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**Paleoecological Investigation of the Effect of Rainbow Trout Predation on Zooplankton  
Body Size and Community Composition**

**Michael Gilray**

**An Honors Thesis**

**Submitted for partial fulfillment of the requirements for graduation with honors in Biology  
from Hamline University**

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## Abstract

Stocking fish for sport may induce food web cascades that impact the trophic state of lakes. Here, the practice of stocking rainbow trout into a Twin Cities metropolitan area lake (Square Lake) was examined to see if changes have occurred in the lake's zooplankton community that could explain its declining water quality. Rainbow trout are size-selective predators that consume large-bodied herbivorous zooplankton (i.e., *Daphnia pulicaria*) and therefore may cause decreased phytoplankton grazing pressure and more eutrophic conditions. A paleoecological study was performed that compared the sediment record of Square Lake to that of a reference lake (Big Carnelian Lake) that has never been stocked with rainbow trout. The primary focus of this study was to use exoskeletal remains (*Bosmina* headshields and *Daphnia* post-abdominal claws) and resting eggs (*Daphnia* ephippia) to determine if changes in the abundances of multiple zooplankton species and the body size of *Daphnia* species have changed from pre-European settlement dates to 1981 onwards, due to the initiation of the trout stocking program. We found that for both proxies (post-abdominal claws and ephippia), *D. pulicaria* mean body size decreased significantly in Square Lake during the post-stocking years (after 1981), but the body size of *D. mendotae* did not decrease. No significant changes in *D. pulicaria* body size were seen in either lake before 1981. A shift in species composition from larger-bodied *D. pulicaria* to smaller-bodied *D. mendotae* after 1981 was not seen until late (~ 2010) in the sediment record. In addition to these findings, data on human population size and land development in the lakes' watersheds were used to understand changes in the sediment and zooplankton community composition. For both lakes, it was found that since pre-European settlement to 1981 the sediment composition has become increasingly dominated by clastic and organic materials, and that the zooplankton populations have shifted in dominance from *Bosmina* to *Daphnia*. Differences in fish stocking practices of each lake appeared to have caused opposite changes in the mean body size of their *D. pulicaria* populations. The rainbow trout stocking in Square Lake has caused the mean body size to decrease, while the stocking of walleye into Big Carnelian Lake seems to have caused it to increase.

## Introduction

Changes in the trophic state of lakes may occur from the "bottom up" via changes in the supply of nutrients to the ecosystem (Brugman & Speziale, 1983; Frisch et al., 2014), or from the "top down" as a result of alterations in predator densities (Sweetman & Finney, 2002) that cascade down the food chain to the primary producers (planktonic algae). Human impacts (e.g.,

sewage effluent, farm fertilizer runoff, storm drain effluent) that increase the loading of nutrients that commonly limit primary production (e.g., P and N) are the most common cause of eutrophication (Schindler, 2006). However, trophic cascades initiated by changes in predator populations (Carpenter et al., 1985) are also known to promote eutrophication. Trophic cascades can occur naturally through events such as winter fish kills (Balayla et al., 2010) and toxin exposures (Burkholder et al., 1995) that decrease the abundance of predators. However, fisheries management practices can also induce these cascades (Shapiro & Wright, 1984; Hembre & Megard, 2005). For example, when zooplanktivorous fish (e.g. rainbow trout) are stocked into a lake ecosystem they consume zooplankton. This decrease in zooplankton then reduces the algae grazing pressure, resulting in increased algae growth and more eutrophic conditions.

Discerning the cause of eutrophication (top-down or bottom-up) is critical for making lake management decisions to reverse eutrophication trends. Square Lake (Washington County, MN) is an example of a lake that has experienced a eutrophication trend over the past several decades, and several previous studies have investigated potential bottom-up and top-down causes for the trend (Engstrom & Saros, 2001; Hembre, 2016). Historically, Square Lake has been one of the clearest lakes in the Twin Cities Metropolitan Area (Metropolitan Council, 2013). This clarity has made Square Lake highly valued recreationally for activities such as scuba diving certification, swimming, water skiing, and fishing. Square Lake's clarity and depth provide suitable habitat for rainbow trout, which require cold ( $< 21\text{ }^{\circ}\text{C}$ ) and well oxygenated ( $> 5\text{ mg/L}$ ) water (Matthews & Berg, 1997) and because of this the Minnesota Department of Natural Resources (MDNR) initiated a program in 1981 in which 5,000 yearling trout were stocked to the lake annually. However, since 1981 when rainbow trout were first stocked, the lake's water

quality has declined (higher levels of phytoplankton and lower water clarity). Average summertime water clarity (secchi depth) was between 7-8 m in the 1970s and decreased to about 5 m by 2012 (MPCA water quality database). Concern about the degradation in the lake's water quality led the MDNR to impose a moratorium on rainbow trout stocking in 2013 so that the impact of the stocking practice could be assessed. A monitoring study (Hembre, 2016) compared data from two years (2010 & 2012) in which the lake was stocked with trout, to three years of data from the moratorium period (2013-2015). The goal of this study was to determine if the decreased water quality of Square Lake was due to a top-down trophic cascade, induced by the stocking of rainbow trout. Rainbow trout are zooplanktivorous visual predators that target larger and more visually apparent species over smaller and harder to detect species (Brooks & Dodson, 1965; Sørnes & Aksnes, 2004). In Square Lake there are two *Daphnia* species: the large-bodied *Daphnia pulicaria* and the smaller-bodied *Daphnia mendotae*. It was predicted that in years when rainbow trout were added to Square Lake they would select against the large-bodied *D. pulicaria* causing this species to be less abundant and smaller in body size as compared to the moratorium years. Large-bodied *D. pulicaria* can filter a larger volume of algae than the smaller bodied *D. mendotae* (Burns, 1969). Therefore when their abundances and mean body size decrease, they are able to filter less algae, leading to lower water clarity. The moratorium study (Hembre, 2016) found that the removal of the rainbow trout allowed *D. pulicaria* mean body size and population size to increase. This then led to more algae being filtered and clearer water in Square Lake.

While these results may be conclusive, there are some important limitations of the moratorium monitoring study. Most importantly, this study only lasted for five years, two years

of pre-moratorium monitoring and three years of monitoring during the moratorium. With respect to the period of Square Lake's history when that lake was stocked with trout (since 1981), this is a relatively limited glimpse at the conditions of the lake. Additionally, the moratorium study only investigated Square Lake and did not include a reference lake for comparison.

To expand on the findings of the moratorium monitoring study and to address some of the shortcomings of that study, a paleoecological study of Square Lake and Big Carnelian Lake (a reference lake) was initiated in 2015. Big Carnelian Lake was selected to serve as a reference lake due to its close proximity to Square Lake, its similar size and depth, and the fact that it has never been stocked with rainbow trout. In this paleoecological study we use zooplankton remains (exoskeletal fragments & ephippia) to provide an understanding of the long-term history of changes in these lakes (Brugam & Speziale, 1983; Sweetman & Finney, 2003; Korosi et al., 2008). When paired with the reference lake concept, a paleolimnological approach allows for large time scale comparisons between lake ecosystems (Frisch et al., 2017).

Predictions were made for each of the two study lakes. For Square Lake the initiation of the trout stocking program in 1981 was predicted to increase the size-selective predation pressure on the *D. pulicaria* population. Predation by rainbow trout was expected to be reflected in the sediment record as a decrease in the abundance of *D. pulicaria* relative to the *D. mendotae* population. Additionally, we expected the size-selective predation of the trout to cause a decrease in the body size of *D. pulicaria* (assessed through measuring the sizes of ephippia and post-abdominal claws in sediment samples). For Big Carnelian Lake, the reference lake without any history of rainbow trout stocking, we predicted that there would not be significant changes in

the *Daphnia* species composition or *Daphnia* body sizes through time. An additional objective of this study was to evaluate whether the two proxies for *Daphnia* size and abundance, exoskeletal remains and ephippia, agree with each other. This has been a concern for researchers who use zooplankton remains from sediments to understand historical changes in lakes (Nykänen et al., 2009) because of the differences in how these two types of remains are generated. Ephippia are only produced when the population is reproducing sexually and only by the individuals which have grown large enough to participate in sexual reproduction. Additionally, in larger lakes (like Square and Big Carnelian) with year-round populations of *Daphnia*, sexual reproduction does not reliably occur at a particular time of year and populations may reproduce solely through asexual reproduction for protracted periods (Hembre & Megard, 2006). Exoskeletal remains, on the other hand, are produced by all *Daphnia*, regardless of size and stress conditions whenever the animals molt. Therefore, our investigation of both ephippia and exoskeletal remains provides a more holistic assessment of changes in the *Daphnia* populations in the two lakes and enables us to discern whether records of the two proxies are consistent with each other through time.

