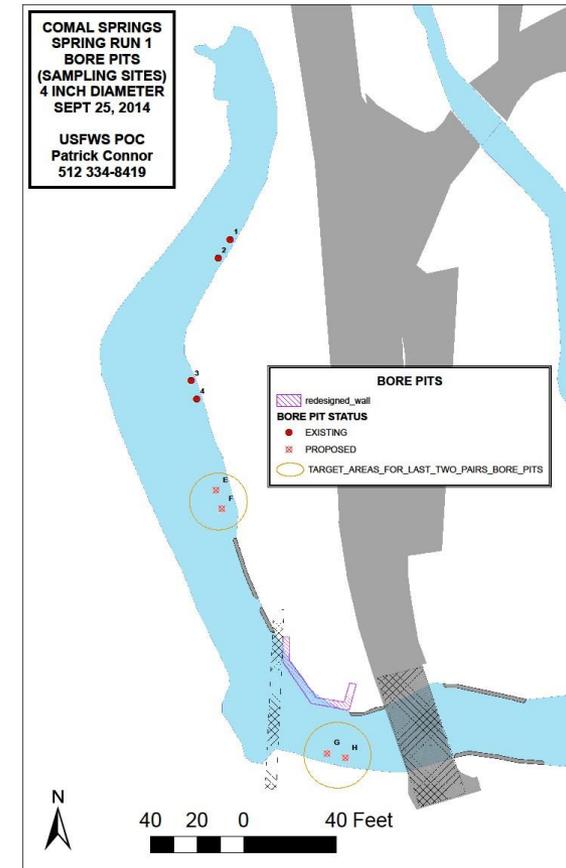
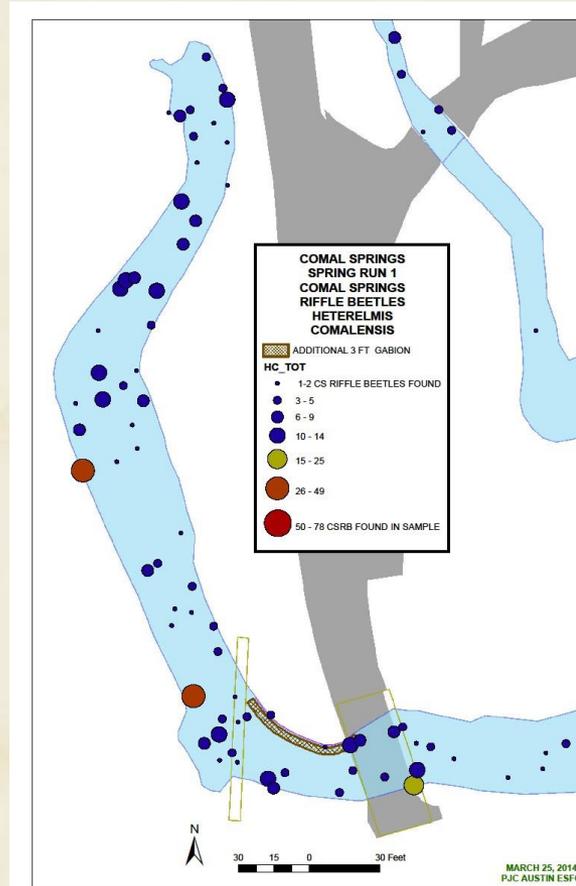
- Inserts with lure material and spacers to prevent mixing.
- Sediment influx, removed by electric and hand pumps.
- WQ: Temperature decreased 2 °C at furthest point.
- No CSRB captured. Adults and larvae of *Microcylloepus* captured in upstream pits. Downstream pits anoxic.



Comal Springs Run 1 - Bore Hole Sampling

Recommendations

- Sample in areas of typical CSRIB habitat with spring outflow and low silt.
- Sample Run 3, western shore, Spring Island
- Hand bury slotted pvc pipe or drive sampling points (Bou-Rouche)
- Sample WQ from deeper section of pit while lure inserted





J. N. Am. Benthol. Soc., 1999, 18(3):344-362. Hyporheic invertebrates-the clinal nature of interstitial communities structured by hydrological exchange and environmental gradients MATTHIAS BRUNKE1 AND TOM GONSER

- Hyporheic invertebrates: 1) different hydraulic gradients: infiltration, exfiltration, and horizontal advection;
- 2) different sediment depths: 20, 50, 100, and 150 cm from the sediment surface;
- 3) different environmental gradients: physico- chemical and particle variables; and
- 4) different interactive combinations between subsurface hydrology, sediment depth, and environmental gradient.
- **Suggest similar study on different types of springs to quantify abiotic vertical conditions wrt to CSR. Many studies show no recovery following floods from hyporheic, although this is a common hypothesis.**

J. N. Am. Benthol. Soc., 1993, 12(1):84-93. Experimentation in the hyporheic zone: challenges and prospectus. MARGARET A. PALMER



Challenge	Technical/conceptual development	Examples of critical experimental work
Conceptualizing the boundaries of the hyporheic zone	<p>Shift research focus from a search for universal hyporheic "models" to reliance on between-system heterogeneity to inspire experiments.</p> <p>Increase replication of experimental units.</p>	<p>Identify key parameters leading to major between-stream differences in the nature and extent of the hyporheic zone.</p> <p>Determine the functional relationship between these key parameters and local biological and physical processes.</p>
Developing tools for collecting quantitative and unbiased samples	<p>Design sampling devices that can penetrate deep into the stream-bed.</p> <p>Develop devices for ensuring intact sample retrieval.</p> <p>Prevent sample contamination and sampler avoidance.</p> <p>Calibrate sampling devices and quantify sampling zone of the device.</p>	<p>Assess the relative efficiencies of different sampling devices across substrate types.</p> <p>Evaluate the effect of an experimental manipulation or sampling device on the process under study.</p>
Evaluating the impact of bed movement on hyporheic processes	<p>Design devices for quantifying near-bed and deep-bed substrate movement.</p> <p>Design experiments that can be extrapolated across hydrological conditions</p>	<p>Determine the effect of bed movement on nutrient exchange between the hyporheic, groundwater, and surface water zones.</p> <p>Determine the relationship between carbon storage in the hyporheic zone and bed movement.</p> <p>Assess the role of bed movement in constraining biotic processes in the hyporheic zone.</p> <p>Determine if transport events modify hyporheic community dynamics.</p>
Measuring and manipulating subsurface flows	<p>Develop techniques for measuring subsurface flows over small spatial scales</p> <p>Design artificial streams and hyporheic incubation chambers that adequately simulate subsurface flows.</p>	<p>Determine how subsurface flows influence: nutrient uptake and storage, primary production, food availability and secondary production, solute exchange, faunal dynamics, retention of organic matter.</p>
Accounting for exchanges between the water column, the hyporheic zone, and the groundwaters	<p>Develop field techniques for manipulating or controlling these exchanges; at a minimum, incorporate measurement of these exchanges into field protocols.</p>	<p>Determine the relative importance of surface water vs. groundwater inputs on hyporheic processes.</p> <p>Determine the effect of local conditions on the flux of water and material between the surface waters, the hyporheic zone, and the groundwater.</p>



CSRB Life History

- Asynchronous
- Multivoltine
- Detritivore/Herbivore:
Shredder/Scraper? (Examine
Mandibles, Isotopes, microbial biomass)
- K- Strategist (< growth rates, longevity,
reproduction)

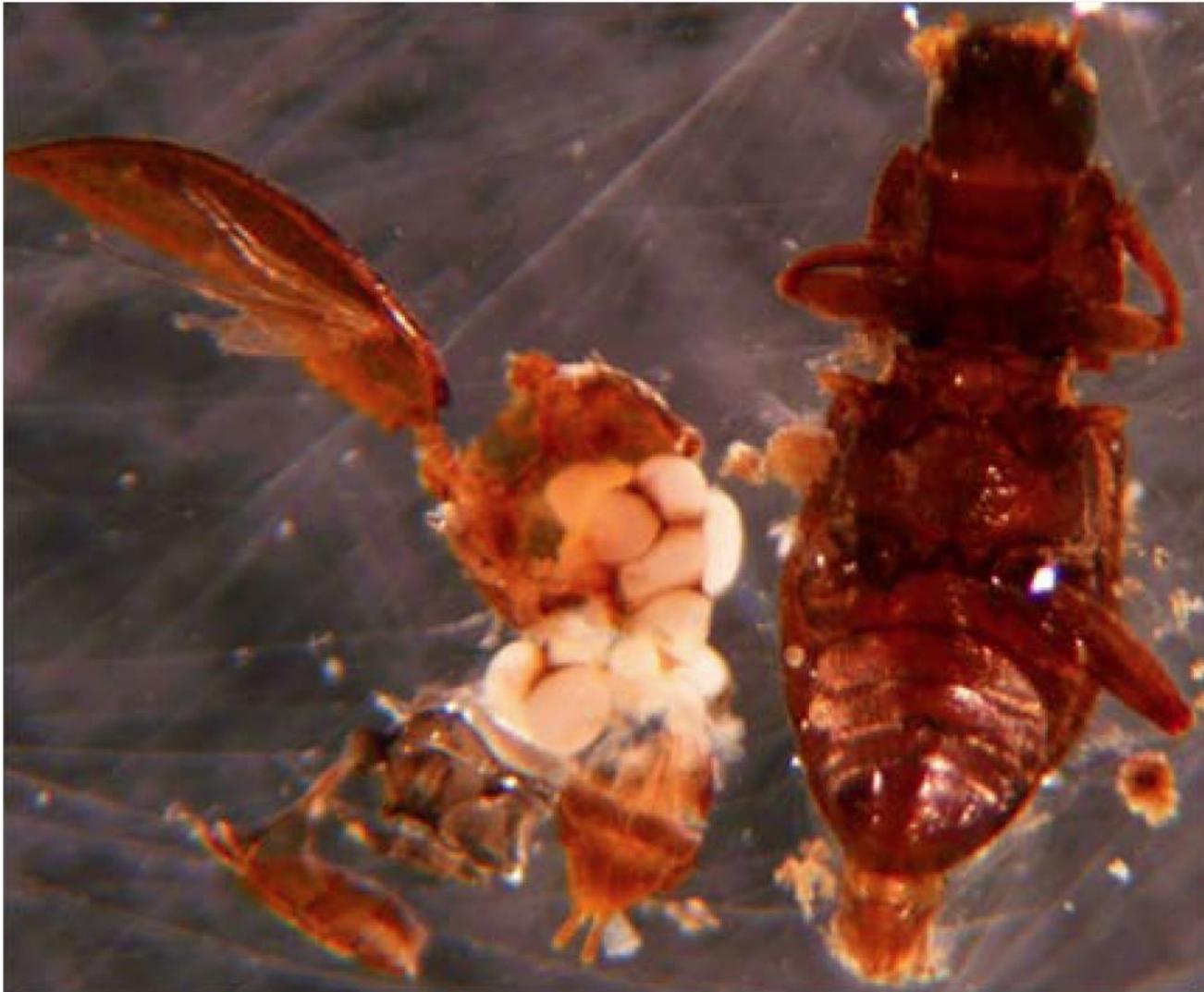


Figure S1. Photograph of a dissected single female in captivity that was found to be carrying around 10 relatively large eggs

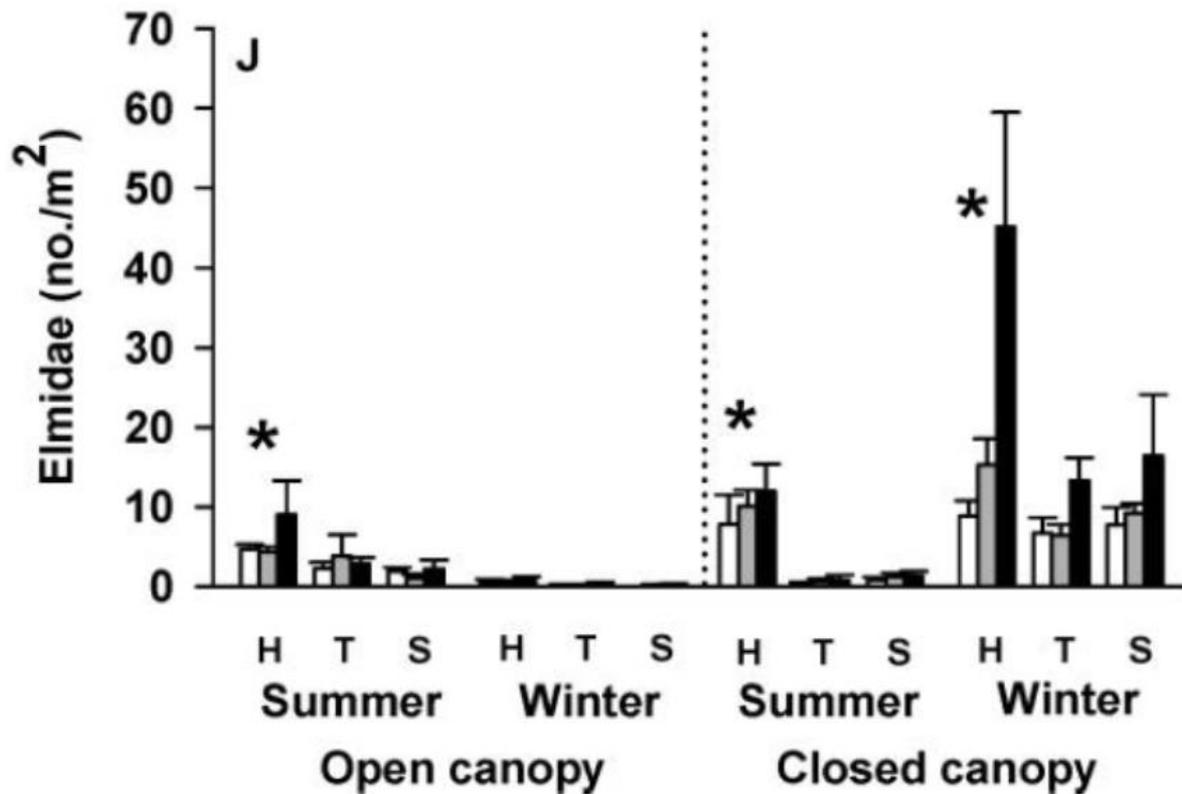
CSRB Questions for Evaluation

- **Separation of natural variability from perturbation-induced variability for population estimates**
- **What is appropriate spatial scale for sampling CSRB for biological goals? surface/hyporheic; spring; rock; wood; M2; cotton lure**
- “if the appropriate spatial scale for a study cannot be determined a priori, ecological field studies should be conducted across a variety of spatial scales (Ives et al. 1993). Use of plotless designs and related spatial statistics have rarely been pursued by benthic ecologists, although the technical and statistical machinery needed are widely available (e.g. Muotka & Penttinen 1994; Cooper et al. 1998).”

Sampling Frequency on CSRB Abundance

- Many studies (flood, drought, pollution) show invertebrate recovery times of a few months to several years.
- Recovery a function of life history traits (r-K, temperature, growth rates....).
- Recovery a function of disturbance characteristics (intensity, frequency...).
- Recovery a function of combination of disturbance events.
- Recovery from disturbance of CSRB is likely slow.
- Is hyporheic zone a refugia? Need spatial, temporal and vertical characterization. Frequent hypoxia of cotton lures with depth suggest hyporheic is limited away from spring orifices.

Scales of patchiness in the response of lotic macroinvertebrates to disturbance in a regulated river/
C. T. Robinson (5, 10, None, 3 brick types)





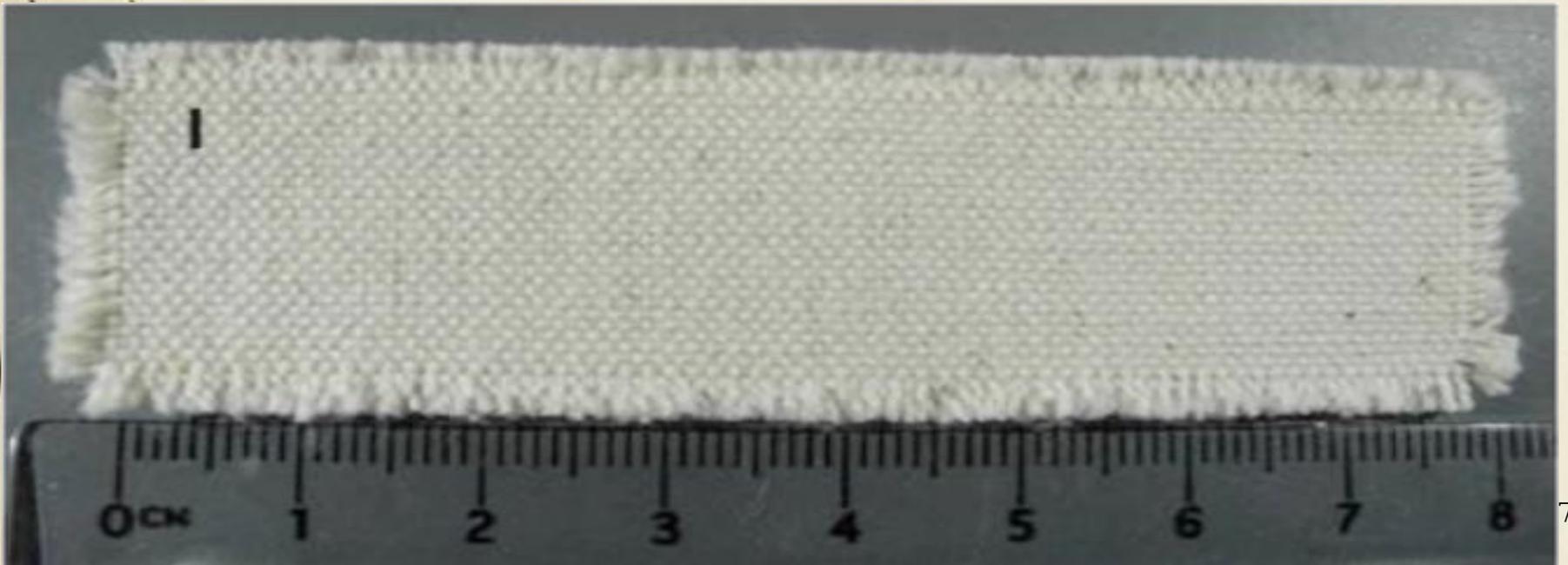
Cotton Lure as Method

- Okay as a refugia collection technique, but
- What does it tell us about CSRB
 - Population (density, abundance)
 - Area sampled
 - Conditioning (microbial colonization)
 - Alternative Assay
 - Distribution Potential w Statistics

Ecological Indicators 32 (2013) 131–139

A standardized cotton-strip assay for measuring organic-matter decomposition in streams

- In field trials, we incubated cotton strips made of artists' fabric in 49 streams in the Midwest (USA), northern Michigan (USA), and in New Zealand to: (1) test the assay under field conditions, (2) provide an initial population of data to which future studies can be compared, and (3) assess some environmental conditions that might influence cotton-strip decay.



