

Effects of Rotenone on Zooplankton Communities

Summary Report

Center for Lakes and Reservoirs

Department of Environmental Science and Management

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by

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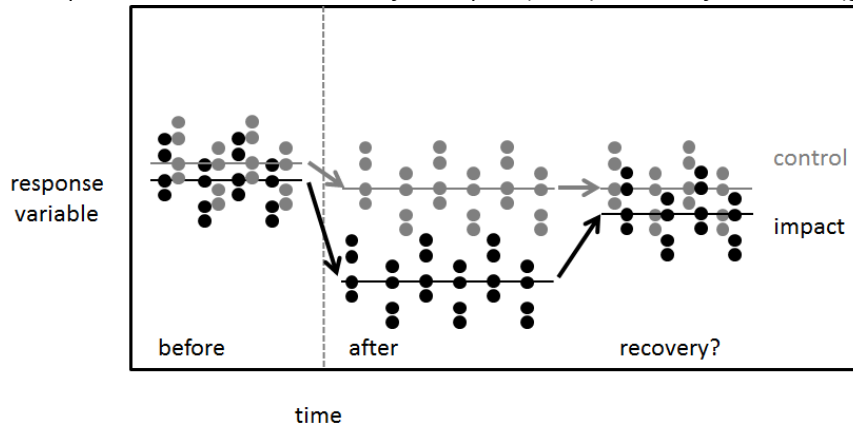
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Question: How does rotenone affect zooplankton communities in lakes in eastern Washington?

Approach: This question was evaluated by comparing the recovery of rotenone-treated lakes to non-treated reference lakes by analyzing zooplankton samples from before and after impact, using a Before-After-Control-Impact design (BACI). BACI is a commonly-used study design to test the effects of environmental impacts after change-inducing events (Underwood 1991; Underwood 1993). In its simplest form, a single site is monitored before and after an environmental event for changes in a response variable (e.g., nutrients, temperature, biomass); however, the single-site design reduces the opportunity to observe and analyze a larger variety of possible variables. The addition of a control site provides an untreated baseline comparison over the course of the study. In addition, single sites or single sampling periods may not be representative of reference conditions. This is particularly true for biotic responses, which may be quite variable. Therefore, the best design for a BACI study is to have multiple control and impact sites that are monitored several times before and after the impact to examine changes in the response variables (Underwood 1994). This design is illustrated in Figure 1.

Figure 1. A response variable is measured in four impact (black) sites and four control (grey) sites both before (n=4



dates) and after (n=10 dates). In this conceptual diagram, both the control sites and impact sites decrease after the environmental impact, perhaps because of some other confounding factor; however, the change in the impact sites is much more substantial. This highlights the need for having multiple control sites and sampling prior to the impact in both the control and impact to understand the baseline variability. Recovery will be determined when the control and impact sites are no longer significantly different.

The impacts on seven rotenone lakes were compared to seven reference lakes (Figure 2). Rotenone was applied to lakes in the fall of either 2014 or 2015. Lakes were sampled monthly when conditions permitted from June 2014 to September 2016 (n=231 total samples). Herein we examine four key metrics of zooplankton communities: total community abundance, species diversity, abundance of three major taxonomic groups (calanoid copepods, cyclopoid copepods, cladocerans), and species composition. For additional details on study design, lakes, sampling methodology, and statistics, we refer the reader to McGann (2017).