## **Methods**

### *Study sites*

Square Lake and Big Carnelian Lake are located in Washington County, Minnesota. European settlement of this area began in the late 1830s (Anderson et al., 1996). As the human population in the county grew, a significant portion of the land was developed for agriculture (Anderson et al., 1996). Land devoted to agriculture peaked around 1925 and began to rapidly decline after 1959 (United States Department of Agriculture) (Fig. 1). The human population in

Washington County remained relatively low until about 1930 when it began to rise, nearing 300,000 in 2012 (United States Census Bureau) (Fig. 1).

Square Lake is located in May Township (near Stillwater, MN). The lake has a surface area of 82.2 ha (33% littoral), a maximum depth of 20.7 m, and a mean depth of 9.3 m. Its watershed to lake ratio is 2.5:1. Square Lake is primarily fed by groundwater (70%) with the remainder supplied by precipitation (25%) and surface runoff (5%) (Carnelian Marine St. Croix Watershed District Water Management Plan, 2010) (Table 1). The watershed is comprised of approximately 49% forested land, 26% grassland, 13% residential, 9% agricultural land, and 3% wetlands (Doneux, 2002). The lake's relatively small and modestly developed watershed, and the low contribution of surface water runoff to its hydrologic budget are important factors that contribute to the lake's relatively low nutrient state (Ramstack et al., 2004). The native fish species of this lake predominantly consist of bluegills, sunfish, northern pike, and black crappie. (Table 2). In addition to these native fish species, rainbow trout (*Oncorhynchus mykiss*) were stocked annually in the fall and spring from 1981-2013 at a rate of ~ 5,000 yearling trout/year by the Minnesota Department of Natural Resources (MDNR). A moratorium on trout stocking began in 2013 and is ongoing. The MDNR's trout stocking program was operated as a 'put and take' fishery in which the trout did not naturally reproduce in the lake (because of lack of access to suitable spawning habitat), and the majority of the trout stocked each year were removed through recreational fishing or by other sources of mortality by the end of the summer. Therefore, since the initiation of the stocking moratorium in 2013, rainbow trout are not likely to have persisted in the lake.



Big Carnelian Lake is located less than 1 mile south of Square Lake (Fig. 2), also in May Township. The lake has a surface area of 179 ha (28% littoral), a maximum depth of 19.5 m, and a mean depth of 7.6 m (MPCA, 2014). The lake is part of the Carnelian-Marine Watershed, and is separate from Square Lake's watershed. The headwater lake of the Carnelian-Marine Watershed is Big Marine Lake, which drains to the south through the Carnelian-Marine outlet stream, then passes through several other small lakes before entering Big Carnelian Lake. A stream exits Big Carnelian Lake and then enters Little Carnelian Lake before it outlets to a drainage pipe that discharges to the St. Croix River (Met Council Report, 2014). Relative to Square Lake, Big Carnelian Lake has a large watershed area (6,630 ha) and watershed:lake area ratio (37:1). Similar to Square Lake, the MNDNR have monitored the fish populations of Big Carnelian Lake, and have found the major fish species to be bluegill, sunfish, northern pike, and yellow perch (Table 3). In addition to the resident native fish species in Big Carnelian Lake, the MDNR has stocked walleye (*Sander vitreus*) into the lake for recreational purposes since 2002 at 1000 fry every even numbered year and 2000 fry every odd numbered year. This lake has never been stocked with rainbow trout, nor any other significant amount of zooplanktivorous fish (Table 2). Because Big Carnelian Lake is similar to Square Lake in its size and depth, has experienced the same climatic conditions, but has never been stocked with rainbow trout it is an appropriate choice for a reference lake to which Square Lake can be compared.

#### *Sediment core collection and processing*

Three sediment cores were collected from each study lake on 8 July, 2015. Each set of cores was collected from the center of the lake, at approximately the deepest depth. Sediments were collected using a piston corer with a 7-cm diameter polycarbonate tube. One core from each

lake was extruded in the field with a portable extruder (Glaser & Griffith, 2007). The top 15 cm of these cores was extruded in 0.5 cm increments and in 1 cm increments for core depths greater than 15 cm. The other two cores from each lake were returned to the LacCore laboratory at the University of Minnesota. The sediment-water interfaces of the whole cores were stabilized with zorbitrol to preserve the stratigraphy of the sediments.

A variety of procedures were performed with the cores at the LacCore Laboratory during the week of July 13, 2015. For samples from the field-extruded cores, loss on ignition (LOI) analyses were done using standard methodologies (Dean, 1974) to determine the dry mass and composition (i.e., organic matter, carbonates, non-carbonate inorganic matter) of the sediments. Samples from the field-extruded cores were also freeze-dried to prepare them for subsequent  $^{210}\text{Pb}$  dating. This isotope was chosen because of its half life of 20 years, which gives accurate dating for approximately 100 years. This time frame allowed for a high resolution comparison between the pre-European settlement and the modern conditions of the lakes. Lead-210 dating was performed at the Minnesota Science Museum's St. Croix Watershed Research Station. One core from each lake was also extruded in the laboratory at the same increments as for the field-extruded cores. These samples and the leftover sediments from the field-extruded cores were brought to Hamline University (and stored in a cold room @ 10 °C) until they were used as source material for investigating the zooplankton remains deposited in the sediments of the two lakes. In addition, the third core from each lake was split and digitally photographed, and the split cores were also scanned for magnetic susceptibility. In January 2018, the split cores were also sampled at key depths, depths that would construct a comprehensive understanding of the history of each lake, within one sediment core per lake in order to create smear slides for

qualitative assessment of sediment composition (i.e., % makeup of carbonates, clastics, diatoms, and amorphous organic matter). Smear slides were made by sampling the sediment at 7-8 depths in each core and a single slide was created for each depth. Toothpicks were placed at key depths along each core and a small quantity of sediment was extracted and placed on a microscope slide. Sediment was smeared with a small amount of deionized water to create a thin layer of sediment and slides were examined under a petrographic microscope to assess the sediment composition.

#### *Extraction and examination of zooplankton remains*

Sediment samples were prepared from one core per lake and was sample was prepared for each depth examined. Thirty five depths spanning from pre-European settlement dates were examined for ephippia and twenty five were examined for exoskeletal remains. Preparation of sediment samples for examination of exoskeleton remains closely followed the methods of (Korosi et al., 2011). Briefly, 1.2 mL of sediment from a particular core sample was treated with a 10% KOH solution, heated to 70-80 °C, and stirred gently for 20 min. This solution was then poured onto a 45 µm sieve and washed with DI water to remove any remaining inorganic materials. The remainder was collected in a 15 mL centrifuge tube and treated with both a 1% safranin-ethanol solution and 5-7 drops of glycerin gel to thicken the solution (Korosi et al., 2011). For each sediment sample, two slides were made (each with 200 µL of solution) and sealed with cover slips and stored at room temperature for future examination. Slides were examined under a calibrated compound microscope at 100x magnification. For each slide, *Daphnia* post-abdominal claws were identified by species (Korosi et al., 2011), counted, and measured from tip of the claw to the base. Measurements were made of the first 100 claws or

until the entire the slide was counted (Kitchell and Kitchell, 1980). Claw lengths were converted to body sizes using a regression equation (Kitchell and Kitchell, 1980). Additionally, *Bosmina* headshields were counted throughout the entirety of the slides.

The examination of ephippia followed a slightly different method. Because ephippia are less abundant in sediments than are exoskeletal remains, a greater volume of sediment (2.5mL) was processed for each depth. Additionally, ephippia are larger and better preserved than exoskeletal remains, so the sediment was filtered through an 80 µm mesh screen directly. Samples were then divided into six centrifuge tubes containing a 30% sucrose solution. The tubes were centrifuged at 1085 rpm for 1.5 min to separate the ephippia (which are lipid-rich and less dense than other materials in the sediments) from other sedimentary material (Caceres, 1998). The supernatant from these samples was initially examined under a dissecting microscope to retrieve the ephippia and then transferred to a compound microscope for identification and measuring. Ephippia were identified to the species level based on the presence (*D. pulicaria*) or absence (*D. mendotae*) of spikes on the dorsal ridge of the ephippia. Ephippia were then measured from one end of the dorsal ridge to the other.

## **Results**

### *Lead-210 dating*

Lead-210 dating of the sediment cores provided reliable ages of sediments back to the year 1845 for Square Lake sediments (at a core depth of 38 cm) and 1820 for Big Carnelian Lake (core depth = 46 cm) (Fig. 3). Sediment depths deeper than these were beyond the threshold of the <sup>210</sup>Pb dating, but were approximated by extrapolation using the sedimentation rates near the threshold depths. For Square Lake, sediments at the base of the core (60 cm) are estimated to be

from 1660, while those at the base of the Big Carnelian Lake core (70 cm) are estimated to be from 1695. 1981, the year that rainbow trout were first stocked in Square Lake, is at a depth of about 11 cm in the Square Lake core and about 18 cm in the Big Carnelian Lake core.