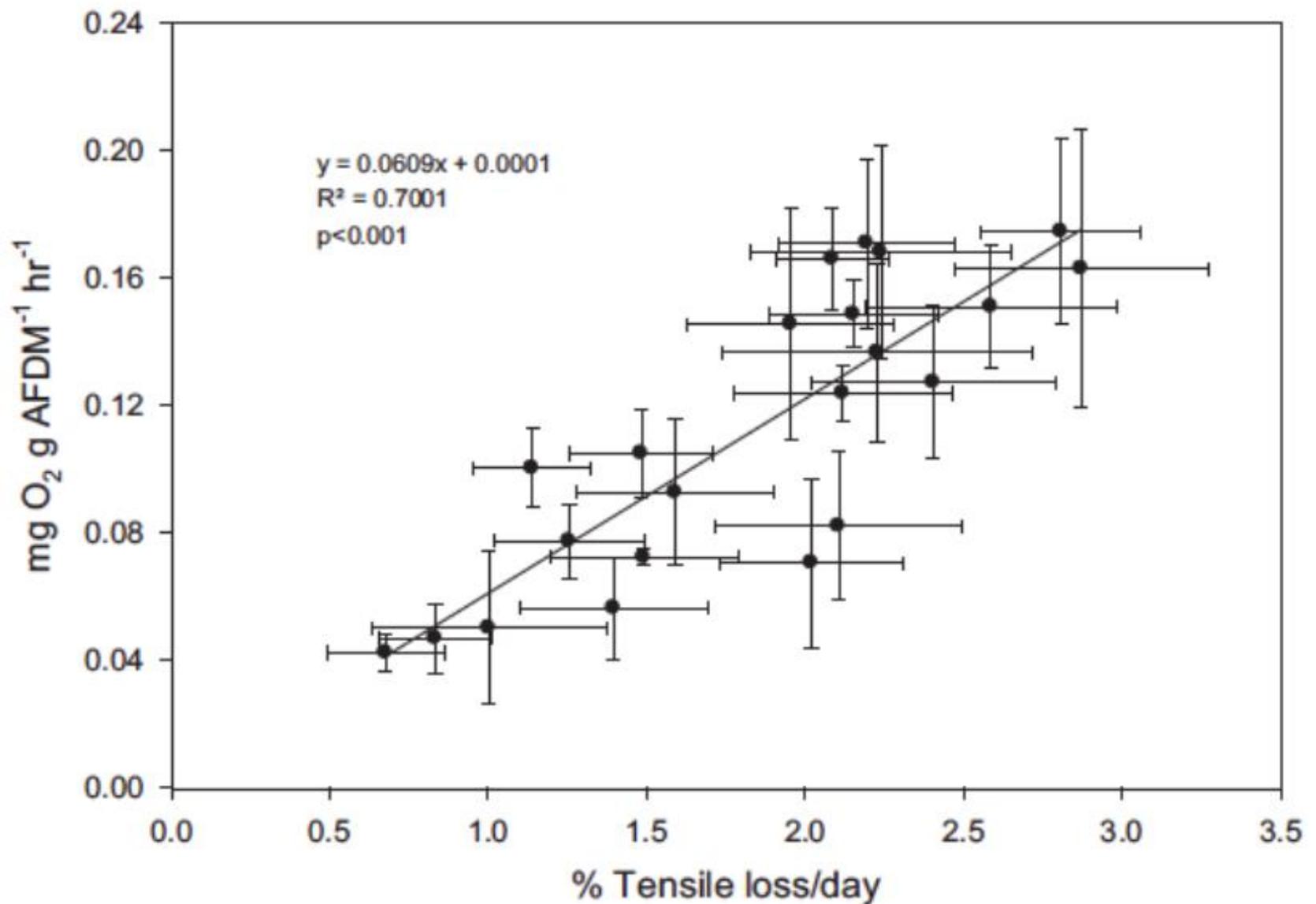


Fig. 4. Positive linear regression between mean cotton-strip tensile-strength loss (± 1 SD) and mean cotton-strip respiration (± 1 SD) for the cotton strips incubated in Michigan streams.



Cotton Lure Modifications?

- Replace cotton lure material with cotton strip fabric to better mimic leaf/wood
- Determine microbial conditioning curve for cotton strip relative to CSRB
- Pre-condition cotton strip and leave at springs for just 2 days and collect.



Other national USFWS listed aquatic invertebrates

- 41 Species (snails, crawling water bugs, amphipods, damselflies, isopods, crayfish, shrimp).
- Most without life history information and biological goals.



JOURNAL ARTICLE

**Modeling Count Data of Rare Species:
Some Statistical Issues**

Ross B. Cunningham and David B. Lindenmayer

Ecology

Vol. 86, No. 5 (May, 2005), pp. 1135-1142



EAHCP CSRB Work Group



September 4, 2019

What did we learn from last time regarding cotton lure?

1. Cotton lure sampling methodology

Is the current cotton lure sampling methodology an appropriate means to monitor abundance at a locale?

If not, what sampling methodologies exist that would provide a better proxy of abundance at a locale?

If the previous two questions cannot be adequately answered without additional study, what would be an appropriate study to answer the questions?

What did we learn from last time regarding cotton lure?

- Potential modifications
 - Examine cotton strip.
 - Determine microbial conditioning curve.
 - Pre-condition, leave at springs for short time.

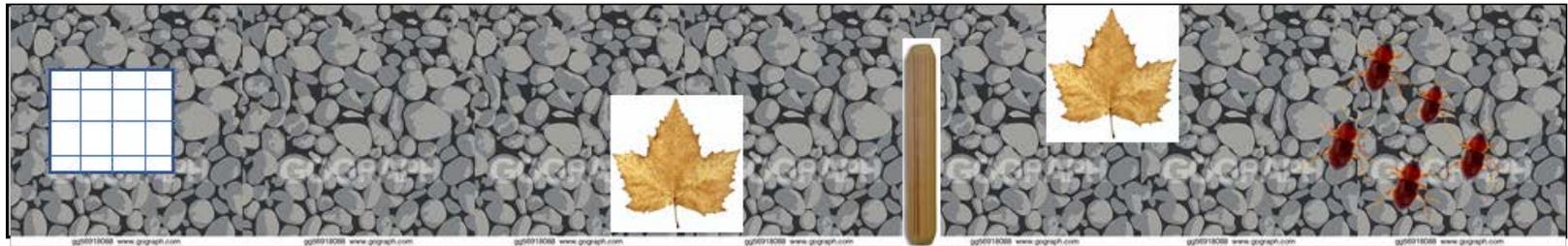
- Explore hyporheic zone
- Natural variability versus perturbation-induced variability
- Spatial scale
- Disturbance recovery
- Data modeling

Proposed research project – CSRB lure efficiency

- What is the efficiency of the cotton lure method?
 - How does the number of beetles on the lure relate to the number of beetles in the immediate area?
 - How does this change with condition of the lure?
- The general concept is to recreate cotton lure sampling in the laboratory.

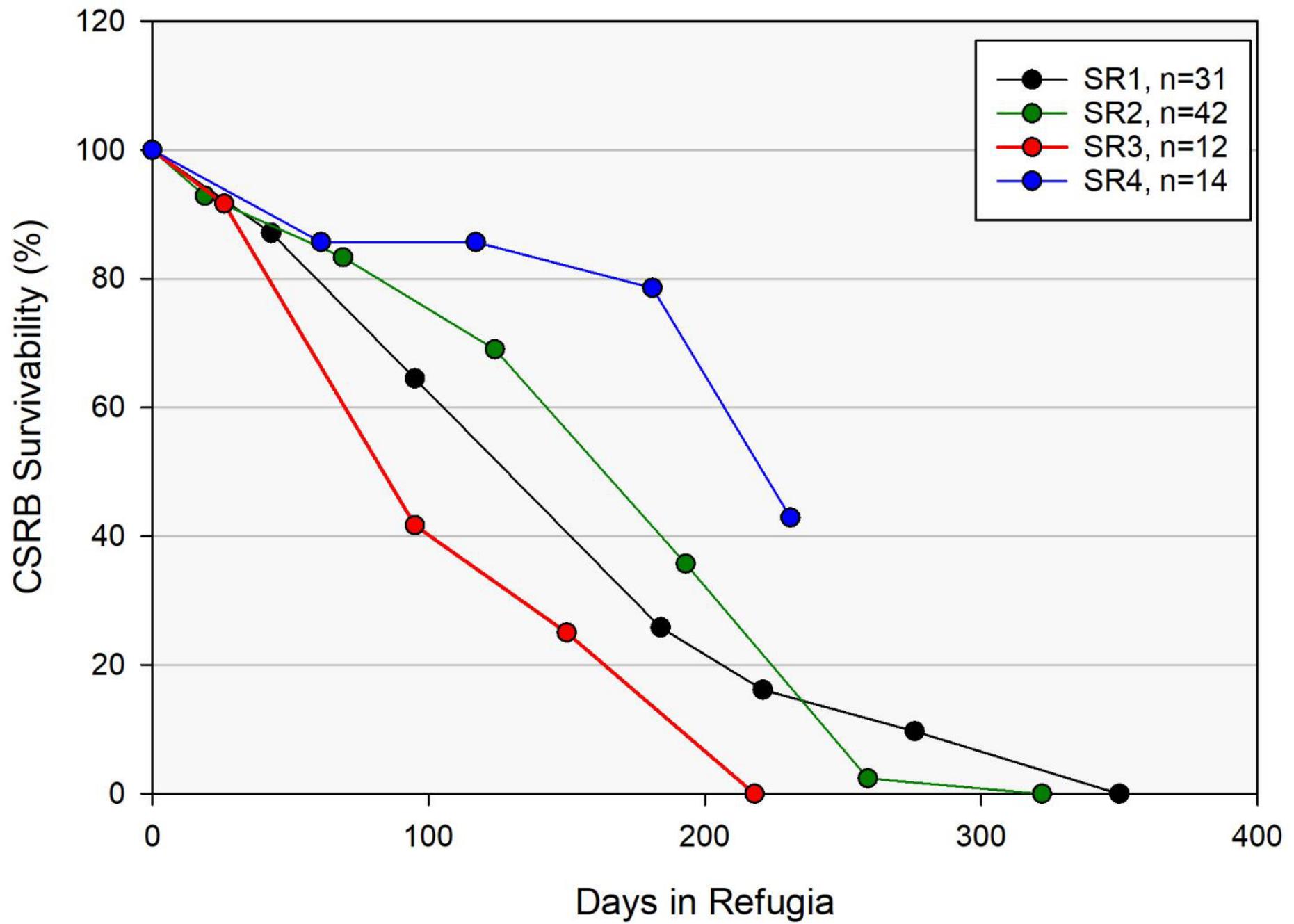
Proposed research project – CSRB lure efficiency

- Construct long (~ 1 m) rectangular plexiglass tanks (resembling Cooke et al. 2015).
- Distribute flow as evenly as possible through drilled out pvc buried in substrate.
- Construct multiple standpipes to recirculate water.
- Fill with substrate including rocks, leaves, woody debris, branches.



Proposed research project – CSRB lure efficiency

- Bury preconditioned and unconditioned cotton lure (separate tanks).
- Place 20 adult CSRB in tanks
- Examine lure on days 3, 10, 20, 30.
- Tear down tanks and note mortalities on day 30.



Proposed research project – CSRB lure efficiency

- How many beetles?
 - How many replicates?
 - How to collect and condition tank material and lures?
 - Frequency to check lures?
 - Where to place lures?
 - Where to place beetles?
- Tank size?
 - What can we measure on the lure itself at conclusion of experiment?

Charge #2. Biological monitoring, Refugia collections, and Applied Research collections

- What changes are recommended for the Biological monitoring sampling program? What are the stated goals behind those changes?
- What changes are recommended for Refugia removal efforts? What are the stated goals behind those changes?
- Are the current and proposed levels of physical activity in the CSRB habitat protective of the species? If not, what level of activity is appropriate?

Goals of Biological Monitoring program

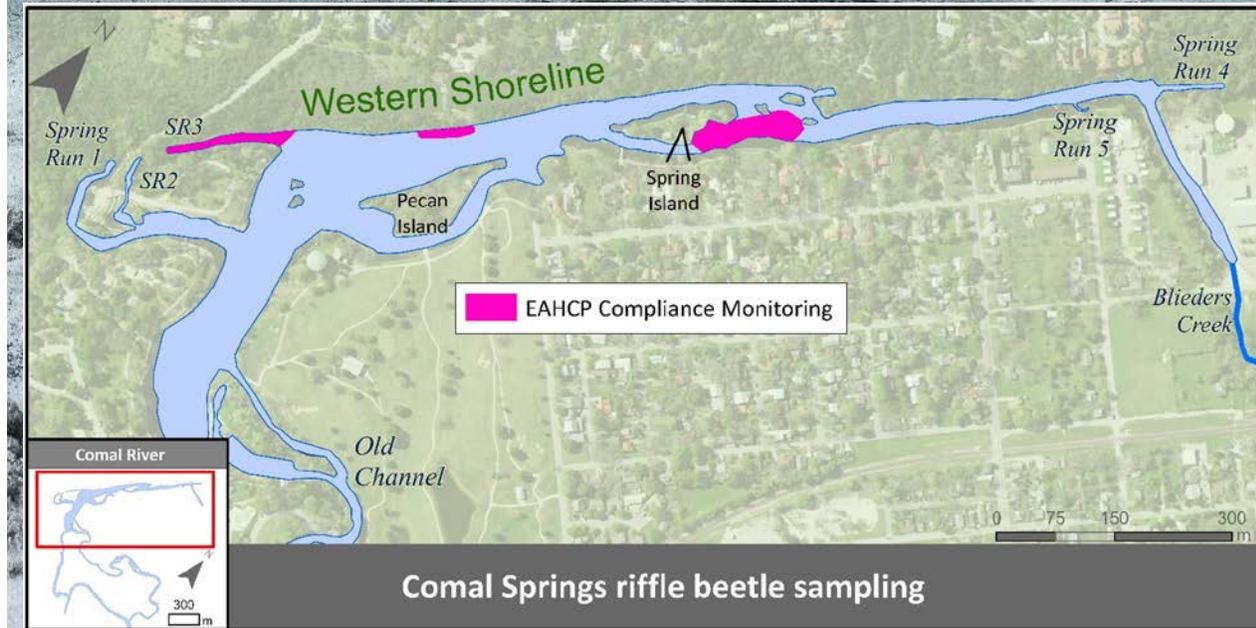
- From HCP Section 6.3.1 on Biological Monitoring
 - “...will provide a means of monitoring changes to habitat availability and the population abundance of the Covered Species...”
 - “...will provide information to effectively determine whether the conservation measures are achieving the biological goals and objectives...”

Texas State CSRB population study

- 85 springs repeatedly sampled 4 times with one week in between cotton lure retrieval and re-deployment.
- Use data to conduct occupancy and abundance modeling.

Biological monitoring program

- CSRB surveys are conducted at least 2x annually since 2004. Critical period surveys conducted as well.
- 60/40 cotton/polyester pieces of cloth are placed into spring openings/upwellings and left for 30 days
- Ten lures are placed at 3 locations. Some springs are repeatedly sampled.
- Standardized metadata are recorded at each lure locations (Hall 2016)
- Since 2004, 41 sampling events have occurred (38 at SI)
- Beetles are returned to collection point



Biological monitoring program

- Should we repeat sampling at the same 10 orifices at 3 LTBG reaches?
- Should we randomize orifice selection?
- Do we want to expand outside of our LTBG sampling area?
- How many lures should we set?
- Is twice a year necessary?
- What should we be measuring that we currently are not?
- Should we be quantifying silt accumulation within the sampling areas?

Refugia Program

- USFWS collections for the EAA Refugia began in 2017.
- Collections are conducted to meet contractually obligated standing stock numbers and conduct research.
- In 2017, ~ 8 cotton lure collection events were conducted among Spring Island and Spring Runs 1, 2, and 3.
- In 2018, ~ 3 cotton lure collection events Spring Island and Spring Runs 1 and 3.
- In 2019, ~ 2 cotton lure collection events Spring Run 3.

Refugia Program Research

- **2015: Development of husbandry and captive propagation techniques for invertebrates covered under the Edwards Aquifer Habitat Conservation Plan**
- **2018: Life history aspects of the CSR B**
- **2019: Two studies examining conditions for optimal pupation and eclosion to adult and a study evaluating captive population nutrition and longevity of the CSR B**

Refugia Questions

- How many beetles should we remove from the system each year?
- Does the 25% rule make sense?
- How often should we leave an orifice alone between collections?
- Should we remove from the same orifices that we use for LTBG collections?
- Should USFWS use similar collection measurements and techniques to Biomonitoring methods?

Are the current levels of physical activity in the system appropriate?