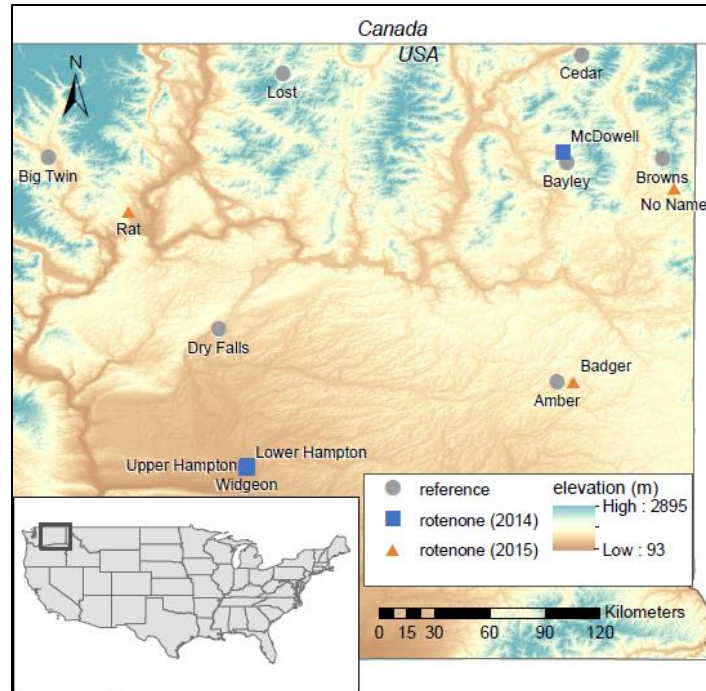


Figure 2. Map of study lakes in eastern Washington based on elevation gradient. Inset shows relative position in the state of Washington.

1. **Short-Term Responses**

- a) *Before treatment*: We compared total abundance, species diversity (the Shannon-Wiener index), abundance of three major taxonomic groups (calanoid copepods, cyclopoid copepods, cladocerans), and species composition between reference lakes and treatment lakes prior to treatment (Figure 3, 4). Linear mixed effects models were used to examine differences between rotenone and reference lakes. Findings are summarized in Table 1.

Table 1. Summary of short-term response of recovery metrics, where significance is assessed at $\alpha = 0.05$.

Metric	2014	2015
total zooplankton abundance	no significant difference	no significant difference
diversity	no significant difference	marginally significant difference
calanoid copepod abundance	no significant difference	no significant difference
cyclopoid copepod abundance	significantly higher in rotenone lakes vs. reference lakes	significantly higher in rotenone lakes vs. reference lakes
cladoceran abundance	no significant difference	no significant difference
species composition	no significant difference	no significant difference