#### *Sediment composition*

The composition of the sediments throughout time was both visually examined as well as measured using LOI techniques (Figs. 4-6). Trends in percentage composition of clastic, organic, and carbonate materials were largely consistent between these two methods. In Square Lake, the percentage of organic matter in sediments was relatively consistent historically, but increased around 1980 (Figs. 4 & 6). Carbonates were historically (1700-1870), the largest component of sediments. However, this changed starting around 1880, when clastics became more proportionally abundant than carbonates in the sediments (Figs. 4 & 6). The influx rates of all sediment components displayed trends similar to those of the percent compositions (Figs. 6 & 7). In contrast to the Square Lake record, the compositional makeup of Big Carnelian Lake sediments is highly consistent throughout the entirety of the core. Clastic materials are consistently the major sediment component, organic materials are moderately abundant, and there is minimal carbonate presence (Figs. 5 & 6). Influx rates of these sediment components largely matched the trends in percent composition, however, around 1970 the influx rates of organic and clastic materials peaked, even though the percent composition didn't significantly increase (Figs. 6 & 7).

#### *Proportional abundance and fluxes of zooplankton remains*

The patterns for the rate of delivery of *Daphnia ephippia* to the sediment (influx rate) were similar for both lakes (Fig. 8). *D. pulicaria* influx rate began to increase around 1980 in

both lakes. *D. mendotae* displayed the same general trend with *D. pulicaria* in Big Carnelian Lake, however, after 1980 in Square Lake *D. mendotae* began to demonstrate a lower influx rate than *D. pulicaria*.

Trends through time for the proportion of *D. pulicaria* ephippia relative to all *Daphnia* ephippia (*D. mendotae* + *D. pulicaria*) differed between the two lakes (Fig. 9). In Square Lake, the relative abundance of *D. pulicaria* ephippia increased from the older to younger sediments until 2010. After 2010 the *D. pulicaria* ephippia relative abundances decreased. Big Carnelian Lake did not display a such a discernable pattern. Starting around 1980 the relative abundance of *D. pulicaria* ephippia was highly variable between years, though similar to Square Lake the most recent dates had lower relative abundances than those around 1980.

Influx rates of *Daphnia* post-abdominal claws remains, as well as *Bosmina* headshields, were found to differ between the two lakes (Fig. 10). In the oldest sediments (1780-1960) the influx rates for all species' remains were relatively similar between lakes, with *Bosmina* having higher influx rates than both *Daphnia* species. However, after 1960 the influxes of the three species begin to differ in each lake. The influx rate of *Bosmina* began to decline dramatically after 1960 in Square Lake, but in Big Carnelian Lake the *Bosmina* influx rate remained the highest of all of the zooplankton. *D. mendotae* influx rate, after 1960, increased at a slower rate in Square Lake than in Big Carnelian Lake, though in the most recent year (2015) the *D. mendotae* influx rates were comparable. *D. pulicaria* influx rate was consistently higher than *D. mendotae* in Square Lake and increased at a similar rate as *D. mendotae* from 1960 to 2015. The *D. mendotae* influx rate for Big Carnelian Lake was nearly identical to that of *D. mendotae* from 1960 onward. Additionally, the influx rates of both *Daphnia* species were higher in Big Carnelian Lake than in

Square Lake during these years. These influx findings were corroborated by the differences in remain abundances for the two lakes. The proportional abundances of exoskeletal remains differed dramatically between lakes (Fig. 11). In Square Lake after ~ 1900, *D. pulicaria* claws were more abundant than *D. mendotae*. In contrast, for Big Carnelian Lake, *D. mendotae* claws were more abundant than *D. pulicaria* from ~1945 to present day. However, these trends in exoskeletal abundance were further contextualized with their relationship to *Bosmina*. In Square Lake after ~1977 *D. pulicaria* was the most abundant of the exoskeletal remains. Before this date, *Bosmina* headshields were the dominant zooplankton remain. Interestingly, in Big Carnelian Lake *Bosmina* headshields were consistently the most abundant zooplankton remains, more so than both of the two *Daphnia* species combined.

#### *Daphnia* body size through time

In addition to the abundances of exoskeletal remains, the changes in the size of the remains were found to have different trends between the two lakes. To inform why there were differences between the claw-derived and ephippia-derived body sizes, the distributions of the body sizes were compared. Body sizes generated from both ephippia and claws were normally distributed (Figs. 12 & 13). However, the mean body sizes derived from ephippia were, on average, larger than those derived from post-abdominal claws. Additionally, the claw data yielded a wider range of body sizes than the ephippia data. Independent sample t-tests were run in order to determine if the mean body lengths of the two proxies (ephippia and claw) of historic populations were statistically different in each lake. *D. pulicaria* mean body size in both Square Lake ( $t = -8.55$ ,  $df = 2254.9$ ,  $p < 0.001$ ) and Big Carnelian Lake ( $t = -7.76$ ,  $df = 686.8$ ,  $p < 0.001$ ) was found to differ significantly between the ephippia and claw proxies. *D. mendotae* mean body

size was found to be significantly different between proxies for Big Carnelian Lake ( $t = -3.33$ ,  $df = 1020.9$ ,  $p < 0.001$ ) and not significantly different for Square Lake ( $t = -0.46$ ,  $df = 669.02$ ,  $p = 0.64$ ).

The mean body sizes per year of both *D. pulicaria* and *D. mendotae* were calculated from post-abdominal claw lengths (Figs. 14 & 15) and ephippia lengths (Figs. 16 & 17). These mean body sizes were grouped as belonging to pre-trout stocking years (before 1981) and post-trout stocking years (1981-2013) and linear regressions were run on these subgroups for each lake. In Square Lake and Big Carnelian Lake, *D. mendotae* body size (extrapolated from claw lengths) did not change significantly during the pre or post-stocking years (Figs. 14 & 15, lower panels). In contrast, *D. pulicaria* body size (Fig. 14, upper panel) was relatively stable in pre-stocking years, but decreased significantly during the trout stocking era ( $p = 0.032$ ). *D. pulicaria* in Big Carnelian Lake did not change significantly during neither the pre or post-stocking years (Fig. 15, upper panel).

Mean body size of both *Daphnia* species was also calculated using the ephippial lengths, to determine whether there were any differences between the two methods of estimating historic zooplankton populations. Similar to the patterns for claw-derived body lengths, Square Lake *D. pulicaria* body size did not significantly change over time in pre-stocking years, but did significantly decrease during the trout stocking time period ( $p = 0.017$ ) (Fig. 16, upper panel). *D. mendotae* body size extrapolated from ephippia measurements showed an increase over time, but the linear regressions for the pre-stocking and stocking time periods were not statistically significant ( $p > 0.05$ ) (Fig. 16, lower panel). For Big Carnelian Lake, ephippia-based body sizes of *D. pulicaria* were stable before 1981, but increased significantly after 1981 ( $p = 0.008$ ) (Fig.



17, upper panel). *D. mendotae* throughout all examined times did not demonstrate significant changes in body size (Fig. 17, lower panel).

## Discussion

### *Differences in sedimentation rates and sediment composition between lakes*

A thorough understanding of the similarities and differences in the sedimentation rates and compositions of each lake was necessary in order to determine the potential causes of Square Lake's declining water quality. To do this  $^{210}\text{Pb}$  was chosen for sediment dating because its higher resolution made it effective for the 200 year time span that we were examining. Big Carnelian Lake has experienced generally higher rates of sediment accumulation than Square Lake (Fig. 3). This difference between the two lakes is likely due to the substantially larger watershed size of Big Carnelian Lake (Table 1). The sediment composition of both Square Lake and Big Carnelian Lake is consistent with their watershed sizes and hydrological sources. Square Lake is predominantly groundwater-fed (Table 1), and through this process carbonates from the bedrock are brought up into the lake basin where they are deposited. This then explains the high percentage of carbonates found in Square Lake before 1870 (Figs. 4 & 6). The amount of carbonates present is not likely to have decreased after this year, rather it is more likely that a large amount of clastic material was introduced to the lake's watershed around that time, dwarfing the proportion of carbonate sediment make up. The increase in clastic materials in Square Lake sediment coincides with the increased population of the county from European settlement (Fig. 1). Export of clastic materials into the lake is likely caused by land development

projects (e.g., agriculture, home building), and this is likely why they became the major component in Square Lake sediment at the time, as well as why they decreased after 1990 when agricultural development of the area declined (Fig. 1). The percentage composition of Big Carnelian Lake sediments is relatively consistent throughout time, despite the aforementioned changes in population and land use in that area (Figs. 5 & 6), though influxes of clastic and organic materials increased substantially after ~1920 (Fig. 7). Big Carnelian Lake receives its input from a series of other lakes, and so all these lakes are considered a part of the lake's major watershed. Therefore, sediment changes as a result of changes in land use and population of the county are more closely reflected in the sediments of Big Carnelian Lake, than in Square Lake.

The influx rate of the sediment components illustrate changes that occurred at similar times in each lake. Both lakes had consistent influx rates of organic, carbonate, and clastic materials up to 1870. After 1870 the influx rates of all materials began to increase, and even displayed similar spikes in influx rates around 1990 in Square Lake and around 1970 in Big Carnelian Lake. This delayed response in Square Lake could be the result of differences in development or land use in Square Lake's particular watershed, or the result of Big Carnelian Lake's larger watershed being more sensitive to these types of changes.