Meeting 4 - 10.9.2019

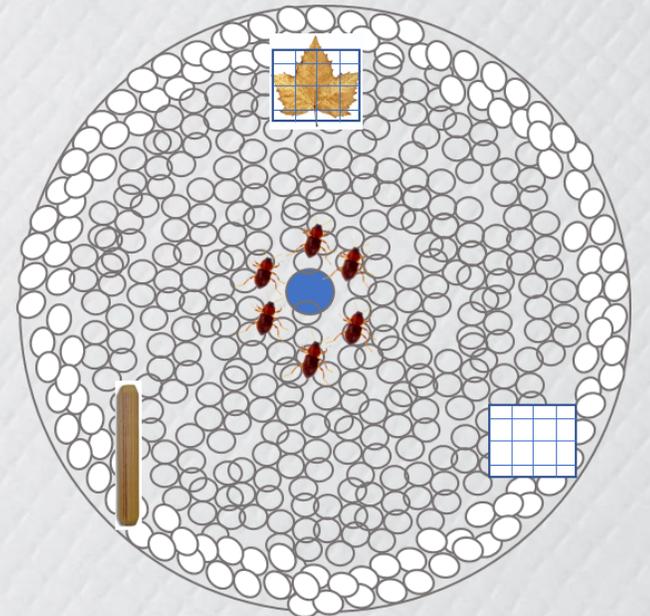
CSRB Work Group

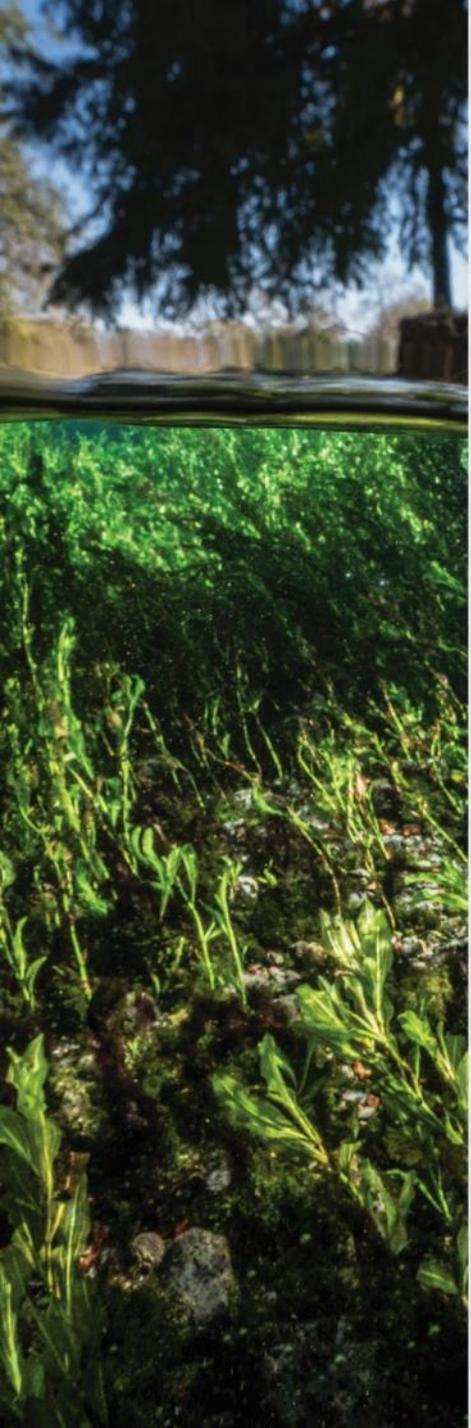


Review of Work Group meeting 3 – tank study

Cotton lure efficiency tank study

- 5 circular tanks, water enters tank from bottom, stand pipe in middle
- Tank material consists of wood, leaves, limestone gravel, and cotton lure
- Wood and leaves collected from wild, conditioned appropriately, and kept in wireframe.
- Cotton lure washed thoroughly with DI
- 20 adult beetles placed in tank
- Lure checked at 10, 20, and 30 day intervals

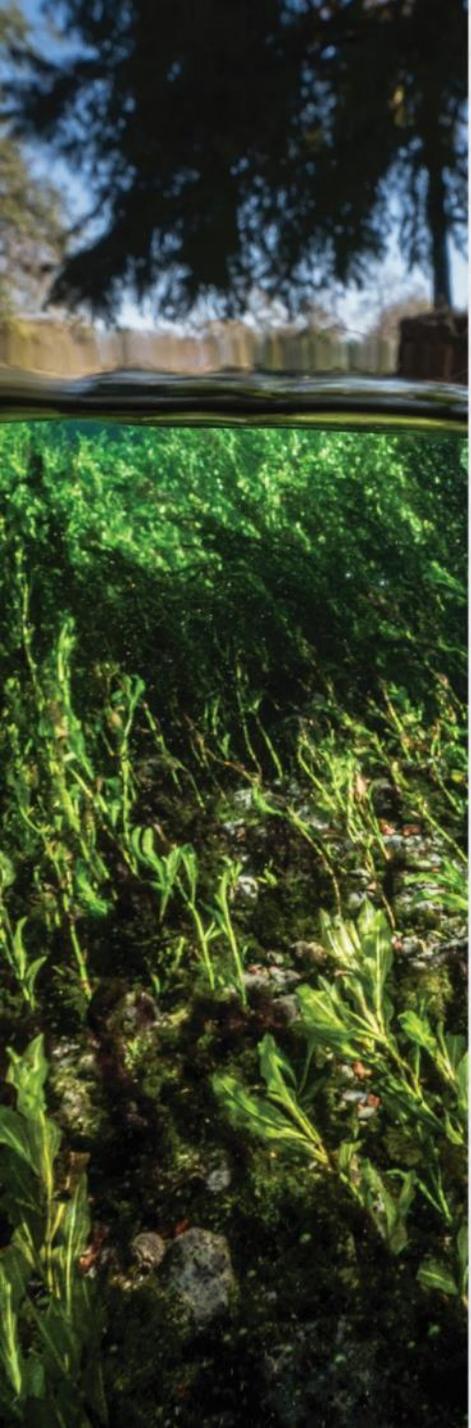




Review of Work Group meeting 3 - Biomonitoring

Goals of biomonitoring

- “...will provide a means of monitoring changes to habitat availability and the population abundance of the Covered Species...”
- “...will provide information to effectively determine whether the conservation measures are achieving the biological goals and objectives...”



Review of Work Group meeting 3 - Biomonitoring

Recommendations from meeting 3

- Continue monitoring in the three LTBG reaches and follow established sampling protocols
- Add regular system wide population surveys



Review of Work Group meeting 3 - Biomonitoring

Recommendations from meeting 3

- Continue monitoring in the three LTBG reaches and follow established sampling protocols
- Add regular system wide population surveys

2019	2020	2021	2022	2023	2024	2025	2026	2027
Nowlin			EAHCP			EAHCP		

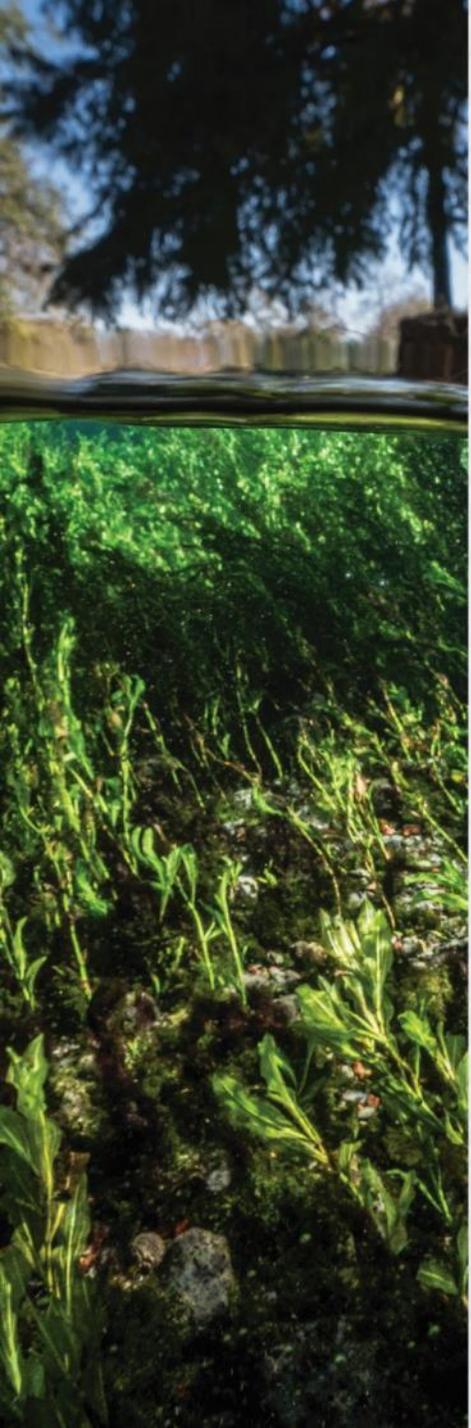
- Have methodology developed by a third party and approved by CSRB WG or Science Committee prior to procurement



Background on Refugia collections

Refugia collections

- What is USFWS tasked with?
- What did they do in 2017, 2018, 2019
- Location of collections
- Partial take off lure



Background on Refugia collections

Refugia collections

- What is USFWS tasked with?
 - “Collection, establishment, and maintenance of standing stocks, refugia stocks, and salvage stocks for the Covered Species”
 - Standing stock = 500 CSRB
 - Salvage stock = 500 CSRB

Background on Refugia collections

Table 1. Biowest CSRB survey results 2004-2018.

<u>location</u>	<u>surveys</u>	<u>lures_set</u>	<u>adults_caught</u>	<u>larva_caught</u>
:-----:	-----:	-----:	-----:	-----:
Spring Island	42	425	2704	1407
Spring Run 3	42	451	4452	573
Western Shoreline	42	464	4321	1228

Table 2. USFWS CSRB survey results 2017 - 2/2019.

<u>location</u>	<u>surveys</u>	<u>lures_set</u>	<u>adults_caught</u>	<u>larva_caught</u>
:-----:	-----:	-----:	-----:	-----:
Spring Island	14	51	498	432
Spring Run <u>1</u>	14	54	634	50
Spring Run <u>2</u>	2	4	60	9
Spring Run <u>3</u>	21	162	2700	675

A photograph of a stream with green vegetation on the bank. The water is clear and reflects the surrounding greenery. The bank is covered in lush green plants and grasses. The background shows a fence and trees under a blue sky.

Background on Refugia collections

Refugia cotton lure encounters in 2017, 2018, and 2019 in Spring Run 3.

- 2017
 - 12 unique 'date.collected'
 - 75 lures set
 - 1896 'adult_plus_larva' encountered
- 2018
 - 7 unique 'date.collected'
 - 60 lures set
 - 929 'adult_plus_larva' encountered
- 2019
 - 2 unique 'date.collected'
 - 26 lures set
 - 550 'adult_plus_larva' encountered

Background on Refugia collections

Refugia cotton lure encounters in 2017, 2018, and 2019 in Spring Run 3.

2017

12 unique 'date.collected'
75 lures set
1896 'adult_plus_larva' encountered

2018

7 unique 'date.collected'
60 lures set
929 'adult_plus_larva' encountered

2019

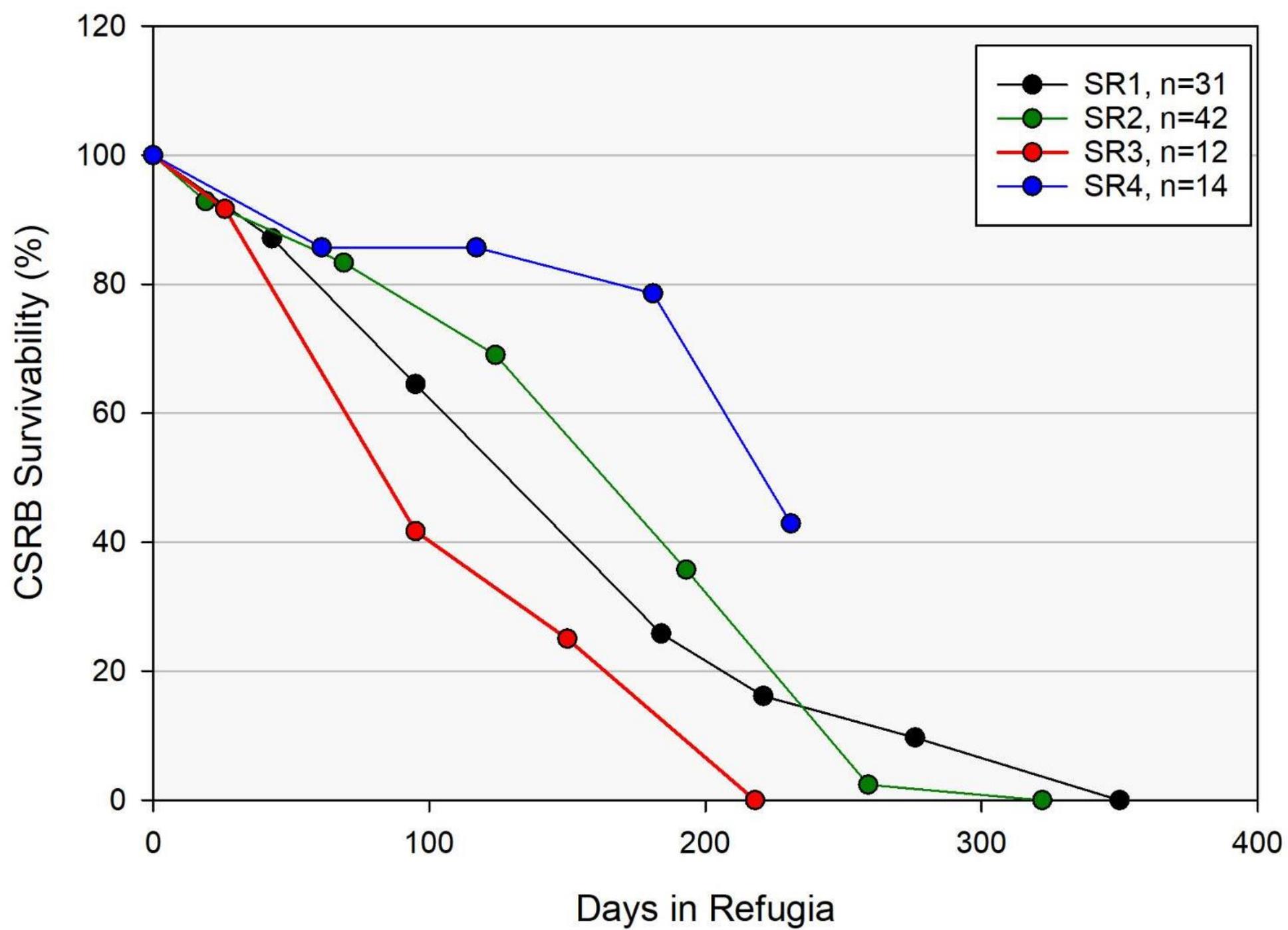
2 unique 'date.collected'
26 lures set
550 'adult_plus_larva' encountered

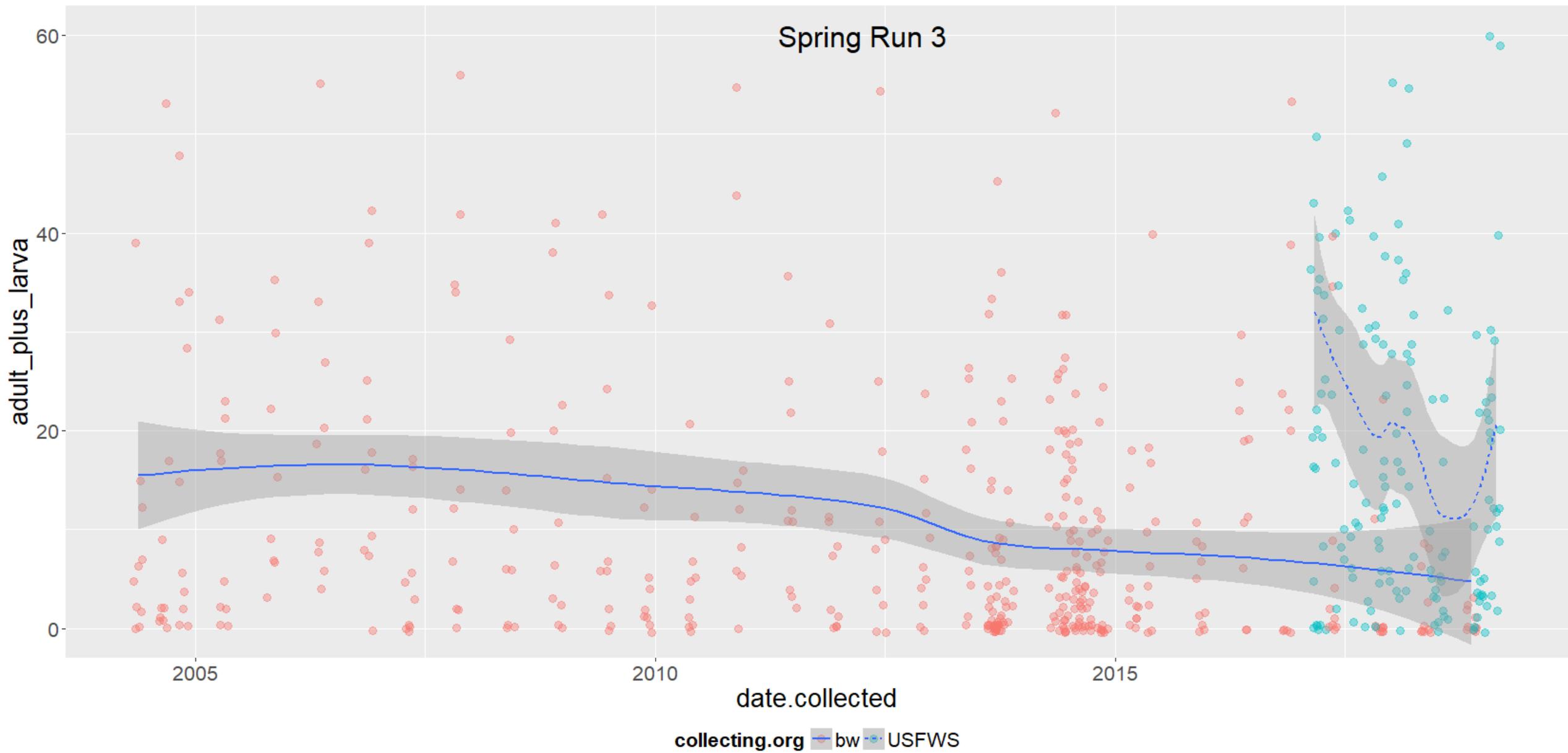
Spring Run 3 CSRB removals		
	cotton lure	wood dowel
2017	347	24
2018	264	44
2019	465	30
	1076	98
		1174



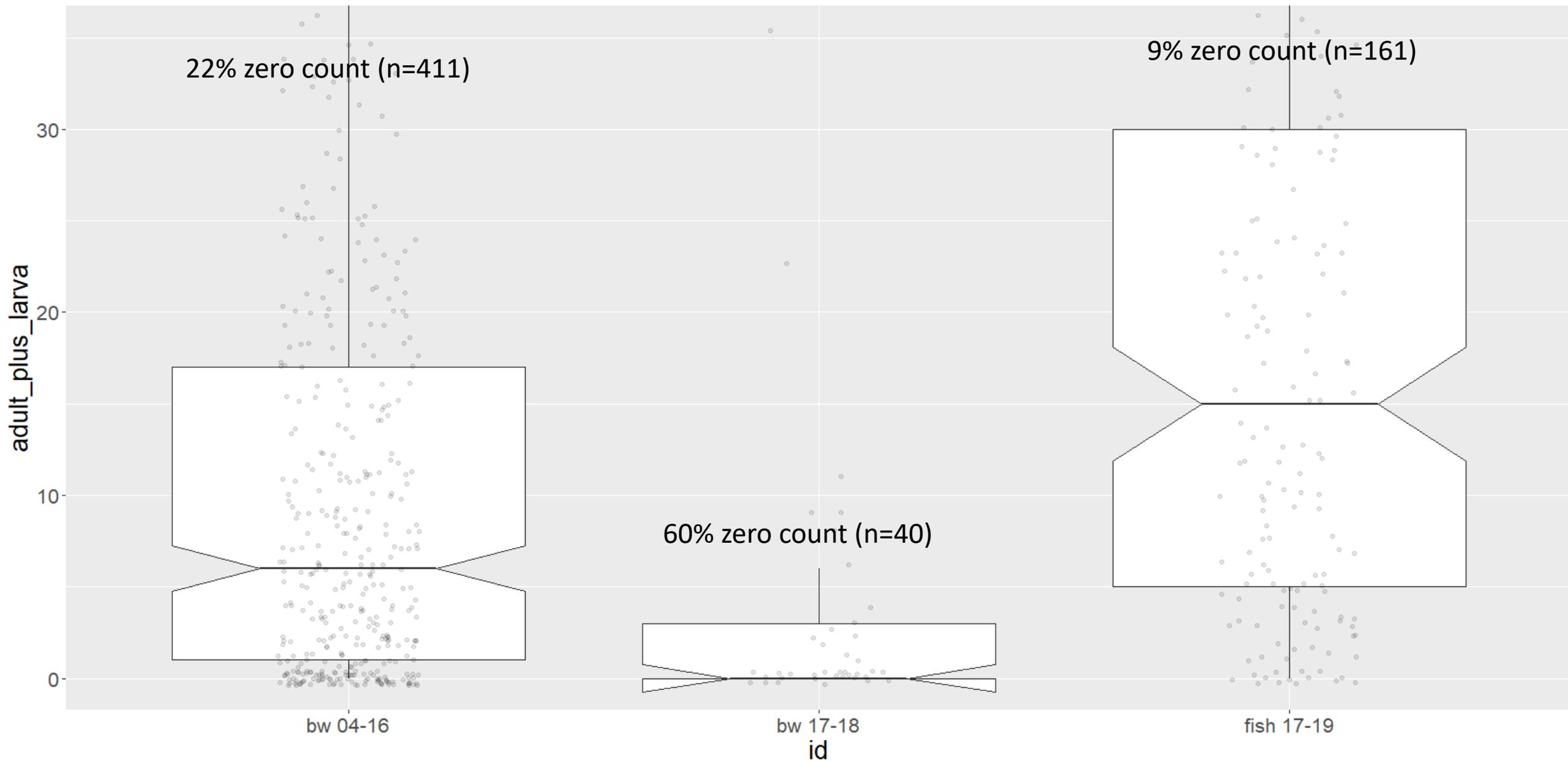
Background on Refugia collections

In 2019 and 2020 the Refugia program is collecting CSRBs to support research purposes rather than standing stock until survivability in captivity is increased and better quantified. USFWS is also using the biomonitoring survey as an opportunity to collect beetles.