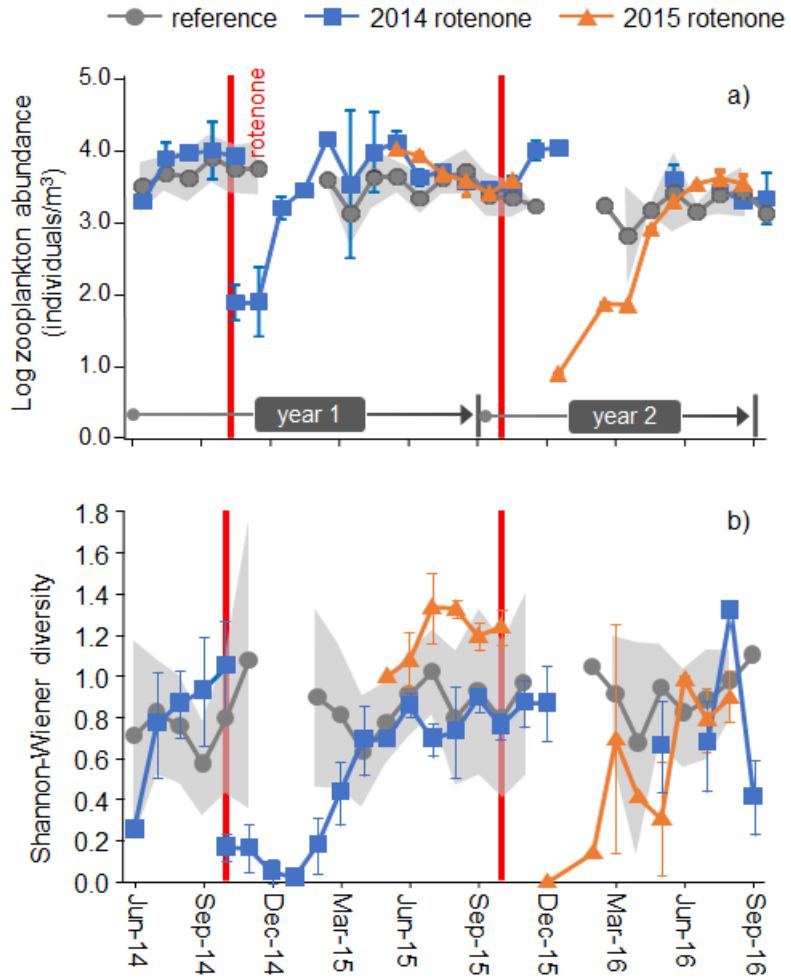


Figure 3. Monthly averaged adult a) log zooplankton abundances (individuals/m³) and b) Shannon-Wiener diversity for 2014 rotenone, 2015 rotenone, and reference lakes. Gray bars represent 95% confidence intervals for reference lakes, error bars for rotenone lakes represent ± 1 standard error, and red lines indicate date of treatment.

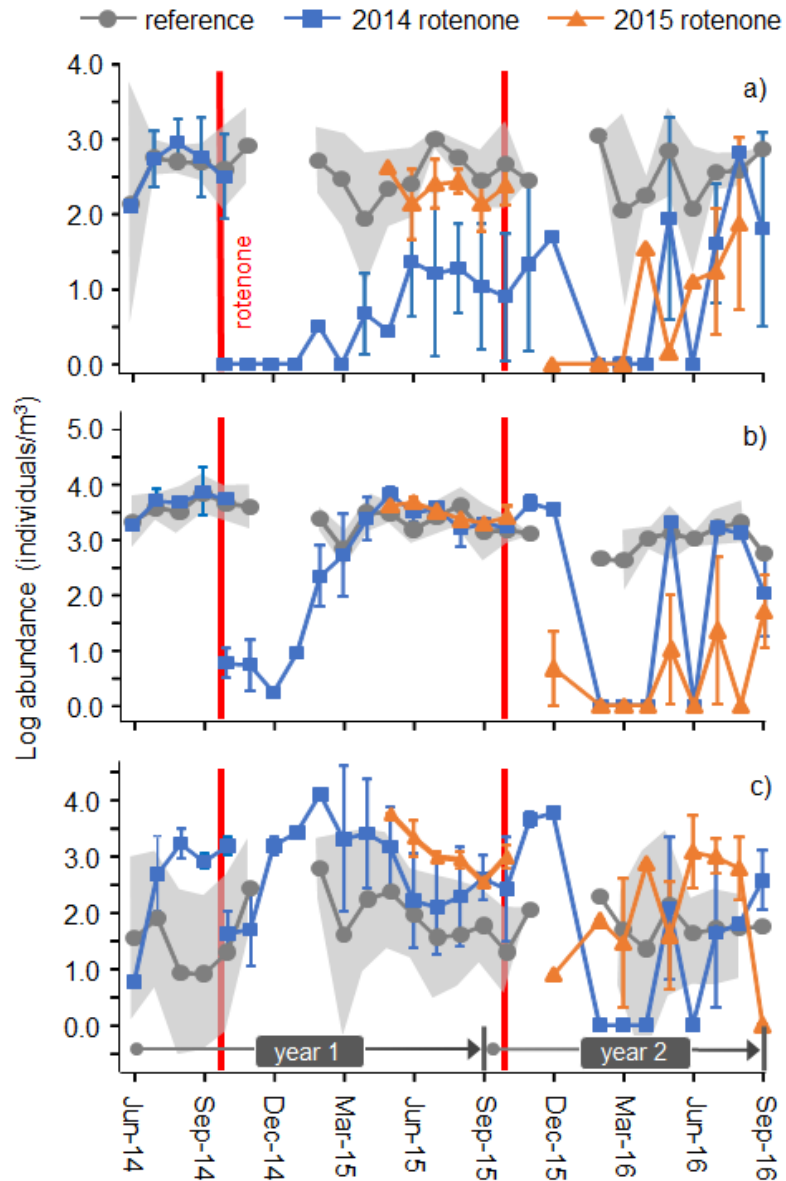


Figure 4. Monthly averaged log adult a) calanoid abundance, b) cladoceran abundance, and c) cyclopoid abundance (individuals/m³) for 2014 rotenone, 2015 rotenone, and reference lakes. Gray bars represent 95% confidence intervals for reference lakes, error bars for rotenone lakes represent ± 1 standard error, red lines indicate date of treatment for 2014 and 2015 rotenone lakes.