#### *Zooplankton body size and community composition*

Important differences were found in the *D. pulicaria* remains deposited in Square Lake and Big Carnelian Lake sediments that help explain the declining water quality in Square Lake. Both ephippia and claw data from Square Lake show that *D. pulicaria* significantly decreased in mean body size after 1981, when rainbow trout stocking began (Figs. 14-17). This strongly supports the expectation that the stocking of rainbow trout into Square Lake and subsequent

size-selective predation pressure would select against the larger *Daphnia*. The decrease in *D. pulicaria* body size would have decreased the filtering ability of the population (Burns, 1969) and is consistent with the trends in monitoring data that show that the lake has become more eutrophic since the 1980s (Hembre, 2016).

The found differences in the influx rates of ephippia and claw remains suggest important differences in the ways that historic populations are derived. The influx rates of the claw remains were, in the most recent years (2010-2015), nearly ten times greater than were the ephippia influx rates for the same years, in both lakes (Figs. 8 & 9). Additionally, claw-derived influx rates were able to be generated for older sediments than ephippia-derived rates. Ephippia are produced by zooplankton for sediment deposit and eventual deposition, yet they degrade faster, and are therefore less abundant in the oldest sediment than the post-abdominal claws, which are simply deposited when *Daphnia* die. This then limits the number of ephippia available in the oldest sediments, which then prevents influx rate calculations to be generated for those dates. Despite these differences, similar trends in the influx rates of the *Daphnia* species in both lakes were found for both proxies (Figs. 8 & 10). The increasing trend in influx rates for both species in both lakes, during the most recent sediment years, indicates that populations of both *Daphnia* species are increasing with time.

Similar population trends were seen through our ephippia and claw-derived relative abundances as well. The relative abundance of *D. pulicaria* did not decrease significantly in Square Lake like we predicted they would. Instead a transition from *Bosmina* dominance in the oldest sediments (1800-1980) to *Daphnia* dominance (1980-2015) was found in Square Lake, while Big Carnelian Lake was *Bosmina* dominated throughout (Fig. 11). Though shifts from

*Bosmina* to *Daphnia* have been seen in other long-term lake studies (Brugman & Speziale, 1983; Tsugeki et al., 2003), other studies have seen the opposite pattern (Kitchell & Kitchell, 1980; Brooks & Dodson, 1965). The shift from *Bosmina* to *Daphnia* is often not associated with eutrophication. Instead, the increased phytoplankton abundance is thought to give an advantage to *Daphnia* because they are the larger bodied genus. *Daphnia* in particular require sufficient levels of phosphorus (P) in order to become established (Acharya et al., 2004). However, a previous study that used diatoms to assess changes in P levels in Square Lake did not find any significant change in P since pre-European settlement (Ramstack et al., 2004). This lack of change in total P of the lake makes the transition from *Bosmina* to *Daphnia* surprising. Further investigation will be required in order to determine a reason for this deviation from expectation. While the relative abundance trends were found to be similar between the two proxies, only the data for the exoskeletal remains included *Bosmina*. This means that the depletion of *Bosmina* populations in the most recent years of Square Lake's history would not have been seen if an ehippia proxy was the only one selected. If only the ehippia-derived abundances were considered it would be inferred that *Daphnia* are the only major zooplankton players in the community, which often isn't the case.

The ehippia-derived body size changes had a higher degree of significance than the claw-derived sizes, implying that there are differences in the reconstructed population data of each proxy. Additionally, the mean body length of *D. pulicaria* in Big Carnelian Lake was only found to significantly increase since the trout stocking years for the ehippia-derived values. The body size increase was thought to be the result of the stocking of the piscivore walleye into the

lake and reducing predation pressure upon *D.pulicaria*, though this does not explain why this increase was only seen in the ephippia derived body sizes.

Differences between body sizes estimated from claw and ephippia data (Figs. 12 & 13) are due to the restricted size range of ephippia-producing *Daphnia*. To produce ephippia, *Daphnia* must grow to a large enough body size to support them (~ 1.3 mm for *D. pulicaria*; Hembre & Megard, 2006). Ephippia-derived body lengths had a smaller range of sizes than claw derived lengths, and the most frequent sizes were larger for ephippia derived lengths as well (Figs. 12 & 13). Therefore, population estimates derived from ephippia are biased toward the larger members of the population, and do not provide comprehensive information about the whole population. This combined with the irregularity of ephippia production, only being produced during seasonal stress conditions, and the aforementioned faster degradation of ephippia relative to post-abdominal claws suggests that the post-abdominal claw proxy is the more accurate technique. However, it is important to mention that the ephippia proxy has the advantage of being much less labor intensive process, and therefore may be suitable for cursory studies that are examining a relatively short amount of history. For studies that require a long time scale, or that necessitate a large pool of data, the post-abdominal claw proxy is the superior choice.

In summary, the results of this study provide strong evidence that size-selective predation by rainbow trout stocked in Square Lake has resulted in a significant decrease in the body size of *D. pulicaria* (Figs. 14 & 16) and implies that this reduction in *D. pulicaria* body size may be responsible for the eutrophication trend observed in the lake (Hembre, 2016). This revelation has brought to light many important concerns regarding lake management. Together with the

findings of the trout moratorium monitoring study (Hembre, 2016), results of this study imply that the seemingly benign practice of stocking trout can have negative repercussions for water quality. Future management practices will need to consider the long-term trophic impacts on lakes stocked with trout. Additionally, this study provides insight into the type of proxy that should be used to understand historical changes in zooplankton populations. Namely, this study demonstrates that ehippia-derived data can introduce bias because ehippia are only produced by the larger members of the population, are only produced sporadically, and do not persist in sediments as long as exoskeletal remains. Future research on the cores collected for this study should focus on generating a more complete assessment of exoskeletal and ehippial remains in older sediments to provide greater resolution of trends in the zooplankton communities from the base of the cores until the beginning of the trout stocking program in Square Lake (1981). This, paired with more historical information on human population densities, land use, and watershed data, would provide additional confidence to inferences made about past conditions, and could provide a basis for predicting ecological changes in the future.

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**Table 1.** Morphometric and watershed characteristics for Square Lake and Big Carnelian Lake. **SA** is the surface area of each lake in hectares, **Z<sub>max</sub>** is the maximum depths of each lake, is the mean depth of each lake, **WA:LA** is the ratio of watershed area to the area of the lake. \*Due to Big Carnelian Lake being apart of a chain of lakes with a very large collective watershed the **WA:LA** is higher for this lake. Additionally this prevented describing the hydrolicial makeup of this lake in the same way as Square Lake.

Lake	SA (ha)	Z <sub>max</sub> (m)	Z <sub>mean</sub> (m)	WA:LA	% groundwater	% precipitation	% surface runoff
Square	82.2	20.7	9.3	2.5:1	70	25	5
Big Carnelian	179	19.5	7.6	*37:1	NA	NA	NA

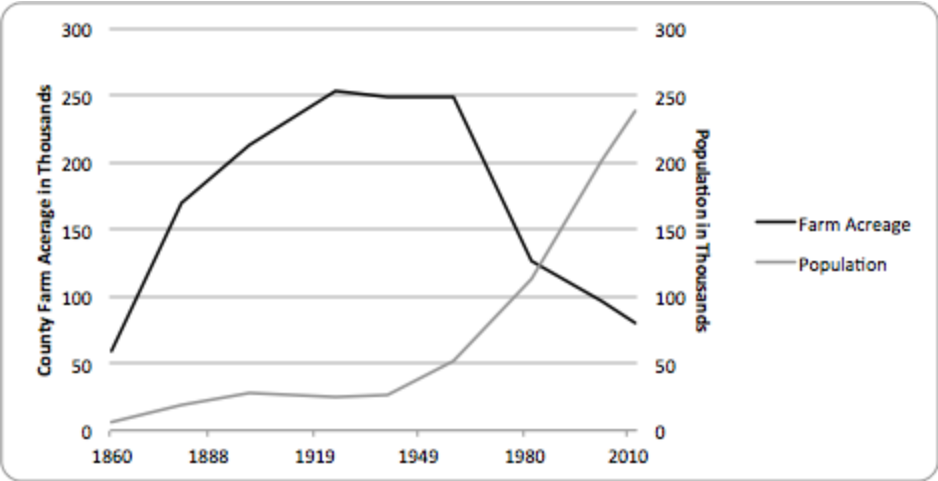
**Table 2.** Square Lake DNR Fish Survey data. The proportion of each major fish species collected through both trap and gill net sampling during DNR monitoring years is presented. Gill nets and trap nets are equipped to capture larger and smaller fish respectively, and so to present a complete sample of both lakes' fish populations data for both methods is shown. \*Sunfish proportions constitute a collection of numerous sunfish species.