CSR encounters on cotton lures at Spring Run 3, 2004 - Feb 2019.



CSRB encounters on cotton lures at Spring Run 3 (left to right) BioWest biannual surveys including critical period monitoring 2004-2016, BioWest biannual surveys 2017-2018, USFWS Refugia 2017-Feb 2019.



Questions regarding Refugia collections

How many beetles should we remove from the system each year and where from?

What is an appropriate standing stock number?

How often should we leave an orifice alone between collections?

Should we always sample the same orifices/spring runs?

Should we collect beetles out of the orifices used for biological monitoring?

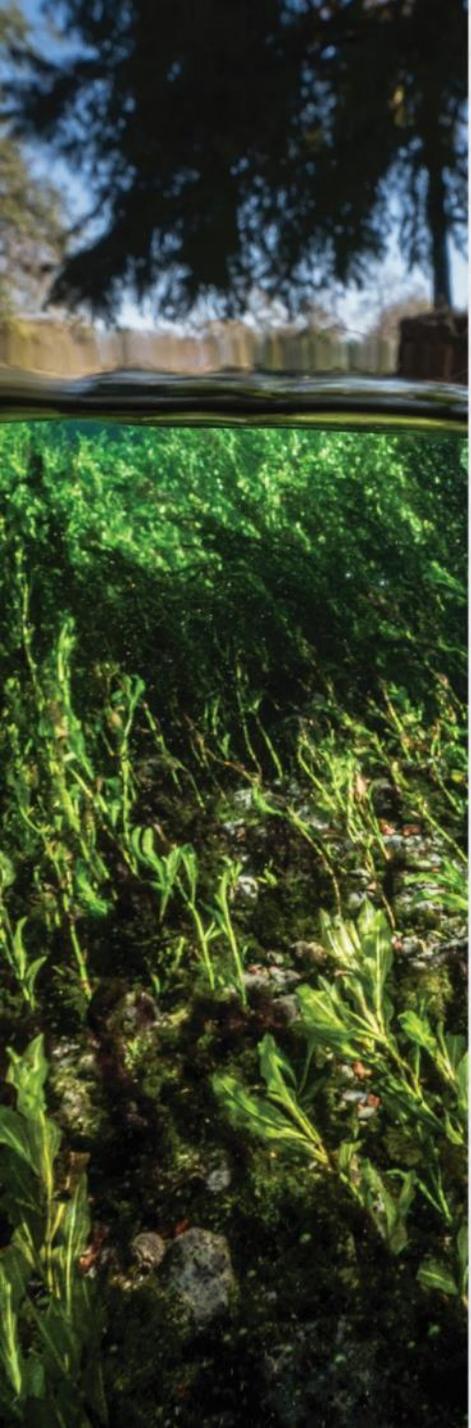
Should we only take 25% off of a lure returning the other 75%?



Suggested recommendations

Recommendations for standing stock collections during non-drought periods

- No more than 200 beetles removed per year for standing stock purposes
- No more than 50 beetles per year from a single orifice
- Maximum 4 collections per year (2 Bio-West – 2 USFWS)
- Track beetles separately by location and collection event
- Recovered at more than one location in the lake



System Disturbance

Are the Refugia and Biomonitoring sampling programs safe for the beetle?

What data metrics can be regularly examined to make this determination?

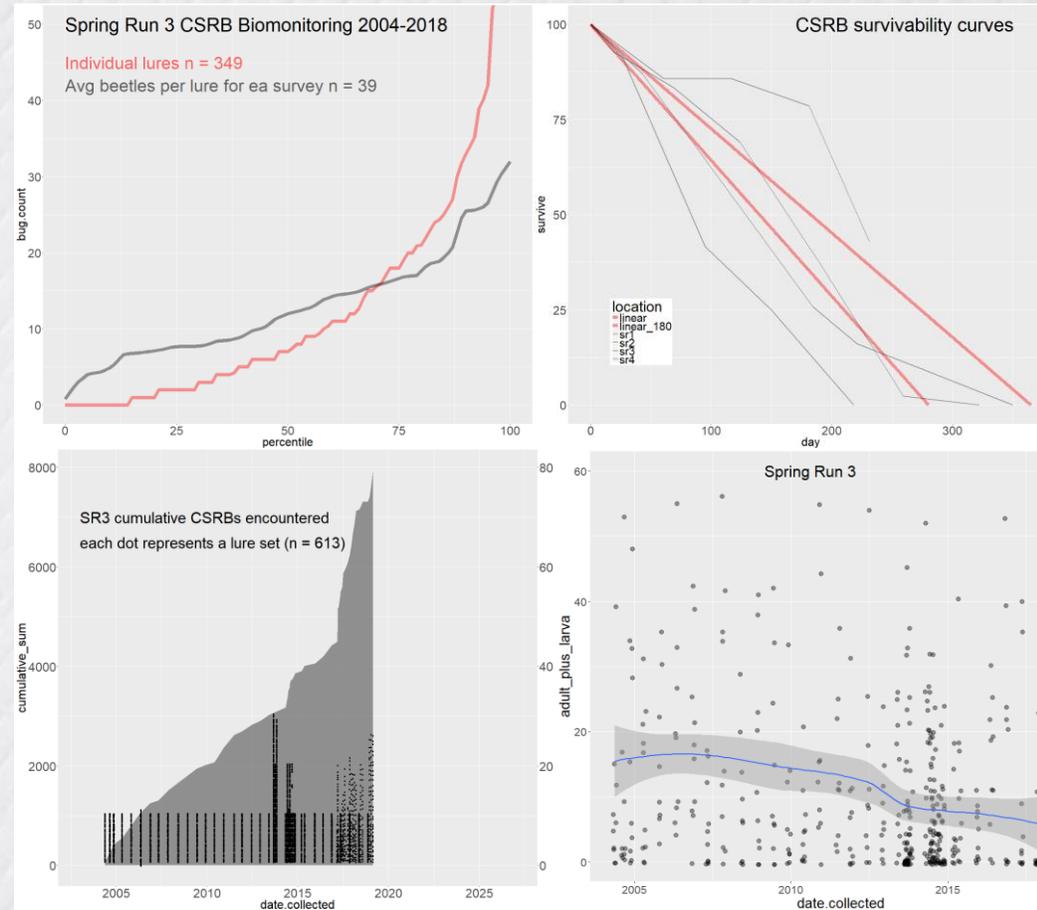


Meeting # 5

Review, discussion, and recommendation on Biological Goals.

Overview of Work Group recommendations.

EAHCP CSRB Work Group meeting 5



10.30.2019



What do we have left to cover?

- Refugia collections (Number – Location)
- Disturbance
- EAHCP Long-Term Biological Goals



Refugia Operations

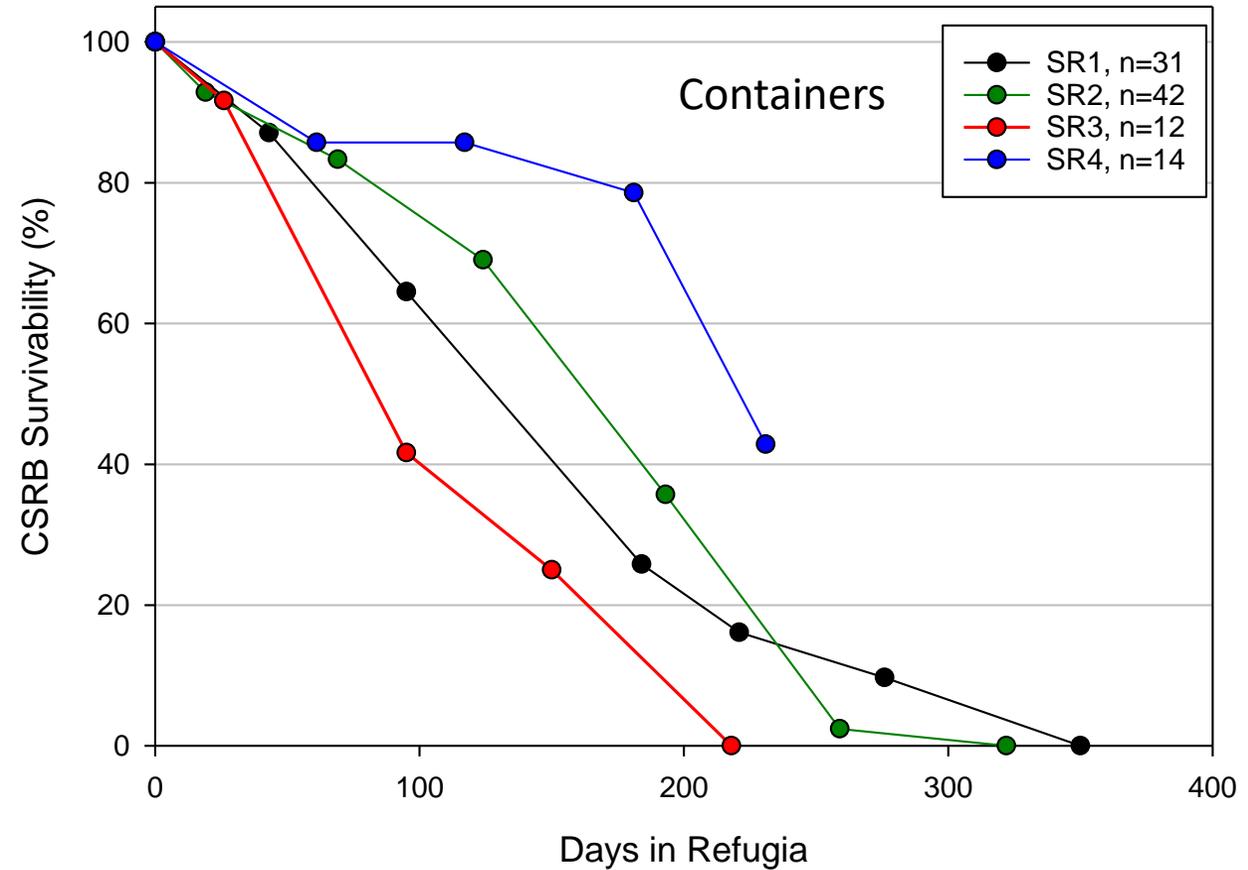
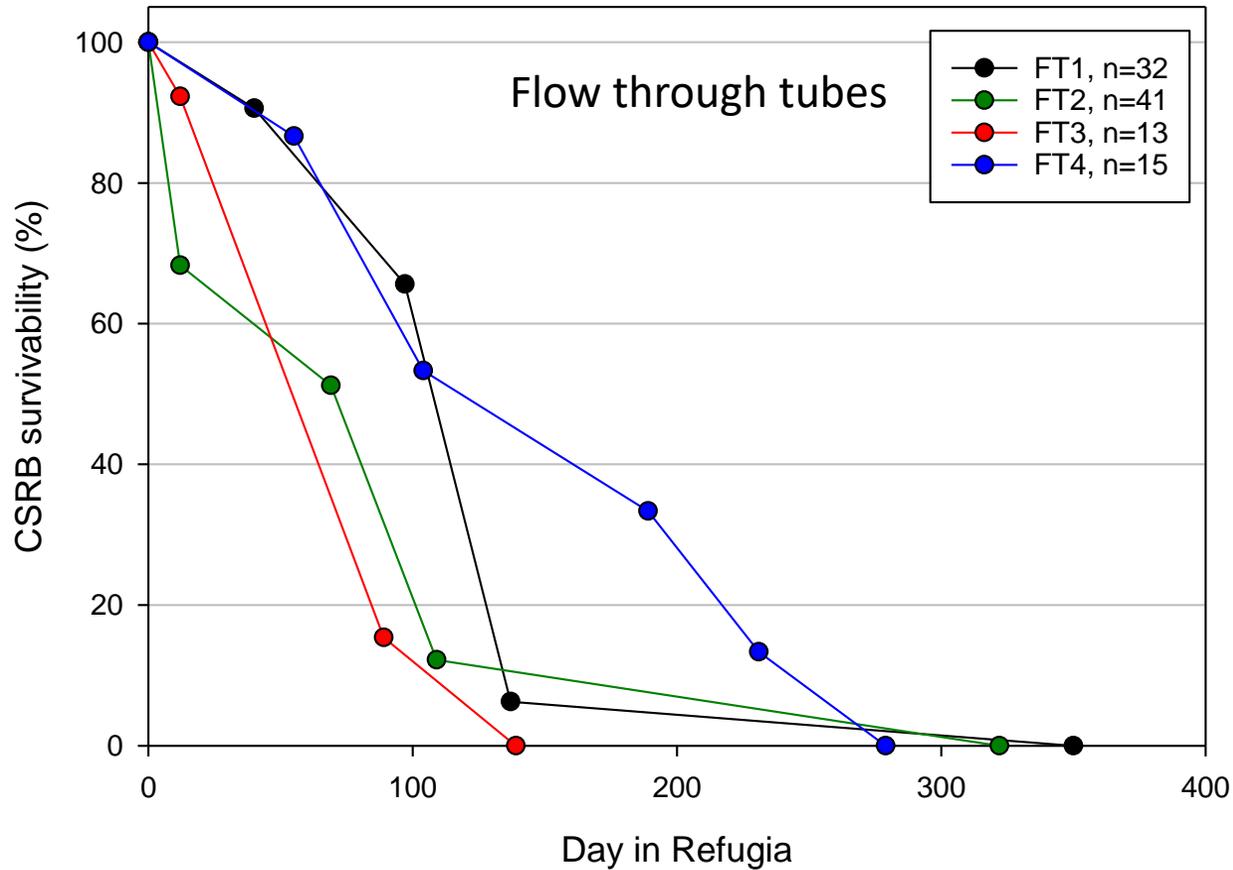
- Collection numbers needed to maintain standing stocks
- Collection efforts needed to meet collection numbers
- Salvage rules

Refugia Operations – collections for standing stocks

- How many beetles need to be collected from the wild to continuously maintain a target stock number?