- b) *Immediately after treatment:* We compared total abundance, species diversity, and abundance of three major taxonomic groups in the two weeks following treatment (n=4: Lower Hampton, McDowell, Upper Hampton, Widgeon) (Figure 3, 4). Values were calculated to determine % change in metrics by:

$$\% \text{ change} = \frac{(\text{value after treatment} - \text{value before treatment})}{\text{value before treatment}}$$

No statistical tests were performed on these data. On average, when comparing rotenone lakes after treatment to before treatment, we found:

- abundance – 98.0% decline
- diversity – 81.4% decline
- calanoid copepod abundance – 100% decline
- cyclopoid copepod abundance – 99.8% decline
- cladoceran abundance – 89.7% decline

2. Long-Term Responses

- a) *Year 1:* We compared total abundance, species diversity, and abundance of three major taxonomic groups between reference lakes and treatment lakes in the first year following treatment (n=14) (Figure 3, 4). Linear mixed effects models were used to examine differences between rotenone and reference lakes. Findings are summarized in Table 2. Values were calculated to determine % change in metrics by:

$$\% \text{ change} = \frac{(\text{value after treatment} - \text{value before treatment})}{\text{value before treatment}}$$

Table 2. Summary of long-term response of recovery metrics (one year), where significance is assessed at $\alpha = 0.05$. Percent change is shown in parentheses.

Metric	2014 Rotenone Lakes	2015 Rotenone Lakes
total zooplankton abundance	marginally significant rotenone effect (21% decline)	no significant rotenone effect (64% decline)
diversity	significant rotenone effect (42% decline)	significant rotenone effect (56% decline)
calanoid copepod abundance	significant rotenone effect (93% decline)	significant rotenone effect (76% decline)
cyclopoid copepod abundance	significant rotenone effect (313% increase, driven by high abundances in Badger Lake)	significant rotenone effect (56% decline)
cladoceran abundance	significant rotenone effect (73% decline)	marginally significant rotenone effect (68% decline)

Following treatment, zooplankton abundance immediately declined but showed a rapid increase to pre-treatment levels by February of the following year (~4 months after treatment), which exceeded pre-treatment levels for 2-3 months, and then remained steady for the remainder of the study duration. Shannon-Wiener diversity decreased immediately following treatment in 2014 and 2015 rotenone lakes, with a general increasing trend until June of the following year (~8 months after treatment).

Calanoid copepod, cyclopoid copepod, and cladoceran abundances all declined following rotenone treatment. Calanoid copepod abundance increased at the slowest rate, while cyclopoid abundances increased at the fastest rate following treatment of all rotenone lakes,

with returns to pre-treatment abundances between February and April of the following year (4-6 months after treatment). Cladoceran abundance increased at a modest rate following the disturbance, returning to pre-treatment abundances by April or May of the following year (6 - 7 months after treatment).

Analysis of zooplankton community composition showed increasing variability in treatment lakes following rotenone treatment when compared to relatively stable community composition in reference lakes (Figure 5). Examining each lake individually (Figure 6), we observed that most lakes shifted to the bottom left quadrant of the ordination plot immediately following treatment (October), corresponding with taxa such as the littoral cladoceran *Chydorus* and the cyclopoid *Acanthocyclops robustus*, but no calanoid copepods. Community composition generally returned to pre-treatment community composition by June of the year following rotenone treatment (~8 months after treatment).