Species	1989		1999		2008	
	%Gill	%Trap	% Gill	% Trap	% Gill	% Trap
Black Crappie	8.6	5	5.1	0	2	4.7
Bluegill	8.6	60	16	50.8	26.8	55.3
Sunfish*	3.4	4.5	3	3	0	16.7
Northern Pike	24.1	5.7	23.1	2.4	15.4	8
Largemouth Bass	6.9	1.4	3.6	0	2	0
Rainbow Trout	0	0	1.2	0	4.7	0
Yellow Perch	0	0	3	0	8.1	0
Walleye	0	0	0	0	0	0
Total Fish per Sample	58	140	169	132	149	150

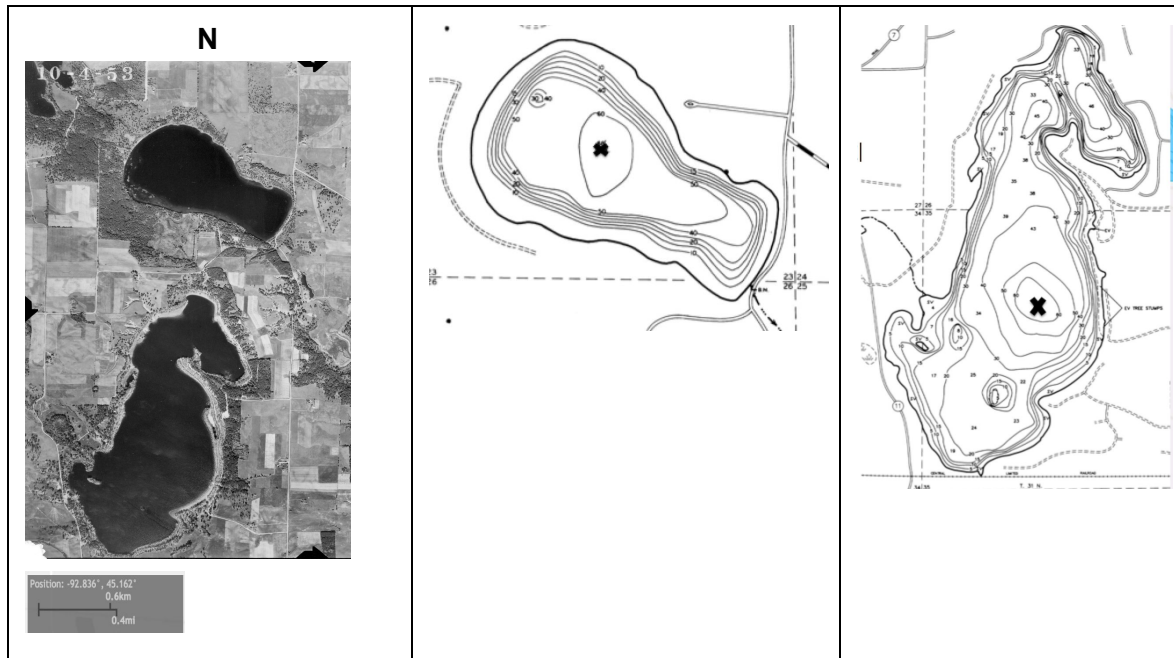
Table 3. BC DNR Fish Survey data. The proportion of each major fish species collected through both trap and gill net sampling during DNR monitoring years is presented. Gill nets and trap nets are equipped to capture larger and smaller fish respectively, and so to present a complete sample of both lakes' fish populations data for both methods is shown. \*Sunfish proportions constitute a collection of numerous sunfish species.

Species	1989		1999		2008	
	%Gill	%Trap	% Gill	% Trap	% Gill	% Trap
Black Crappie	0	6.4	12.8	5	2.6	5.4
Bluegill	47.9	65.3	42.2	47.7	39.3	64.2
Sunfish*	2.9	14.7	0.3	28.7	0	14.7
Northern Pike	24.4	24.4	16.9	0.8	41.9	3.6
Largemouth Bass	1.6	0.1	11.3	2.7	3.1	1.4
Rainbow Trout	0	0	0	0	0	0
Yellow Perch	2.9	1	0.6	2.7	1	4.7
Walleye	0	0	4.4	0	5.2	0.4
Total Fish per Sample	397	806	325	269	191	279

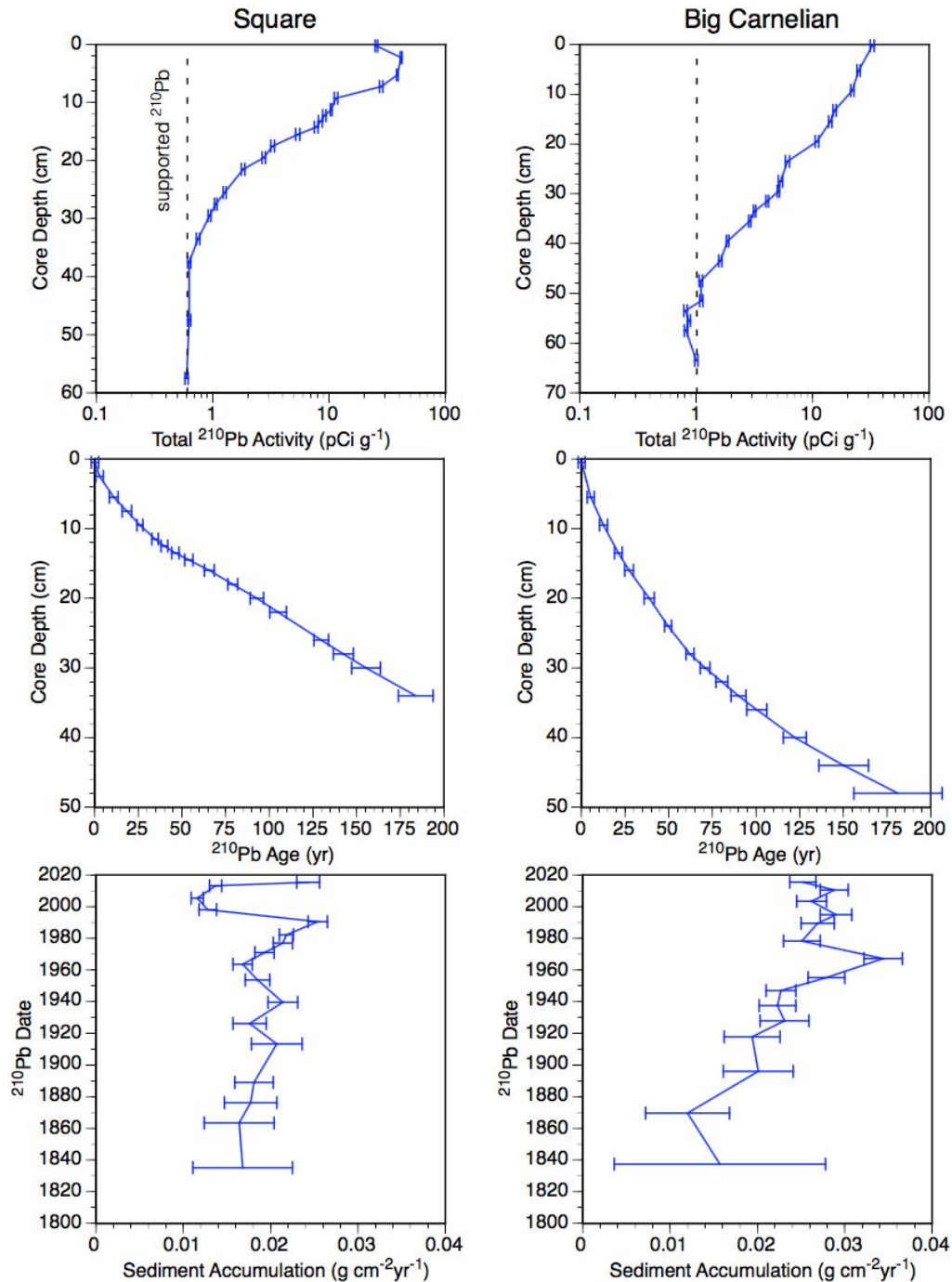
**Figure 1.** Washington County land use and human population size throughout history.