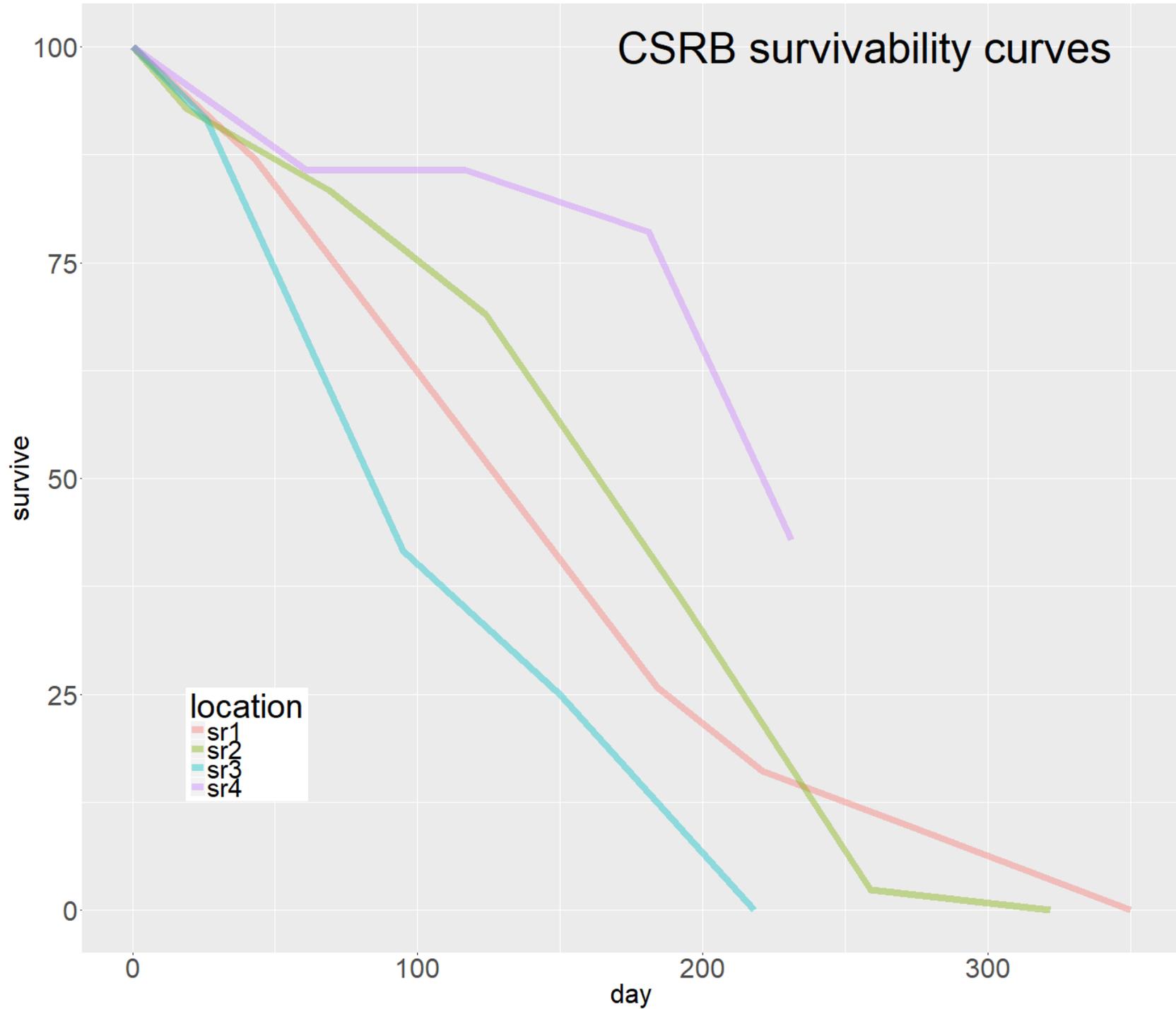


CSRB refugia survivability



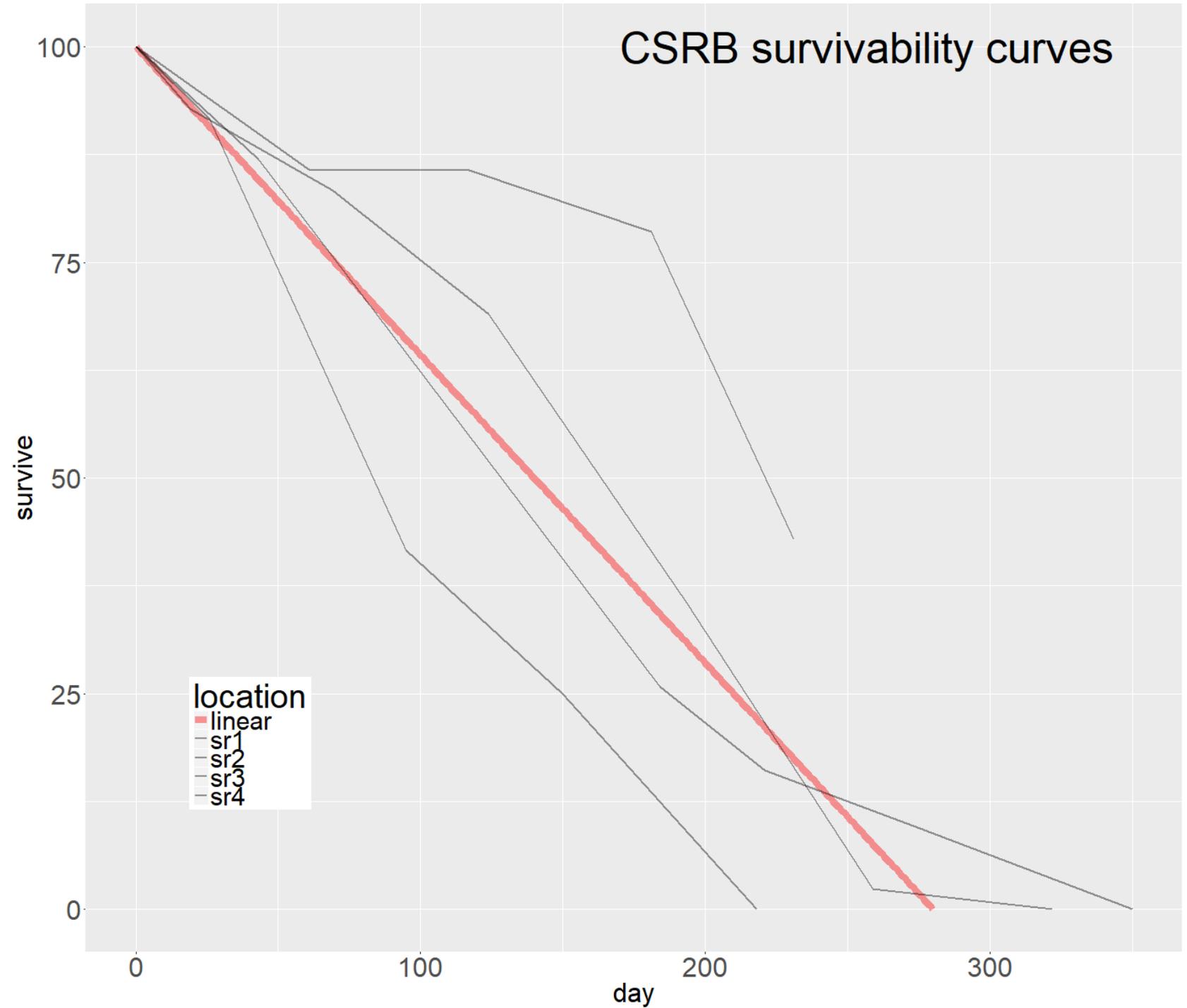
Refugia Operations – collections for standing stocks

- How many beetles need to be collected from the wild to continuously maintain a target stock number?



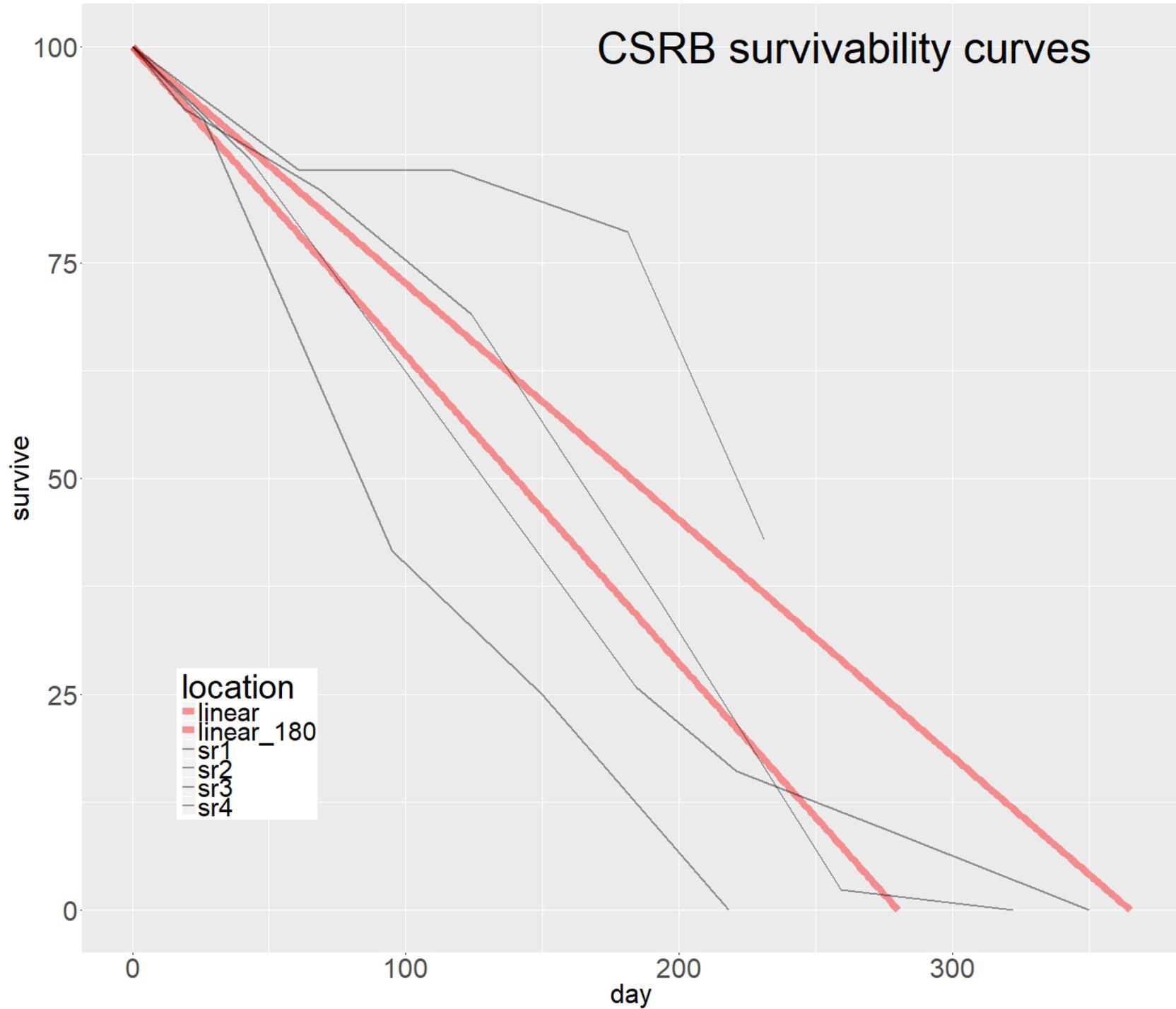
Refugia Operations – collections for standing stocks

- How many beetles need to be collected from the wild to continuously maintain a target stock number?



Refugia Operations – collections for standing stocks

- How many beetles need to be collected from the wild to continuously maintain a target stock number?



Refugia Operations

– collections for standing stocks

- How many beetles need to be collected from the wild to continuously maintain a target stock number?

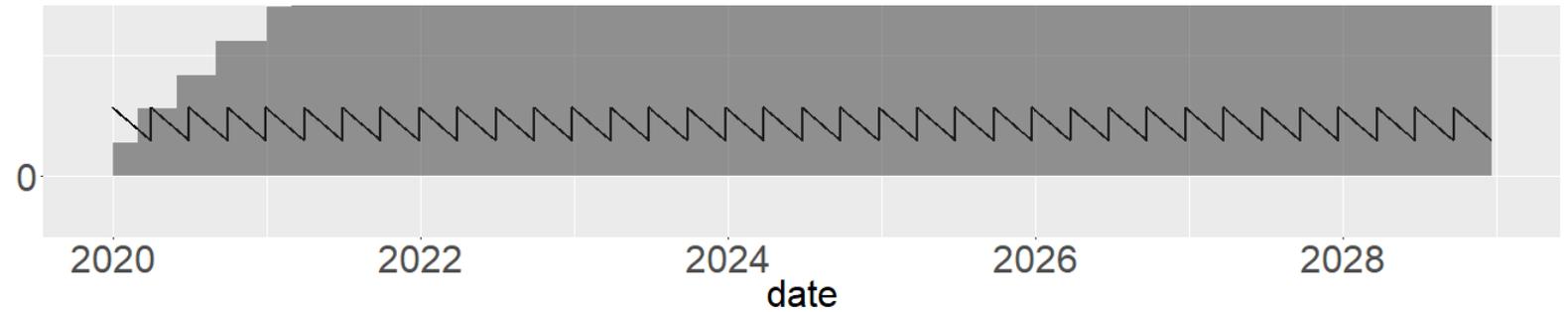
	142 d	182 d
linear survivability	50%	50%
quarterly amounts collected	65	50
max refugium population	133	125
min refugium population	69	75
average refugium population	100	100
annual removals	260	200

$$\frac{200 - 260 \text{ adult CSRBs Removed}}{100 \text{ adult CSRBs in captivity} \cdot \text{yr}}$$

Assumptions: quarterly collections; linear mortality

Refugia Operations – collections for standing stocks

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Refugia Operations – collections for standing stocks



- How many beetles need to be collected from the wild to continuously maintain a target stock number?
- What is the collection effort required to meet the number of beetles for the target stock number?

Refugia Operations – collection effort

- How many adult CSRB beetles can we expect to encounter per collection effort?

Table 1. Biowest CSRB survey results 2004-2018.

<u>location</u>	<u>surveys</u>	<u>lures_set</u>	<u>adults_caught</u>	<u>larva_caught</u>
Spring Island	42	425	2704	1407
Spring Run 3	42	451	4452	573
Western Shoreline	42	464	4321	1228

Table 2. USFWS CSRB survey results 2017 - 2/2019.

<u>location</u>	<u>surveys</u>	<u>lures_set</u>	<u>adults_caught</u>	<u>larva_caught</u>
Spring Island	14	51	498	432
Spring Run <u>1</u>	14	54	634	50
Spring Run <u>2</u>	2	4	60	9
Spring Run <u>3</u>	21	162	2700	675

Refugia Operations – collection effort

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	Refugia	BioWest
Spring Island	10	6
Spring Run 3	17	10
Western Shoreline	-	9
Spring Run 1	12	-

Average adults encountered

10 adult CSRBs
1 lure set



Refugia Operations – Salvage Refugia

- Standing Stock: 500; permanent
- Refugia Stock: 500 – Standing stock; < 120 cfs
- Salvage Stock: 500; < 30 cfs

- Standing + Refugia + Salvage = 1000

Refugia Operations – How many to collect?

Known

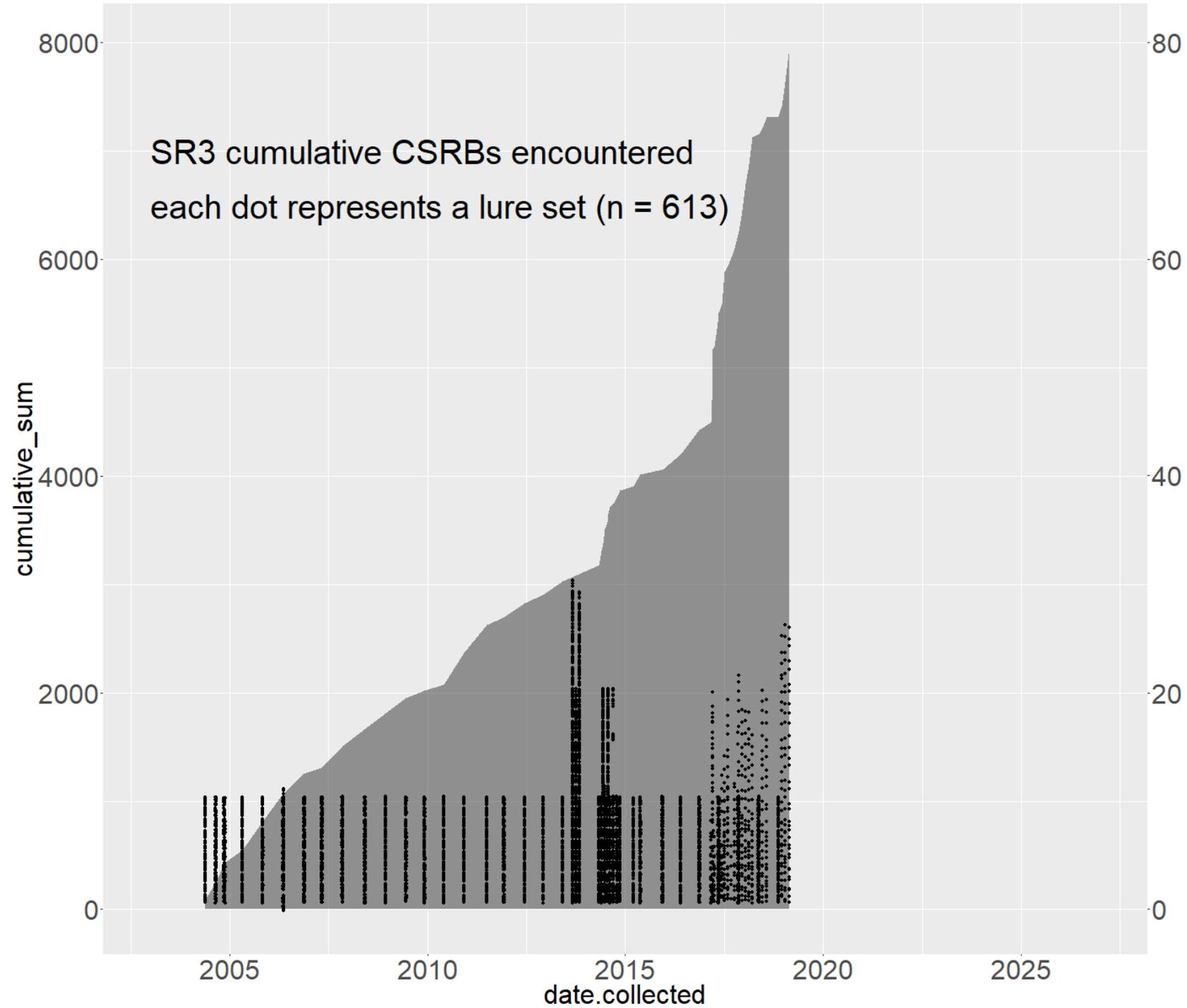
- currently $\frac{260 \text{ adult CSRBs Removed}}{100 \text{ adult CSRBs in captivity} \cdot \text{yr}}$
- $\frac{10 \text{ adult CSRBs}}{1 \text{ lure set}}$
- Not in critical drought
- Husbandry skills can be honed at 50-100 beetles
- Standing + Salvage stocks = 1000 (500 at 120cfs; 500 at 30cfs)

Unknown

- Surface population size
- Effects of disturbance ecology/reintroduction
- How to reliably breed
- How to reintroduce
- Minimum viable population for reintroduction
- Reliable metrics to assess oversampling

Disturbance

- SR3 – Refugia & BioMon
 - 2017
 - 95 lures set
 - 2021 adult+larva
 - 2018
 - 80 lures set
 - 963 adult+larva
 - 2019
 - 46 lures set
 - > 550 adult+larva



Assumptions: quarterly collections; 100% take; linear mortality $\frac{260 \text{ adult CSRBs Removed}}{100 \text{ adult CSRBs in captivity} \cdot \text{yr}}$ $\frac{10 \text{ adult CSRBs}}{1 \text{ lure set}}$

Disturbance

	Refugia
average refugium population	100
max refugium population	133
min refugium population	69
<hr/>	
annual adult removals	260
annual lures set	26
annual adult + larva encounters	350*
<hr/>	
2020-2028 removals	2340
2020-2028 lures set	234
2020-2028 adult + larva encounters	3150

*based off BioWest and Refugia data

^based off BioMon data only

does not include research or system wide surveys

assumes 100% take off lure

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Disturbance

	Refugia	BioMon	
average refugium population	100	-	
max refugium population	133	-	
min refugium population	69	-	
annual adult removals	260	-	
annual lures set	26	60	
annual adult + larva encounters	350*	660^	
2020-2028 removals	2340	-	2340
2020-2028 lures set	234	540	774
2020-2028 adult + larva encounters	3150	5940	9090

*based off BioWest and Refugia data

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Assumptions: quarterly collections; 100% take; linear mortality $\frac{260 \text{ adult CSRBs Removed}}{100 \text{ adult CSRBs in captivity} \cdot \text{yr}}$ $\frac{10 \text{ adult CSRBs}}{1 \text{ lure set}}$

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*based off BioWest and Refugia data

[^]based off BioMon data only

does not include research or system wide surveys

assumes 100% take off lure

	Refugia
average refugium population	250
max refugium population	334
min refugium population	173
annual adult removals	652
annual lures set	66
annual adult + larva encounters	885*
2020-2028 removals	5868
2020-2028 lures set	594
2020-2028 adult + larva encounters	7965

*based off BioWest and Refugia data

[^]based off BioMon data only

does not include research or system wide surveys

assumes 100% take off lure

Assumptions: quarterly collections; 100% take; linear mortality $\frac{260 \text{ adult CSRBs Removed}}{100 \text{ adult CSRBs in captivity} \cdot \text{yr}}$ $\frac{10 \text{ adult CSRBs}}{1 \text{ lure set}}$

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Refugia Operations – How many to collect?