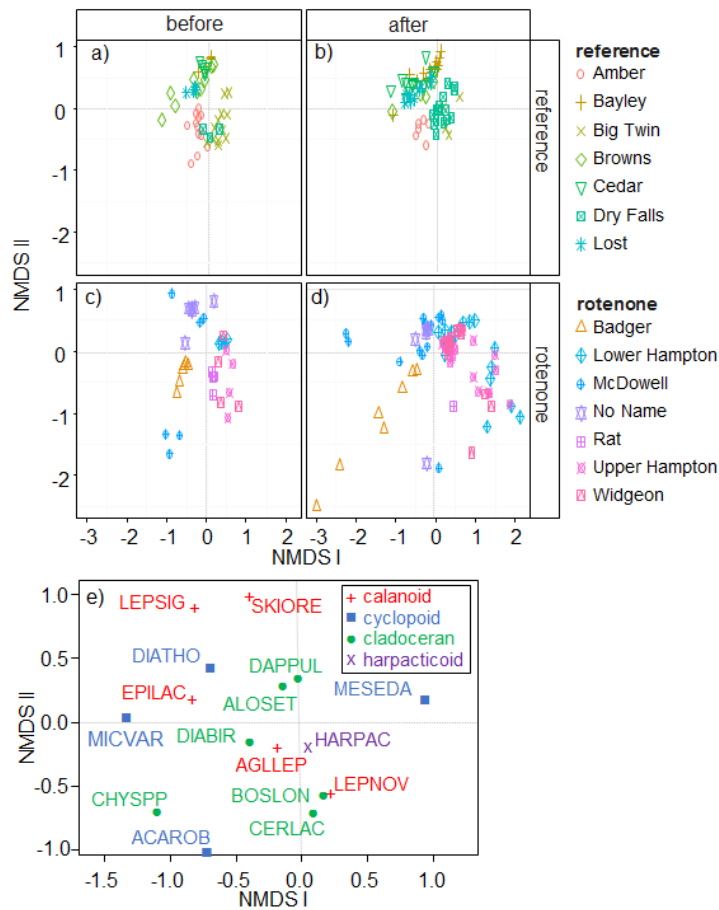


Figure 5. NMDS ordination of zooplankton communities contrasting reference lakes a) before and b) after, with rotenone lakes c) before, and d) after. e) Species scores for common taxa contributing to the ordination plot, with different symbols for major groups.