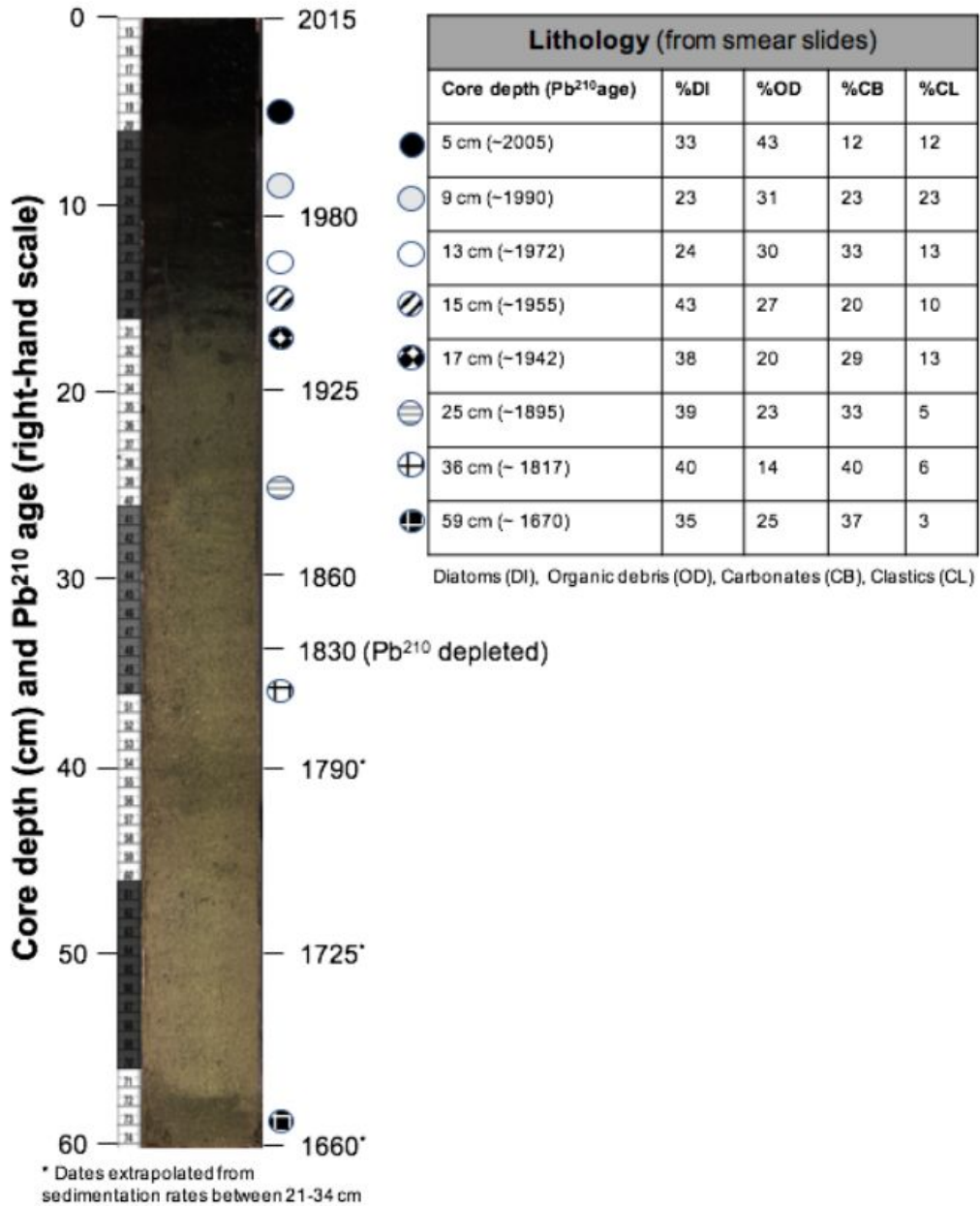
**Figure 2.** Map of Square Lake (middle column) & Big Carnelian Lake (right column) with coring locations identified.



**Figure 3.** Lead-210 dating results for Square Lake and Big Carnelian Lake. The first row of figures show the depths at which  $^{210}\text{Pb}$  was supported in the sediment. The second row displays the age of sediments at different depths throughout each lake. The bottom row of figures display the sediment accumulation rates at different dates in each lake. The error bars are  $\pm 1$  standard error.

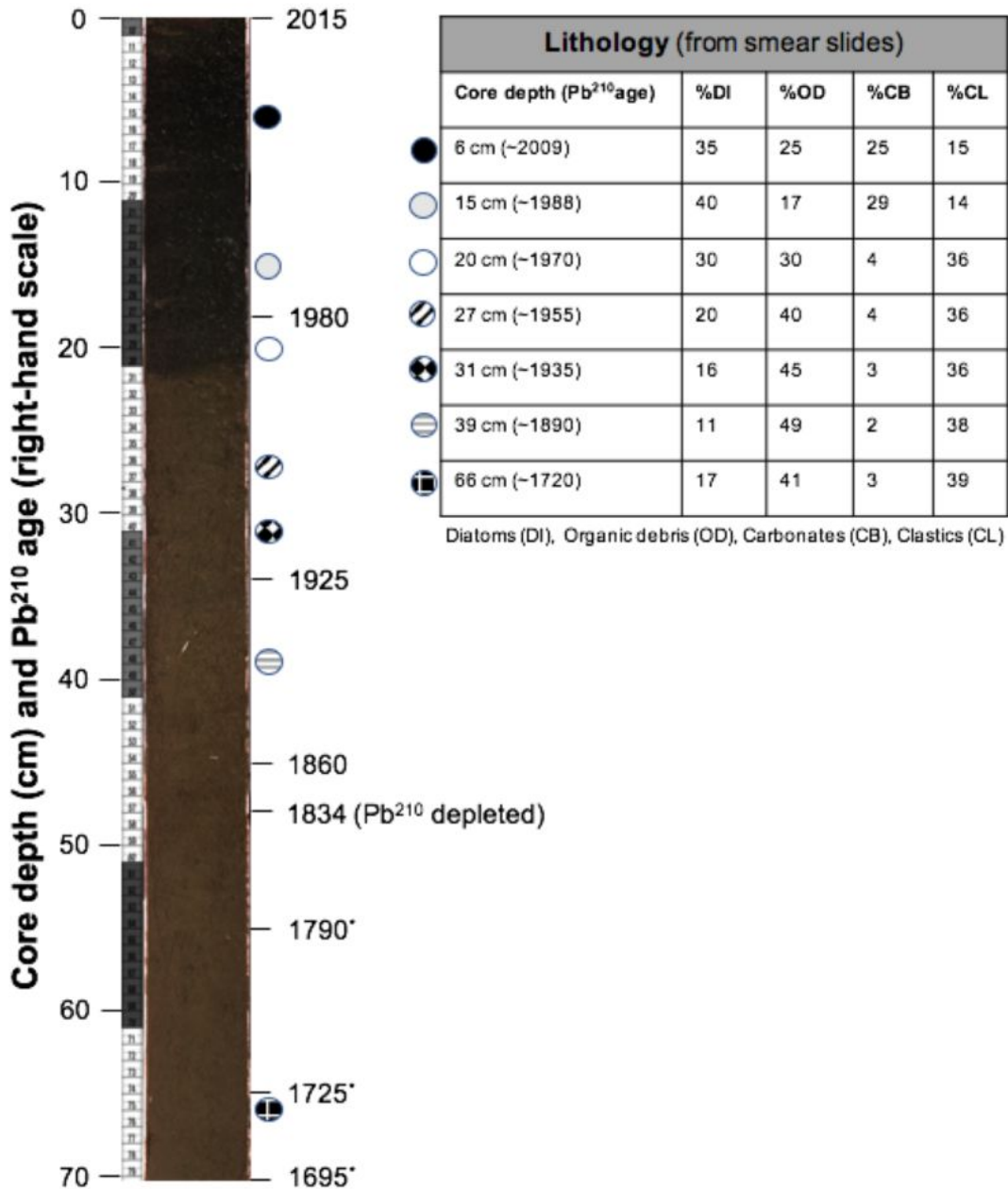


**Figure 4.** Photographic image of Square Lake Core indicating  $^{210}\text{Pb}$  dates and lithological characterization of smear slide samples from various depths in the core. The symbols indicate depths that were examined using smear slides.