Known

- currently $\frac{260 \text{ adult CSRBs Removed}}{100 \text{ adult CSRBs in captivity} \cdot \text{yr}}$
- $\frac{10 \text{ adult CSRBs}}{1 \text{ lure set}}$
- Not in critical drought
- Husbandry skills can be honed at 50-100 beetles
- Standing + Salvage stocks = 1000 (500 at 120cfs; 500 at 30cfs)

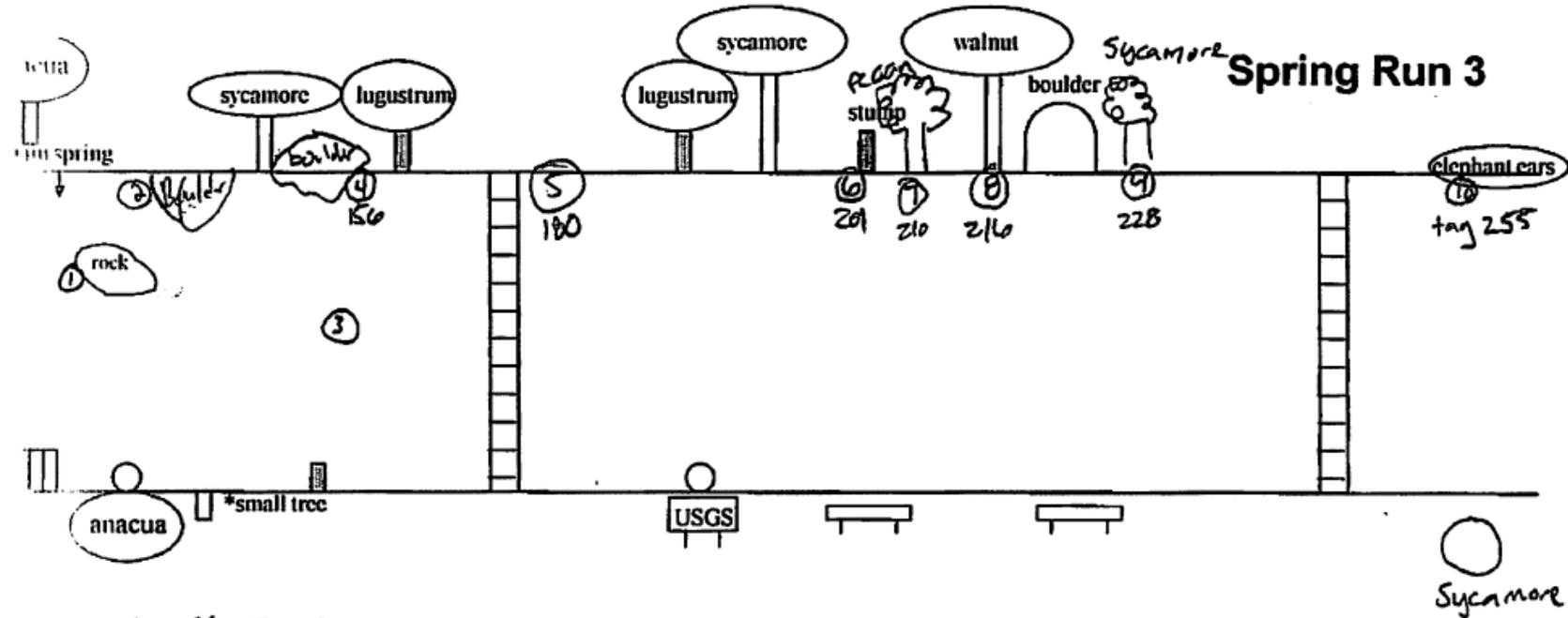
Unknown

- Surface population size
- Effects of disturbance ecology/reintroduction
- How to reliably breed
- How to reintroduce
- Minimum viable population for reintroduction
- Reliable metrics to assess oversampling



Refugia Operations – Where to collect?

- Multiple locations each time
- Separate areas than Biological Monitoring
- Percent take off of lure
- Repeated sampling of orifices



date	depth (ft)	<i>Heterelmis comalensis</i> adults	<i>Heterelmis comalensis</i> larvae	<i>Microcyloepus pusillus</i> adults	<i>Microcyloepus pusillus</i> larvae	<i>S. peckii</i>	<i>S. comalensis</i>
16 April 2018							
15 May 2018							

- 1 Directly out from stage gauge left side of boulder
- 2 Under right side of boulder
- 3 7' down stream from blue pipe under white boulder (no inverts)
- 4 tag 156 under big boulder to right hand side
- 5 tag 180 just to right of bridge
- 6 tag 201 under tag
- 7 tag 210 under pecan tree big tree
- 8 tag 216 directly under tag to left of boulder
- 9 tag 228 under rock pile next to sycamore
- 10 tag 255 directly straight out from sycamore. Lure under horizontal log

recorded
20180521
#



◆ Spring Locations





Long-Term Biological Goals

- Are the current population and habitat LTBGs for the CSRB appropriate? What are the criteria for more appropriate goals?
- What is an appropriate means to monitor the habitat quality goal?
- How can Biological monitoring, Refugia efforts, and Applied Research studies be used to establish new LTBGs?



CSRB Long-Term Biological Goals

- Section 4.1.1.1 of the HCP establishes Long-Term Biological Goals for the Comal Springs riffle beetle.
- “The population measurement goal is to maintain greater than or equal to the median densities observed over the past six years of the EAA Variable Flow Study monitoring.”

**TABLE 4-7
COMAL SPRINGS RIFFLE BEETLE LONG-TERM BIOLOGICAL GOALS**

	Spring Run 3	Western Shoreline	Spring Island Area
Habitat	Silt-free gravel and cobble substrate \geq 90% of each study area		
Density (# of CSRB/	\geq 20	\geq 15	\geq 15

CSRB Long-Term Biological Goals

- Section 4.1.1.1 of the HCP establishes Long-Term Biological Goals for the Comal Springs riffle beetle.
- “The population measurement goal is to maintain greater than or equal to the median densities observed over the past six years of monitoring.”

TABLE 4-9
COMAL SPRINGS RIFFLE BEETLE DENSITY (#/LURE)

2004-2010 data

	Spring Run 3	Western Shoreline	Spring Island Area
Minimum	7	9	11
25 th	12	13	11
Median	17	14	13
75 th	21	20	16
Maximum	32	26	23



TABLE 4-8

NUMBER OF COMAL SPRINGS RIFFLE BEETLES CAPTURED DURING EACH SAMPLING EVENT VIA COTTON LURE METHODOLOGY FROM 2004 THROUGH 2010

Sample Period	Spring Run 3	Western Shore	Spring Island	TOTAL
May-June 2004	88	83	122	293
August 2004	169	143	90	402
Nov-Dec 2004	170	175	146	491
April 2005	119	121	121	361
Nov-Dec 2005	262	201	185	648
May-June 2006	256	195	160	611
Nov-Dec 2006	185	92	125	402
May-June 2007	59	161	119	339
Nov-Dec 2007	204	83	132	419
May-June 2008	155	139	156	450
Nov-Dec 2008	144	133	227	504
May-June 2009	136	226	74	436
Nov-Dec 2009	72	56	198	326
May-June 2010	53	110	20	183
Nov-Dec 2010	298	264	104	666
TOTAL	2,370	2,182	1,979	6,531
Average	158.0	145.5	131.9	458.3

For each survey and each location - calculated average number of beetles per lure (n=15 surveys)

TABLE 4-9
COMAL SPRINGS RIFFLE BEETLE DENSITY (#/LURE)

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Minimum	7	9	7
25 th	12	13	11
Median	17	14	13
75 th	21	20	16
Maximum	32	26	23

Rounded median number to nearest '5' increment

“The population measurement goal is to maintain greater than or equal to the median densities observed over the past six years of the EAA Variable Flow Study monitoring.”

TABLE 4-7
COMAL SPRINGS RIFFLE BEETLE LONG-TERM BIOLOGICAL GOALS

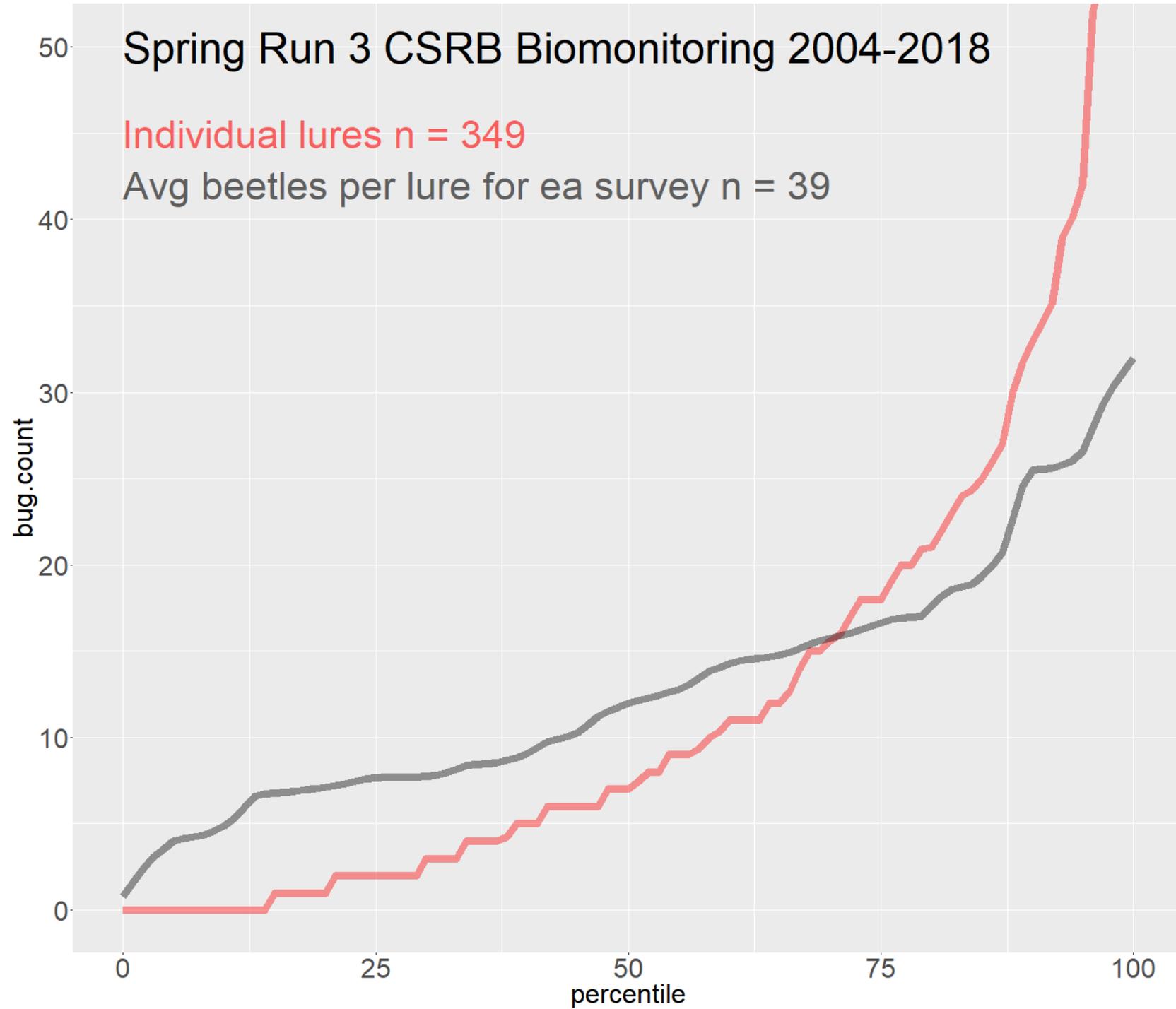
	Spring Run 3	Western Shoreline	Spring Island Area
Habitat	Silt-free gravel and cobble substrate ≥ 90% of each study area		
Density (# of CSR/	≥20	≥15	≥15

LTBGs

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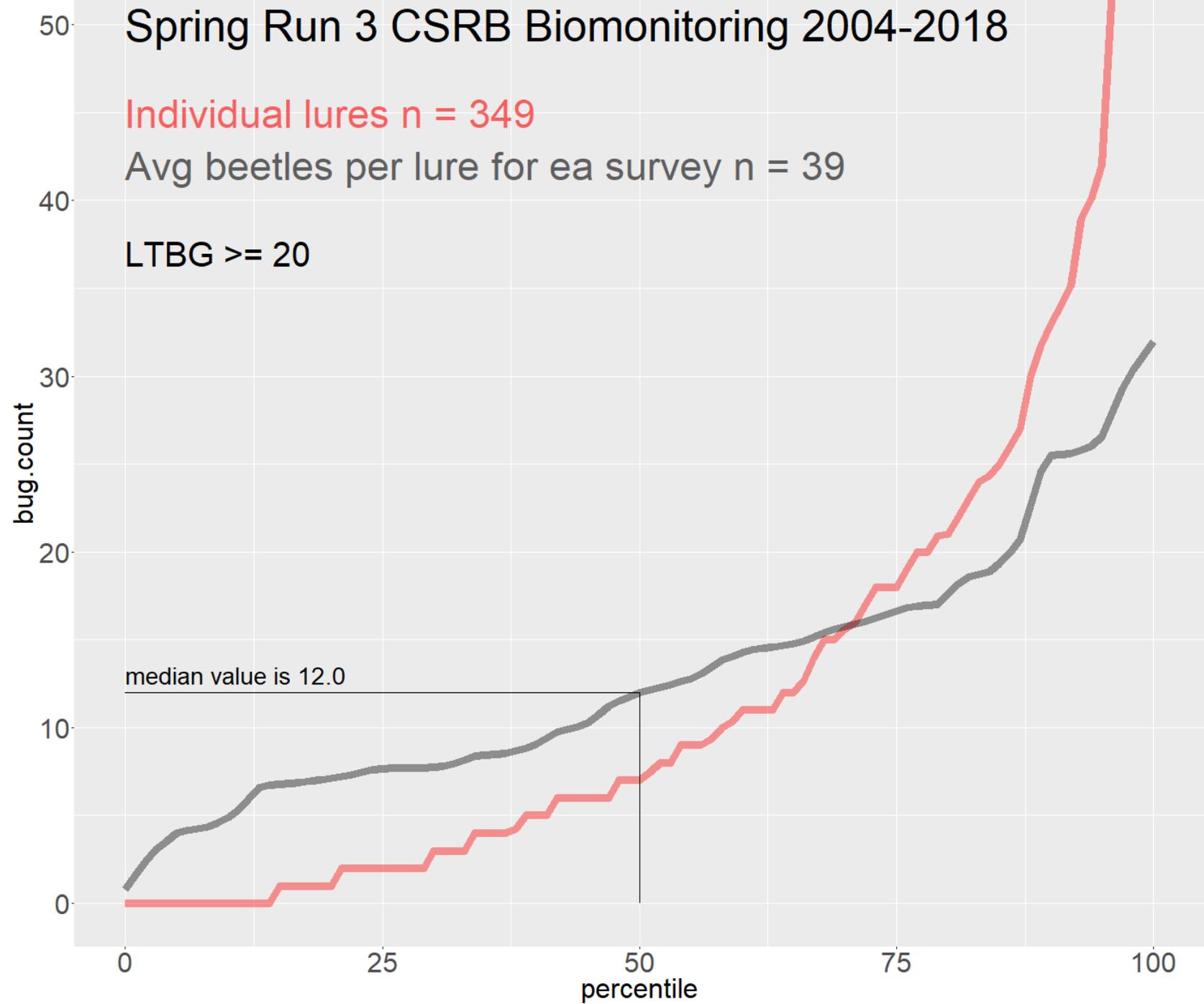


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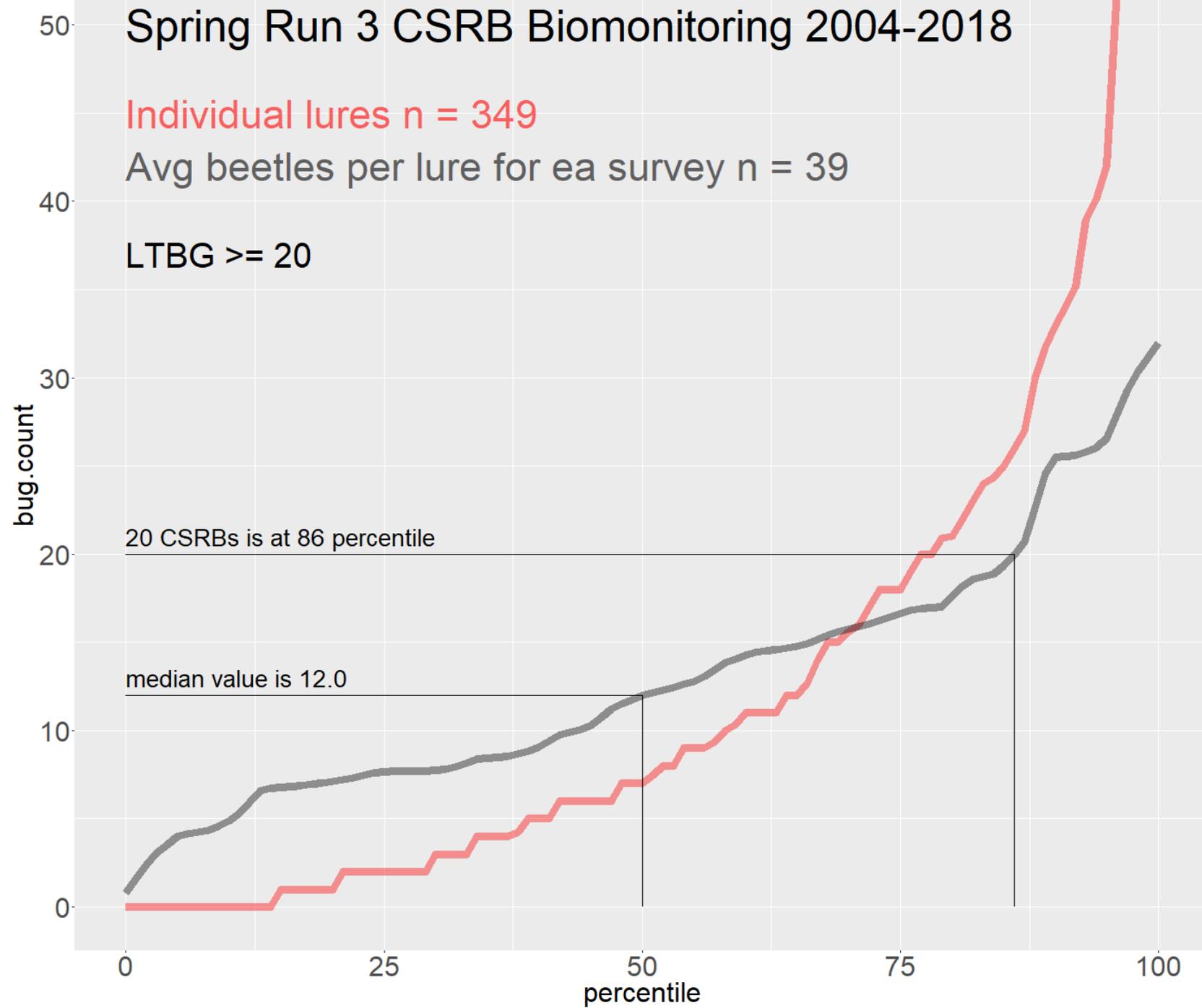