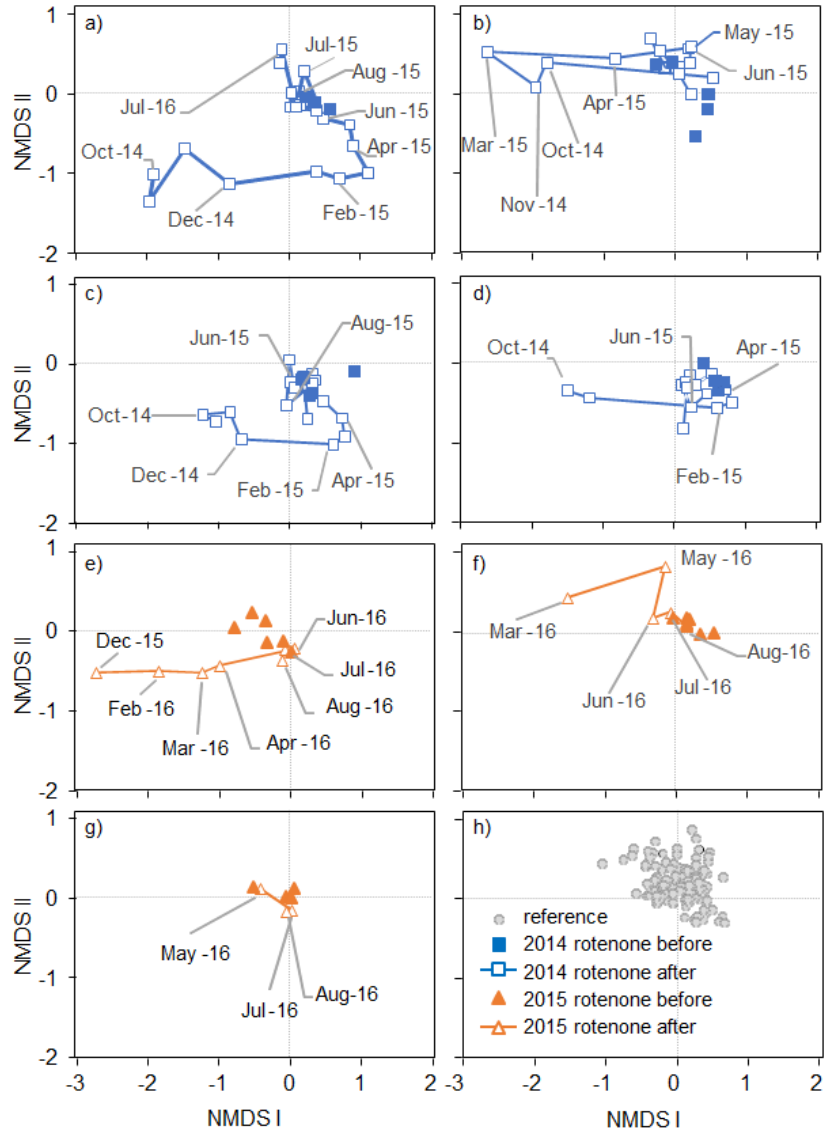


Figure 6. Recovery trajectories of zooplankton communities for all treatment lakes (a-g) and reference lakes (h) from NMDS. a) Lower Hampton, b) McDowell, c) Upper Hampton, d) Widgeon, e) Badger, f) No Name, g) Rat, and h) all reference lakes throughout the study (2014-2016).

- b) *Year 2*: We compared total abundance, species diversity, and abundance of three major taxonomic groups between reference lakes (n=7) and treatment (n=4) lakes in the second year following treatment (Figure 3, 4). Linear mixed effects models were used to examine differences between rotenone and reference lakes. Findings are summarized in Table 3. Values were calculated to determine % change in metrics by:

$$\% \text{ change} = \frac{(\text{value after treatment} - \text{value before treatment})}{\text{value before treatment}}$$

Table 3. Summary of long-term response of recovery metrics (two years), where significance is assessed at $\alpha = 0.05$. Percent change is shown in parentheses.

Metric	2014 Rotenone Lakes
total zooplankton abundance	no significant rotenone effect (58% decline)
diversity	significant rotenone effect (11% decline, excluding an outlier)
calanoid copepod abundance	significant rotenone effect (27% decline)
cyclopoid copepod abundance	significant rotenone effect (26% increase)
cladoceran abundance	no significant rotenone effect (77% decline)

- c) *Overall trends*: Although there was variability between different response metrics, some general conclusions can be drawn. Cyclopoid copepods and cladocerans, as well as total abundance, typically recovered the most quickly following rotenone treatment, recovering to pre-treatment and reference lake conditions near the end of the first year. Calanoid copepods and species diversity recovered more slowly and were still considered not fully recovered at the end of the second year. Species composition was considered largely recovered by the end of the study, though calanoid copepod species were still in low abundances or were absent from rotenone lakes following treatment.

We hypothesize that the lagging recovery of calanoid copepods and some cladoceran taxa (i.e., *Bosmina longirostris*) is the result of failure to achieve critical densities during the summer open-water period (Figure 7). These critical densities are necessary to overcome mate limitation for sexual reproduction (Gerritsen 1980). Other studies have examined the effects of fish stocking and subsequent removal on zooplankton in alpine lakes and observed similar trends: *Daphnia* can recover relatively quickly but calanoid copepods may take decades to recover on their own (Knapp & Sarnelle 2008; Kramer, Sarnelle & Knapp 2008). However, the same lag in recovery may not be observed in lowland lakes. Using data on rotenone treatments that occurred prior to our study, we found that there was a significantly longer time period elapsed of time since last rotenone in reference lakes compared to treatment lakes (average of 42 years vs. 21 years, respectively). Combined with our finding of no

significant difference in calanoid copepod abundance prior to the start of our study, this suggests that calanoid copepod recovery from rotenone in lowland lakes may proceed more quickly than in alpine lakes.