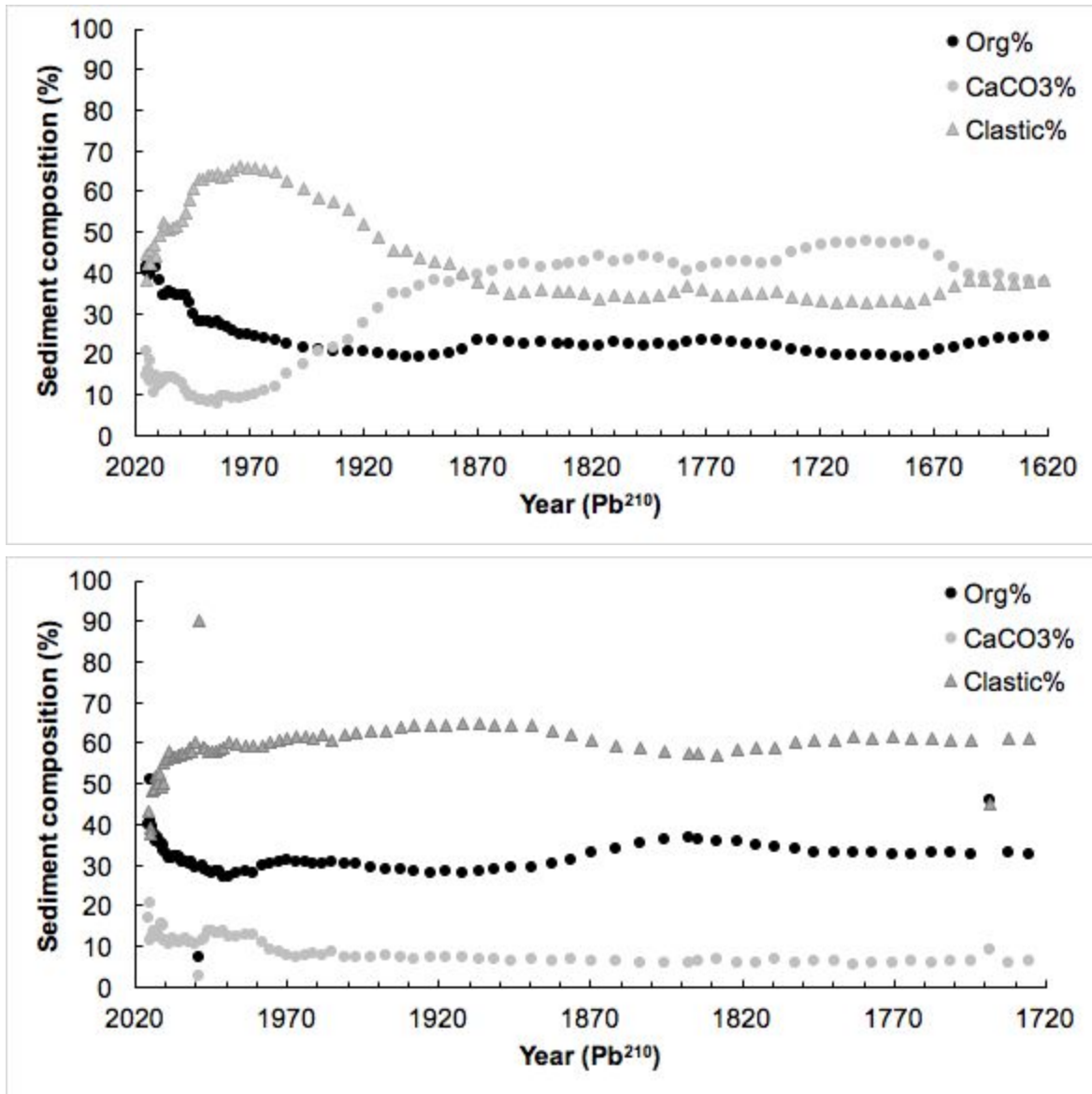




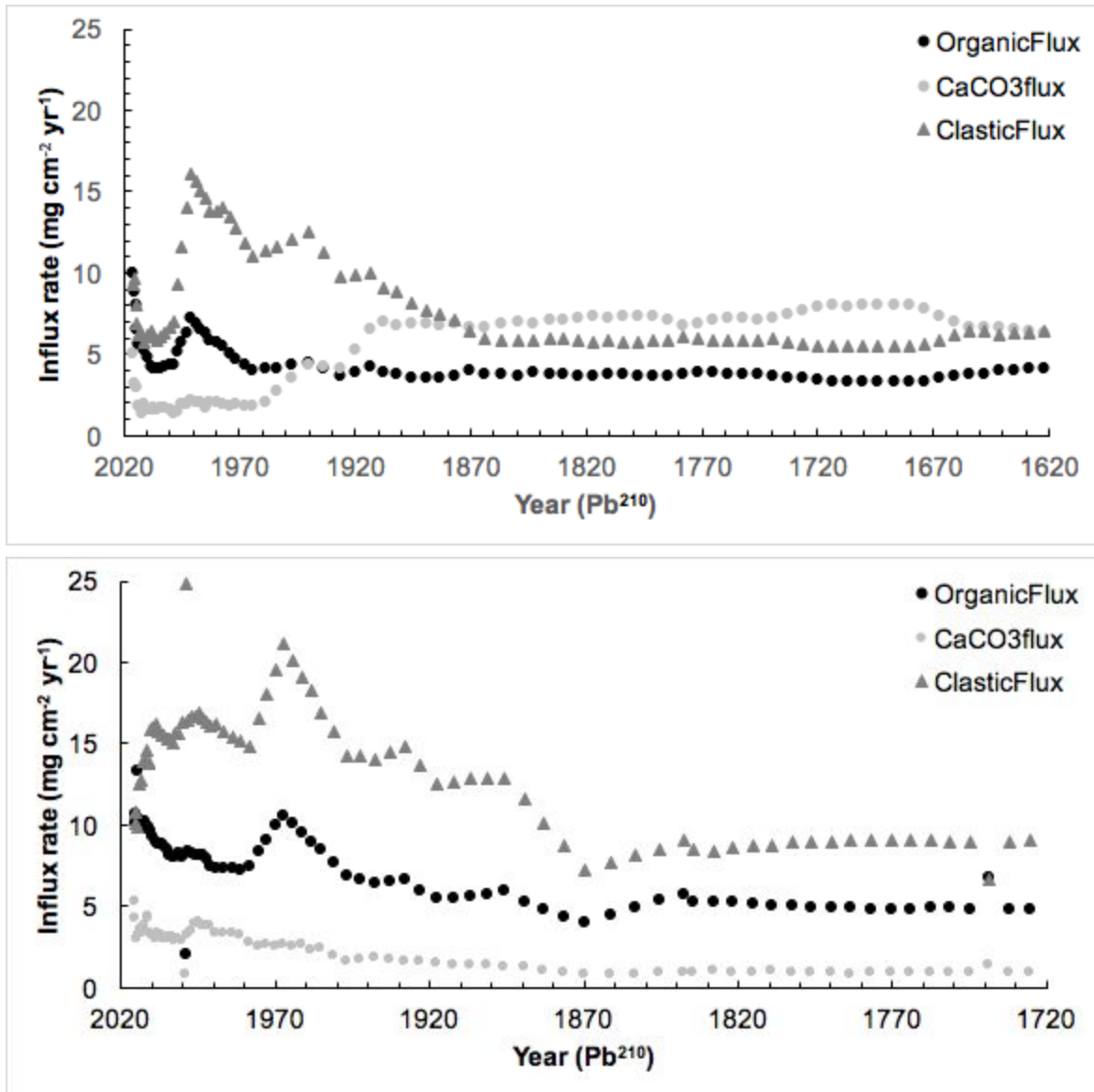
**Figure 5.** Photographic image of Big Carnelian Lake core indicating  $^{210}\text{Pb}$  dates and lithological characterization of smear slide samples from various depths in the core. The symbols indicate depths that were examined using smear slides.



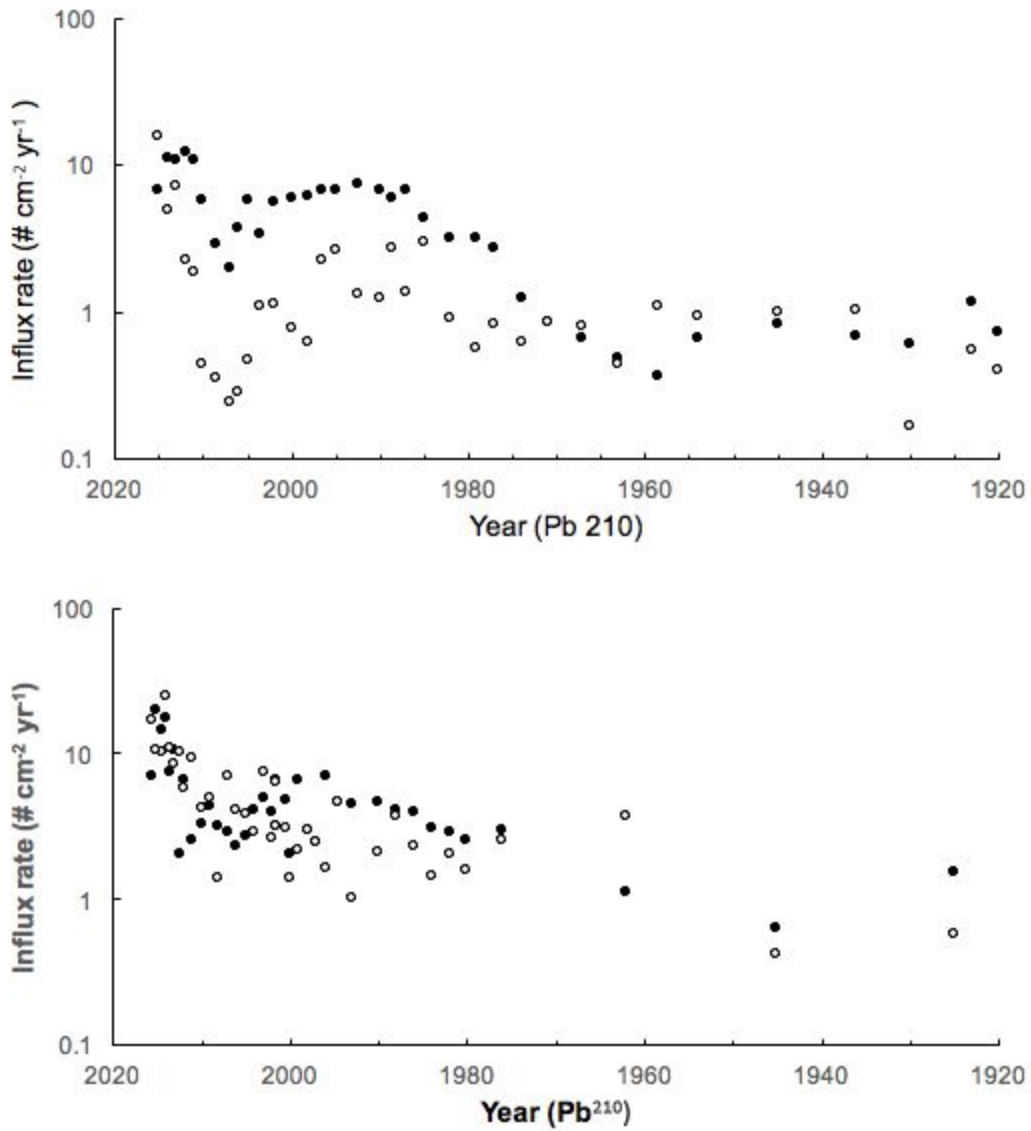
**Figure 6.** Sediment composition (%) through time for Square Lake (upper) and Big Carnelian Lake (lower).