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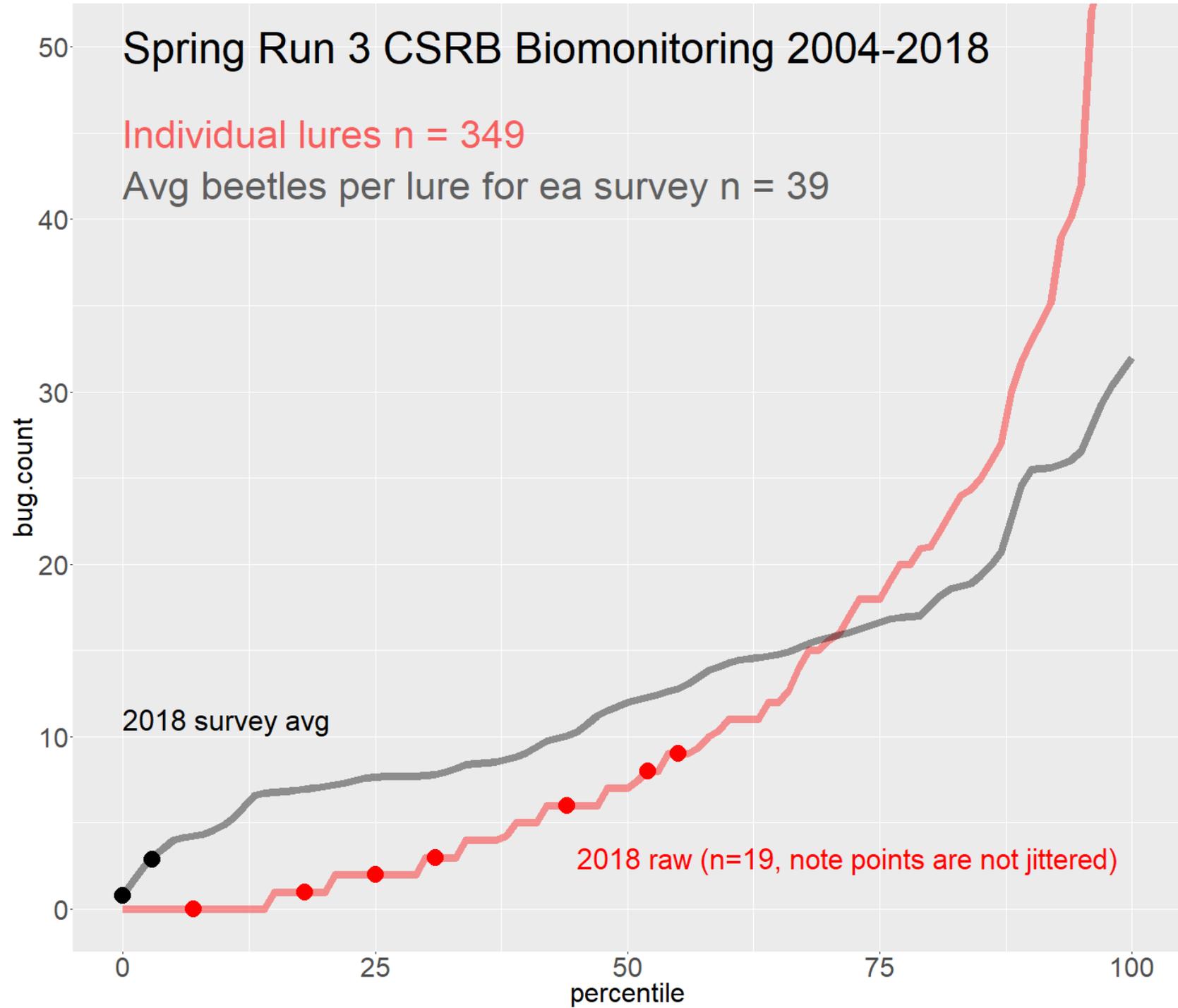


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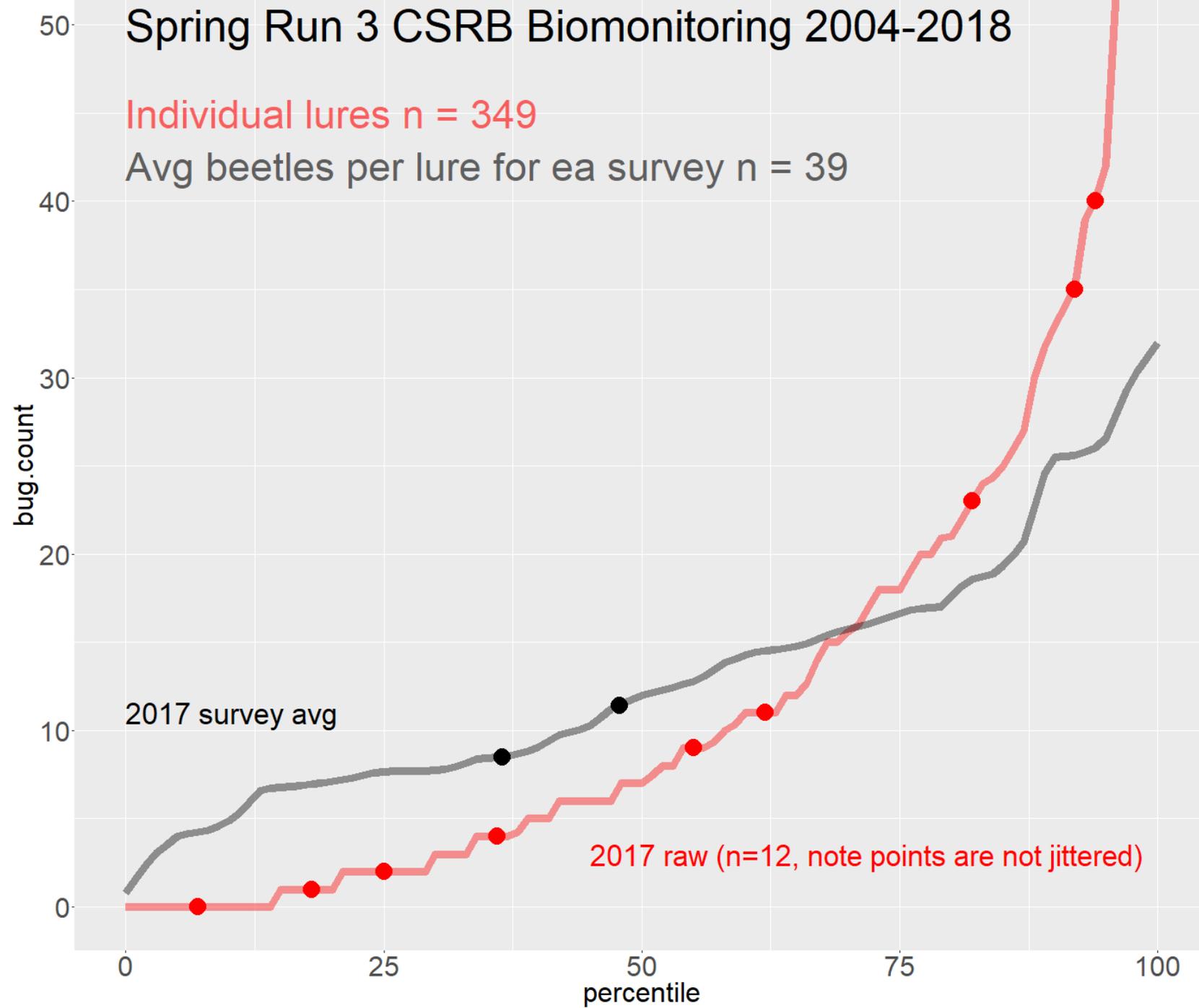


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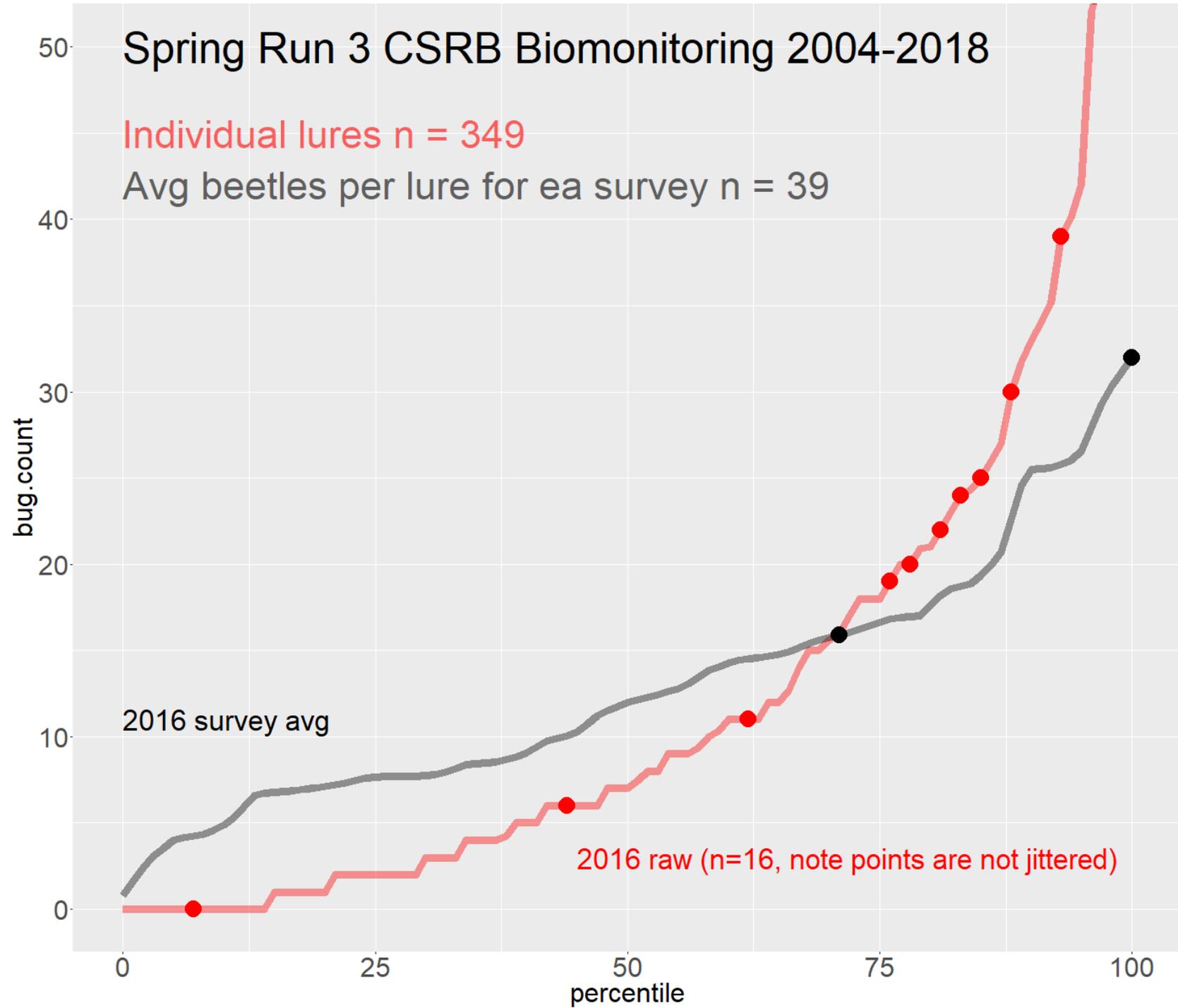


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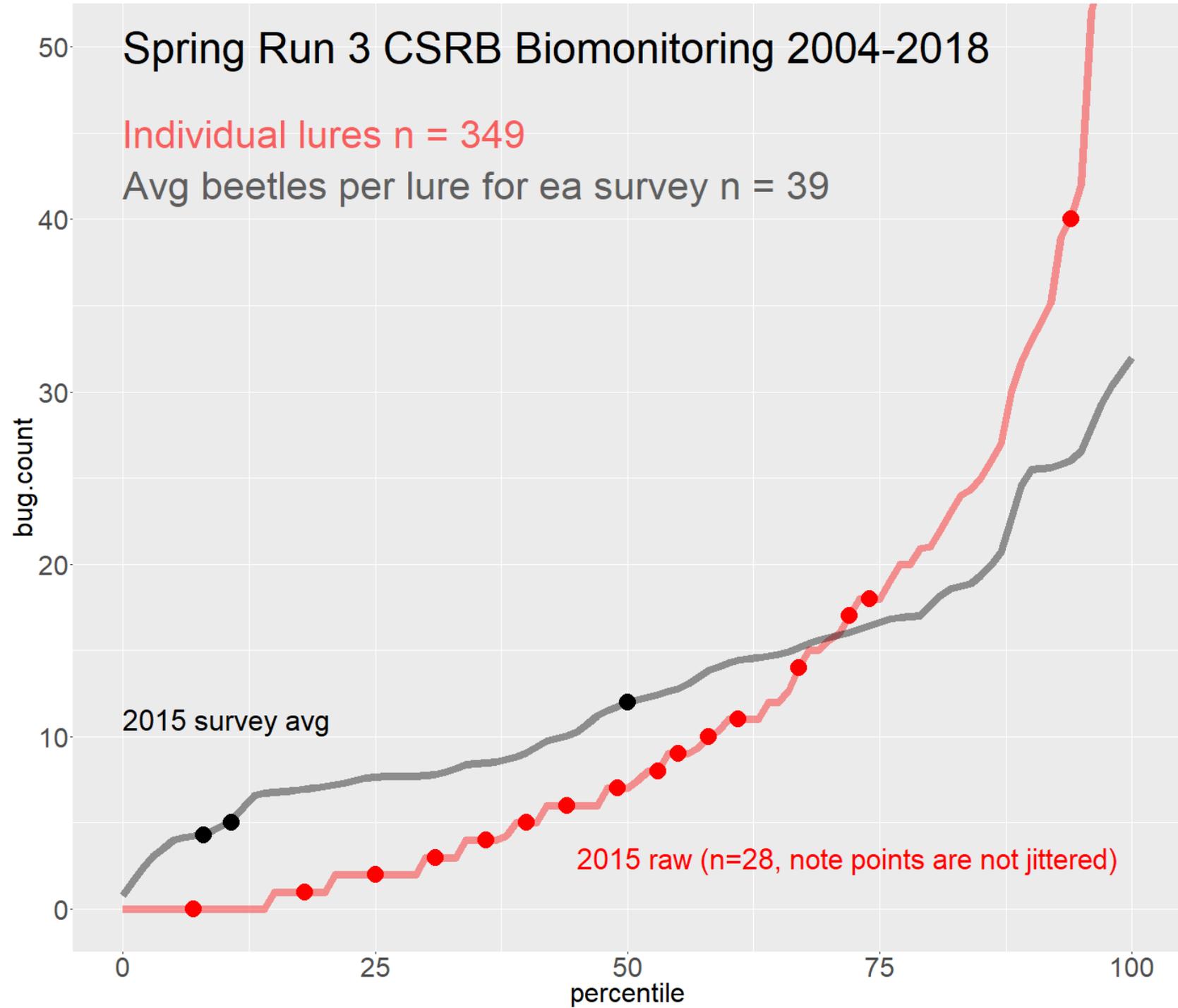


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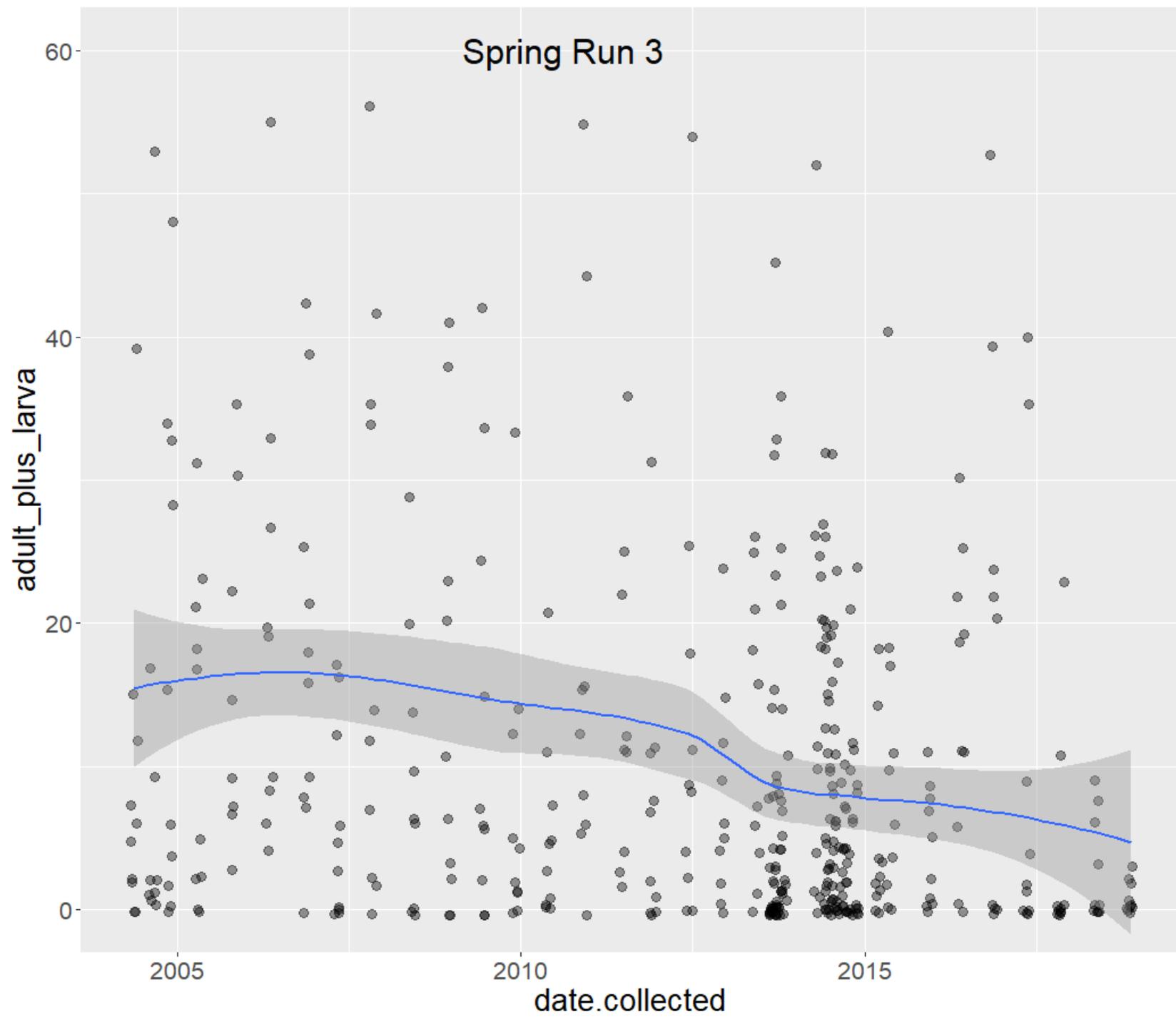