Table 4: Summary of years since last rotenone treatment, with means and standard deviations (SD) for reference and rotenone lakes and results of Welch's two sample t-tests (df=11).

Lake	Years post treatment
Reference	
Amber	30
Bayley	Never
Big Twin	35
Browns	59
Cedar	21
Dry Falls	62
Lost	43
<i>Mean reference (\pm SD)</i>	<i>42 \pm 15</i>
2014 Rotenone	
Lower Hampton	13
McDowell	11
Upper Hampton	13
Widgeon	13
2015 Rotenone	
Badger	15
No Name	68
Rat	12
<i>Mean rotenone (\pm SD)</i>	<i>21 \pm 19</i>
	t 2.03
	p 0.067

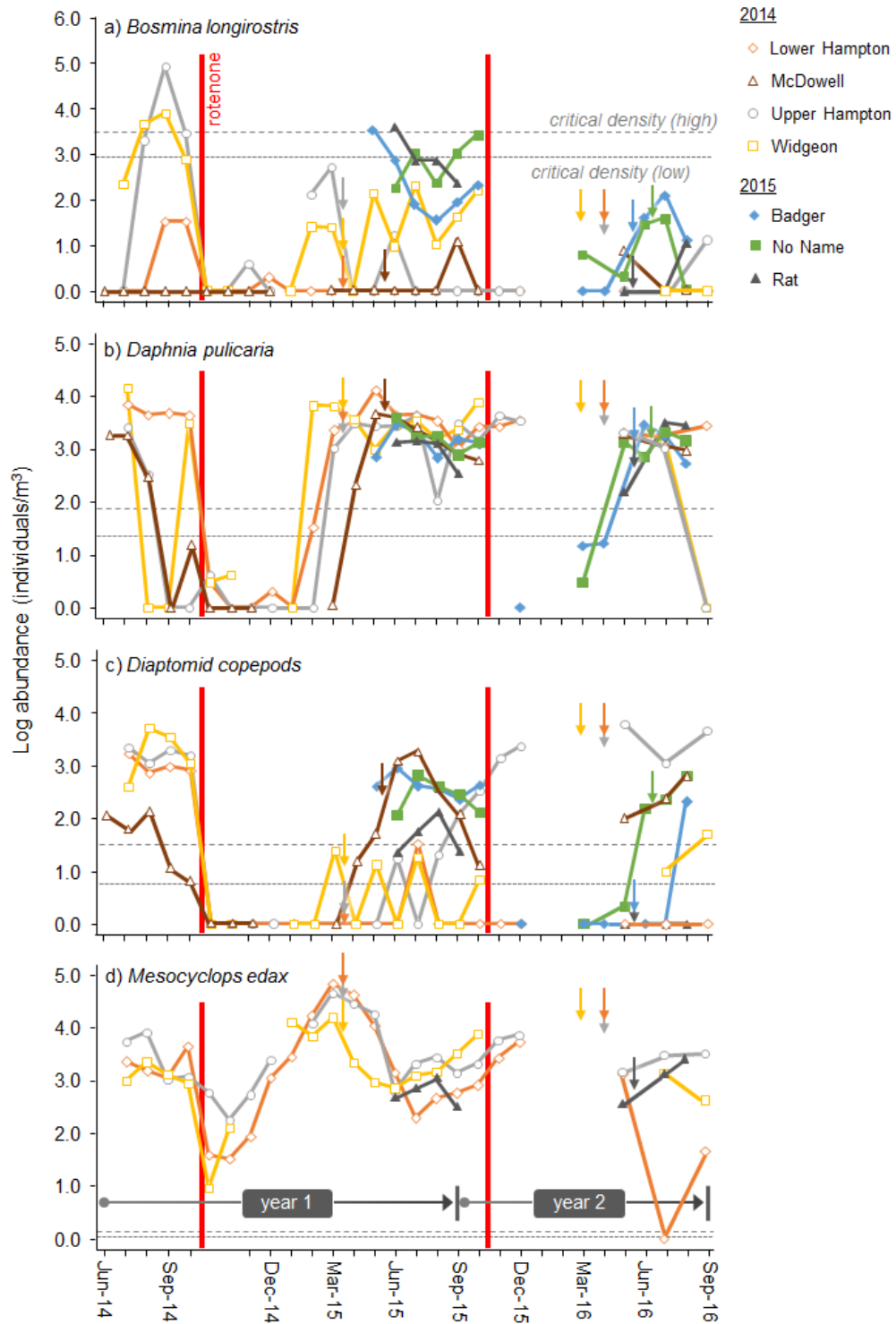


Figure 7. Log abundance (individuals/m³) of a) *Bosmina longirostris*, b) *Daphnia pulicaria*, c) diaptomid copepods (*Agladiaptomus leptopus*, *Leptodiaptomus novamexicanus*, or *Skistodiaptomus oregonensis*), and d) *Mesocyclops edax* across 2014 (open symbols) and 2015 (closed symbols) rotenone lakes. Dashed line indicates the upper critical density for establishment, dotted line indicates the lower critical density for establishment. Red lines indicate date of treatment for 2014 and 2015 rotenone lakes. Vertical arrows indicate dates of trout fry stocking.

We cannot comment on the exact speed of recovery, the effect of fish stocking, and species interactions in the context of our study results as the study was not designed to answer these questions. We examined the effect of climate on zooplankton recovery by comparing mean annual air temperature¹ to the percent change in zooplankton abundance between June, July, and August of the year before treatment to June, July, and August the year after treatment. There were no significant trends (Figure 8).

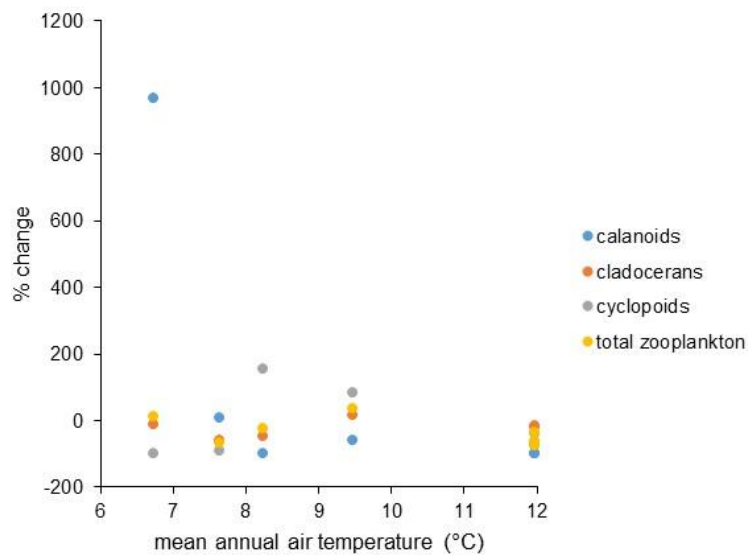


Figure 8. Mean annual air temperature (°C) versus % change in calanoid copepods ($R^2=0.40$, $p>0.05$), cladocerans ($R^2=0.01$, $p>0.05$), cyclopoid copepods ($R^2=0.02$, $p>0.05$), and total zooplankton ($R^2=0.21$, $p>0.05$) abundance ($n=7$).

3. **Future Research Options**

To determine if a more complete recovery of certain species would continue to take place over a longer period of time, if funding and staff were available to continue sampling and analyzing the samples, the following recommendations could be made:

- 1) Monitoring continue following rotenone treatment for a minimum of three years (with a focus on the summer season only in years 2 and 3), given that we did not see complete recovery of either species diversity or calanoid copepods in most lakes. Following this three year period, additional monitoring could subsequently occur every second year to ascertain recovery status.
- 2) Conduct a study on the effects of timing of fish stocking on recovery of zooplankton communities, with a suggestion to keep some lakes unstocked for the duration of the study. Assess when there is sufficient secondary production in lakes to support restarting fish stocking.

4. **References**

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¹ <http://www.prism.oregonstate.edu/>

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