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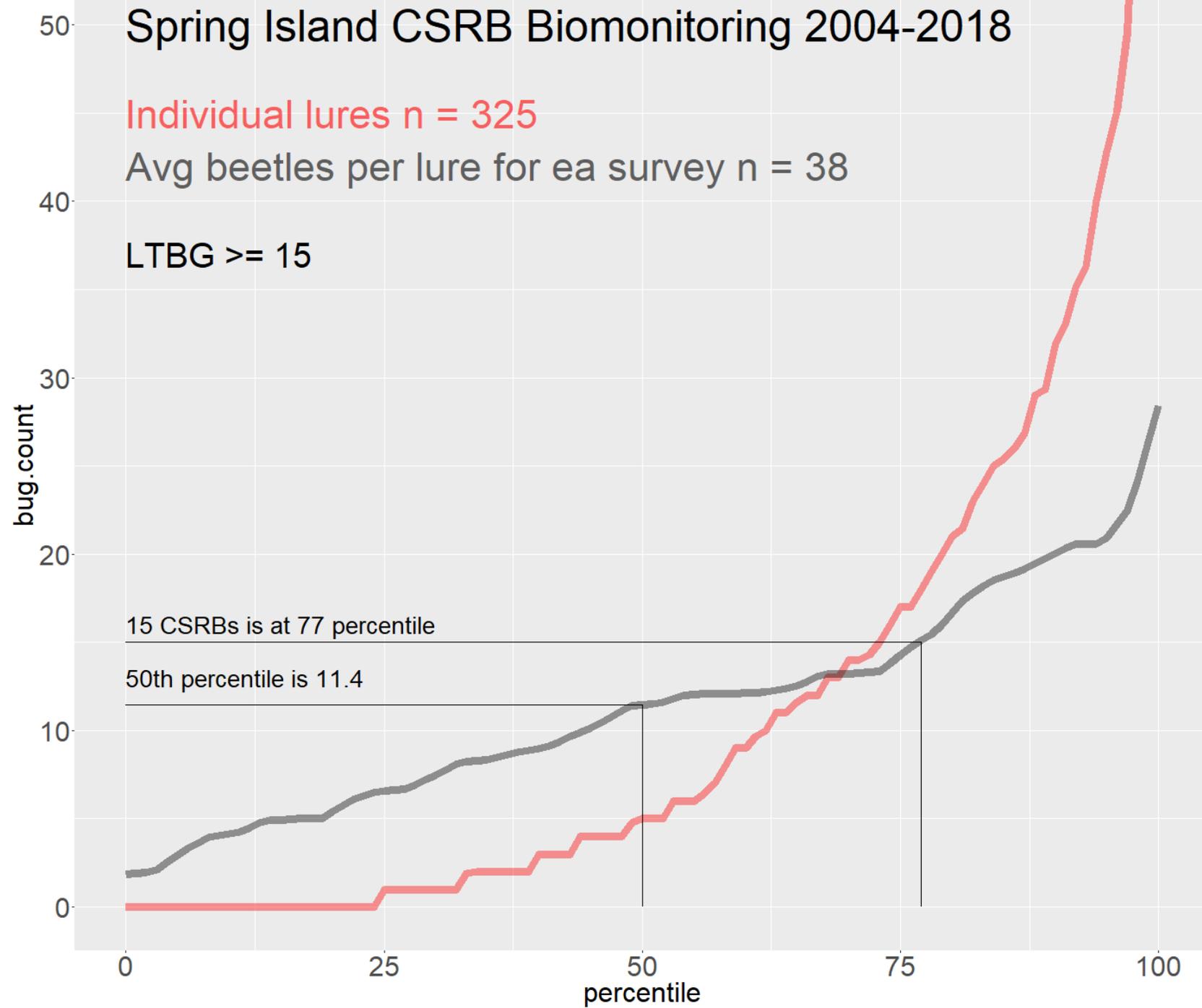


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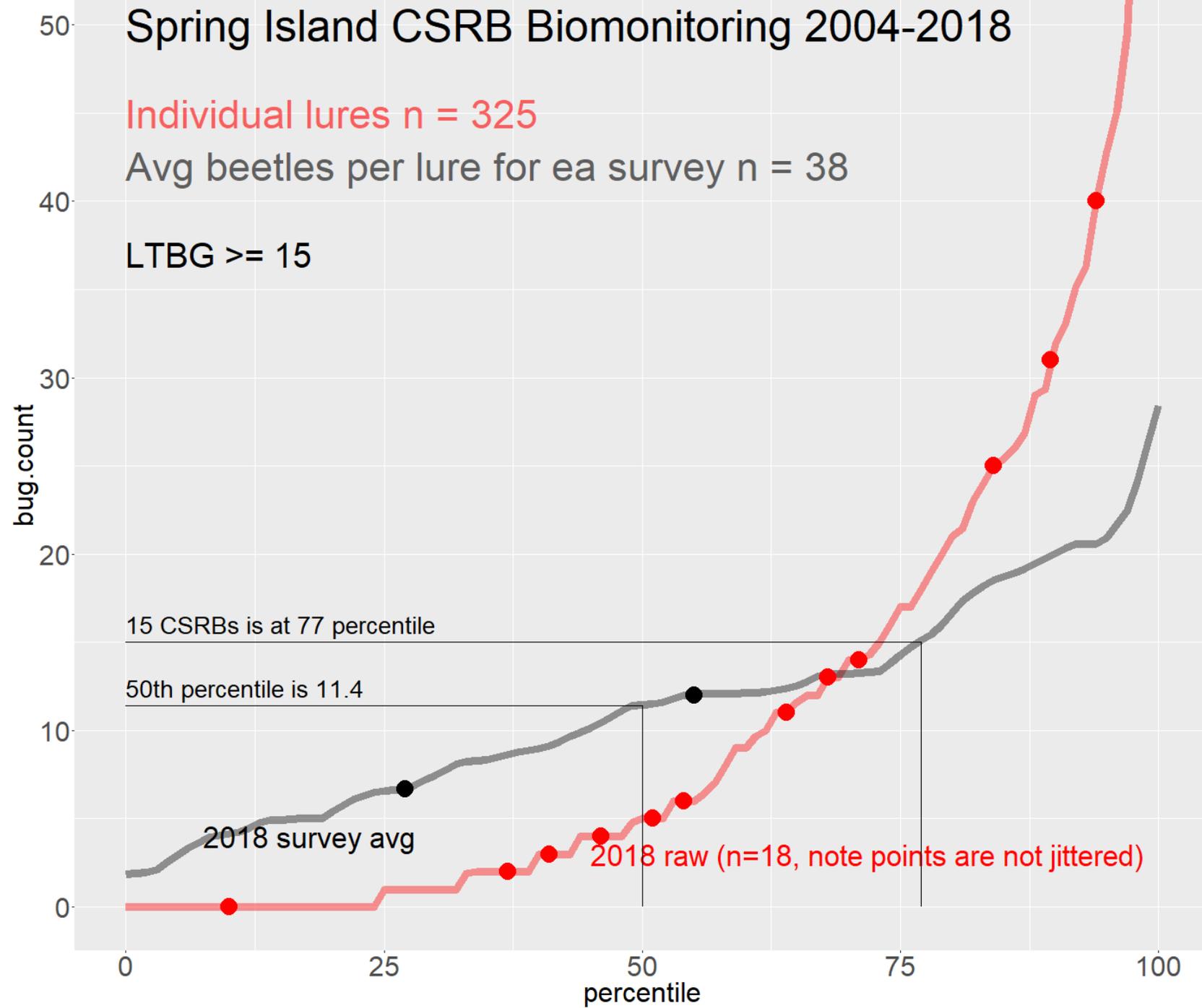


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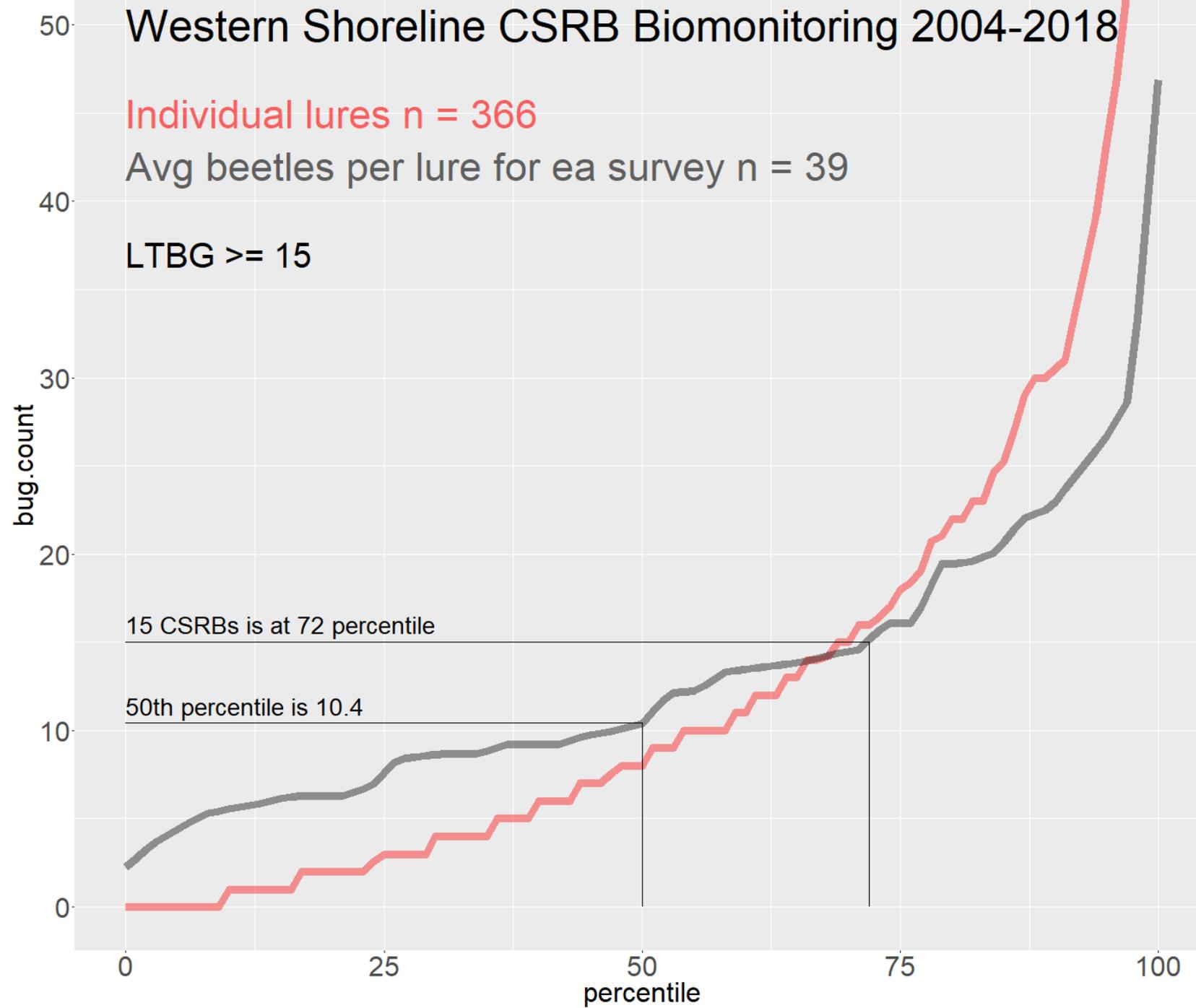


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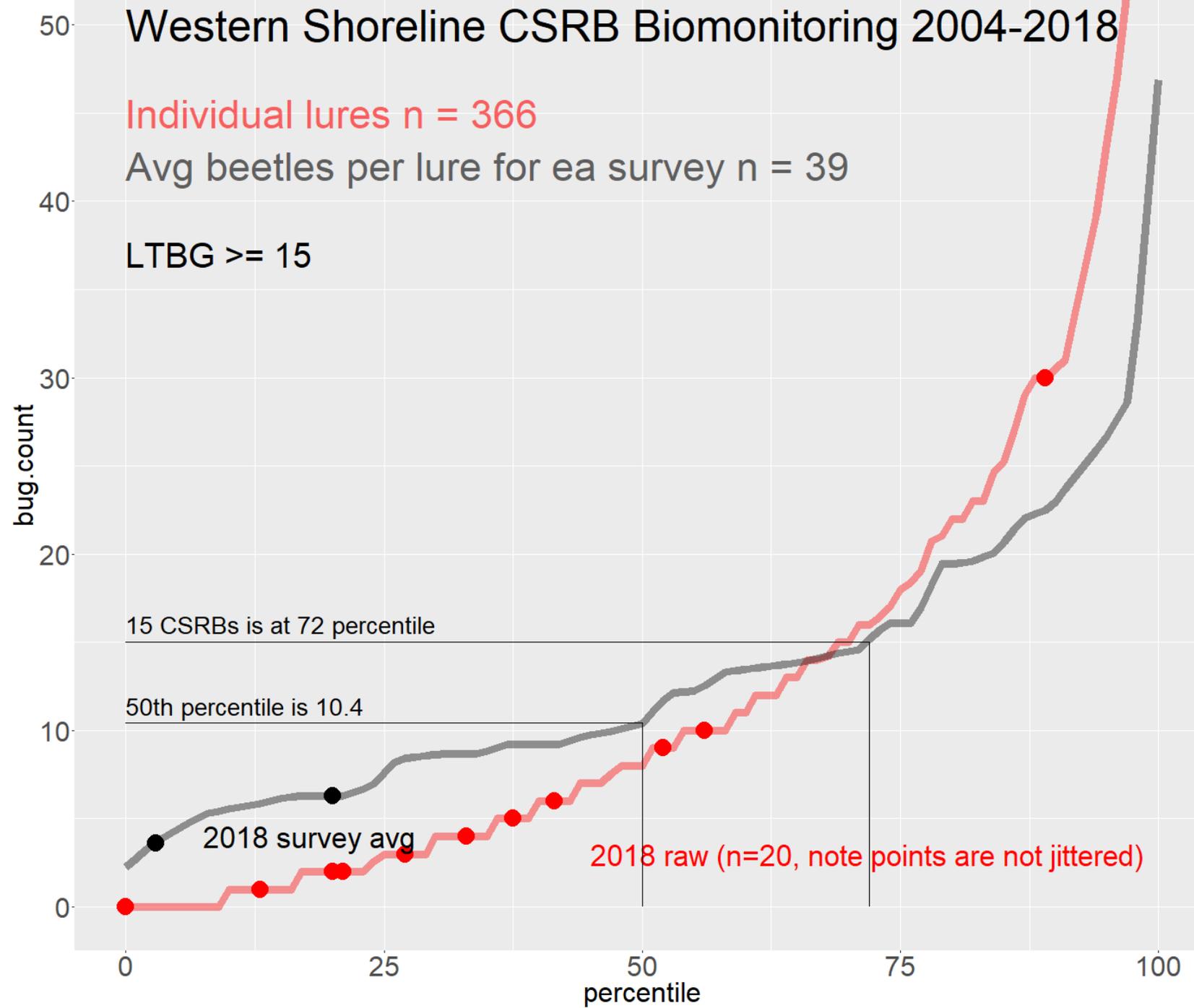


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Long-Term Biological Goals

- What are the purposes of the Long-Term Biological Goals?
 - HCP goals should address the broad biological needs of the species.
 - Maintaining a specific species life history characteristic
 - Providing conditions necessary for an important life history characteristic
 - Restoring something to more desirable conditions
 - Can focus on species itself or habitat
- EAHCP LTBG purpose



Long-Term Biological Goals

- Are the current population and habitat LTBGs for the CSRB appropriate? What are the criteria for more appropriate goals?
- What is an appropriate means to monitor the habitat quality goal?
- How can Biological monitoring, Refugia efforts, and Applied Research studies be used to establish new LTBGs?



Meeting 6 - 12.11.2019

CSRB Work Group



EAHCP CSR B WG Charge

Charge Topic	Topic Question
Cotton lure sampling methodology	Is the current cotton lure sampling methodology an appropriate means to monitor abundance at a locale?
	If not, what sampling methodologies exist that would provide a better proxy of abundance at a locale?
	If the previous two questions cannot be adequately answered without additional study, what would be an appropriate study to answer the questions?
Biological monitoring, Refugia collections, and Applied Research collections	What changes are recommended for the Biological monitoring sampling program? What are the stated goals behind those changes?
	What changes are recommended for Refugia removal efforts? What are the stated goals behind those changes?
	Are the current and proposed levels of physical activity in the CSR B habitat protective of the species? If not, what level of activity is appropriate?
Long-term biological goals	Are the current population and habitat LTBGs for the CSR B appropriate? What are the criteria for more appropriate goals?
	What is an appropriate means to monitor the habitat quality goal?
	How can Biological monitoring, Refugia efforts, and Applied Research studies be used to establish new LTBGs?





Cotton lure sampling methodology

Recommendations

- Continue using cotton lure for CSRB biological monitoring and refugia removals.
- Conduct cotton lure mesocosm experiment using methodology developed during work group meetings.
- EAHCP goal is to conduct and finalize experiment in 2020.

A small fish, possibly a juvenile perch, is shown resting on a light-colored, textured rock. The fish has a silver body with a dark stripe along its side and a prominent eye. The background is a soft-focus natural setting.

Biological monitoring, Refugia and Applied Research collections

Recommendations

- Continue historical Biological monitoring program at 3 locations, twice a year, 10 lures per survey, repeated sampling of individual orifices.
- Conduct two Comal system-wide occupancy and population studies prior to 2028.
 - Potential survey years are 2022 and 2025.
 - It is anticipated study design will be contracted out and raw data from the Nowlin 2019 population study will be used to aid design.

Biological monitoring, Refugia and Applied Research collections

Recommendations

- Reduce Refugia standing stock numbers to 150 adults (75 at each station).
- Allow Refugia collections to retain 100% of beetles captured on a lure.
- Alternate spring orifices between Refugia collection events such that the same spring orifice is not sampled on consecutive collection events.
- Do not overlap Refugia collection locations with Biomonitoring locations.





CSRB Long-Term Biological Goals

- Maintain present Long-Term Biological Goals.
- Revisit Long-Term Biological Goals after population studies and in-situ cotton lure studies have been completed.



CSRB future meetings

- Conduct an annual meeting to review progress and discuss current CSRB topics
- December 9th 2020 – location TBD
- Potential topics:
 - 2019 and 2020 Biomonitoring surveys
 - Refugia collections, captive life spans, and standing stock numbers
 - TSU population survey