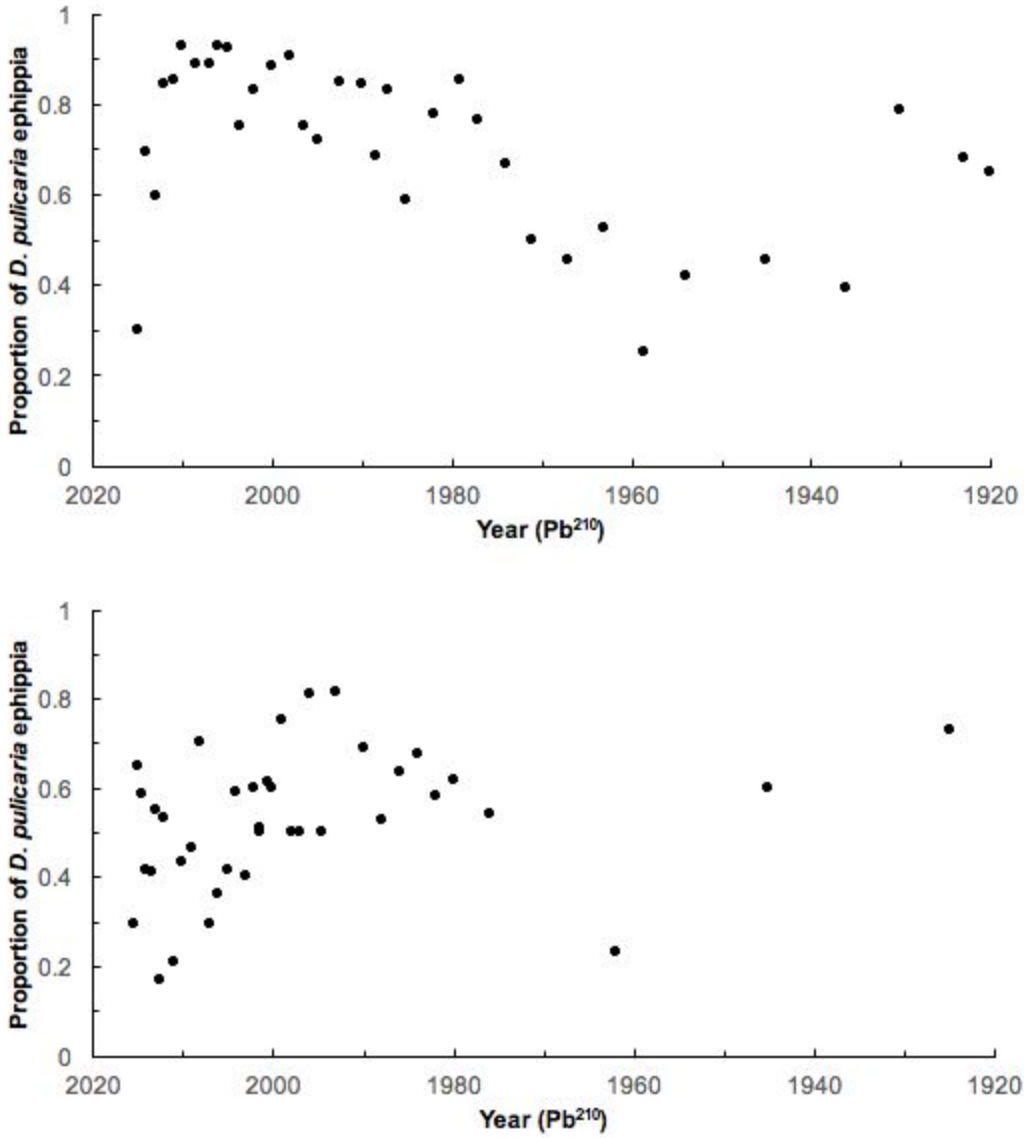
**Figure 7.** Influx rate of sediment components through time for Square Lake (upper) and Big Carnelian Lake (lower).



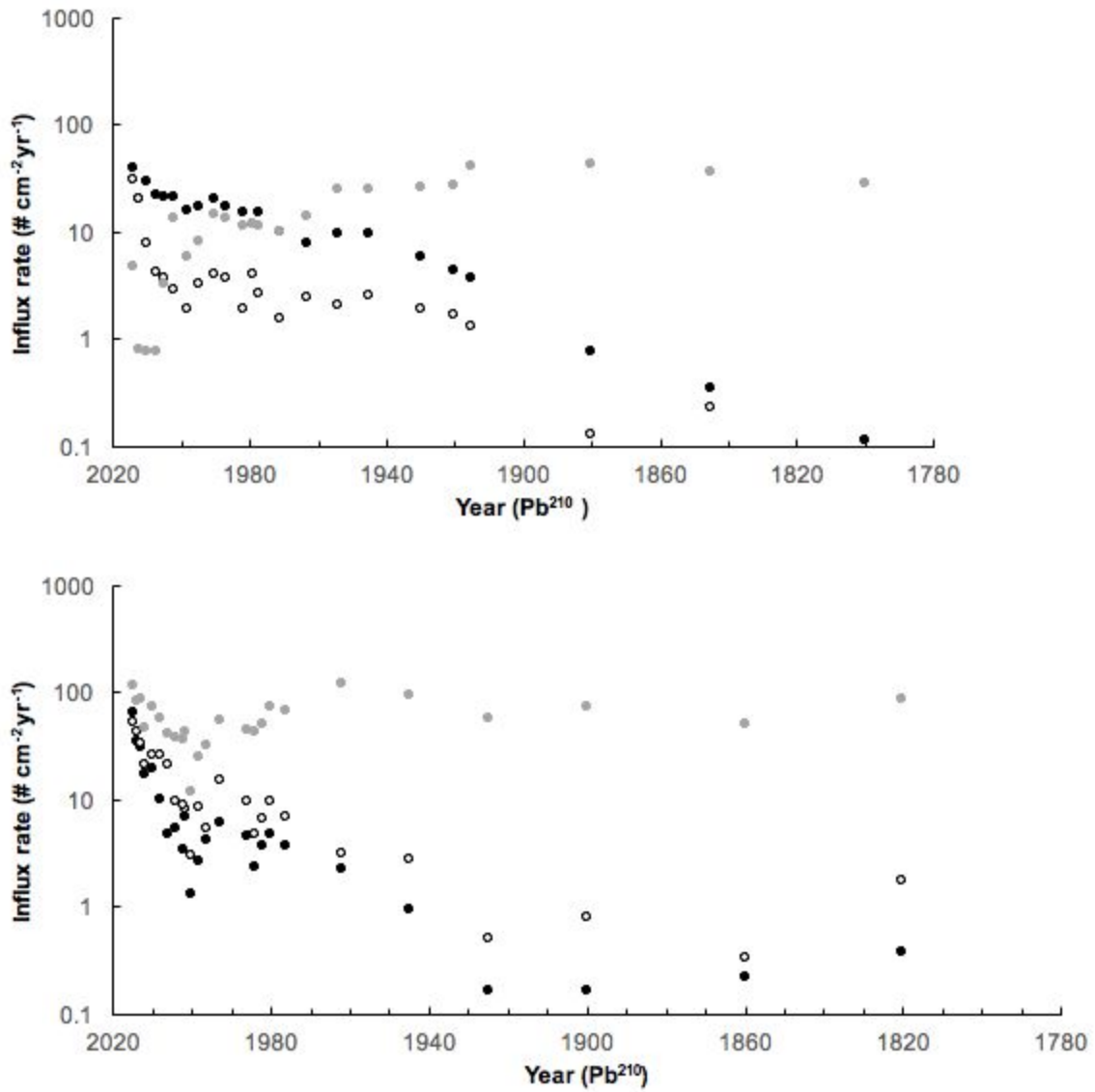
**Figure 8.** *Daphnia pulicaria* (black circles) and *D. mendotae* (white circles) ehippia influx rates over time for Square Lake (top) and Big Carnelian Lake (bottom).



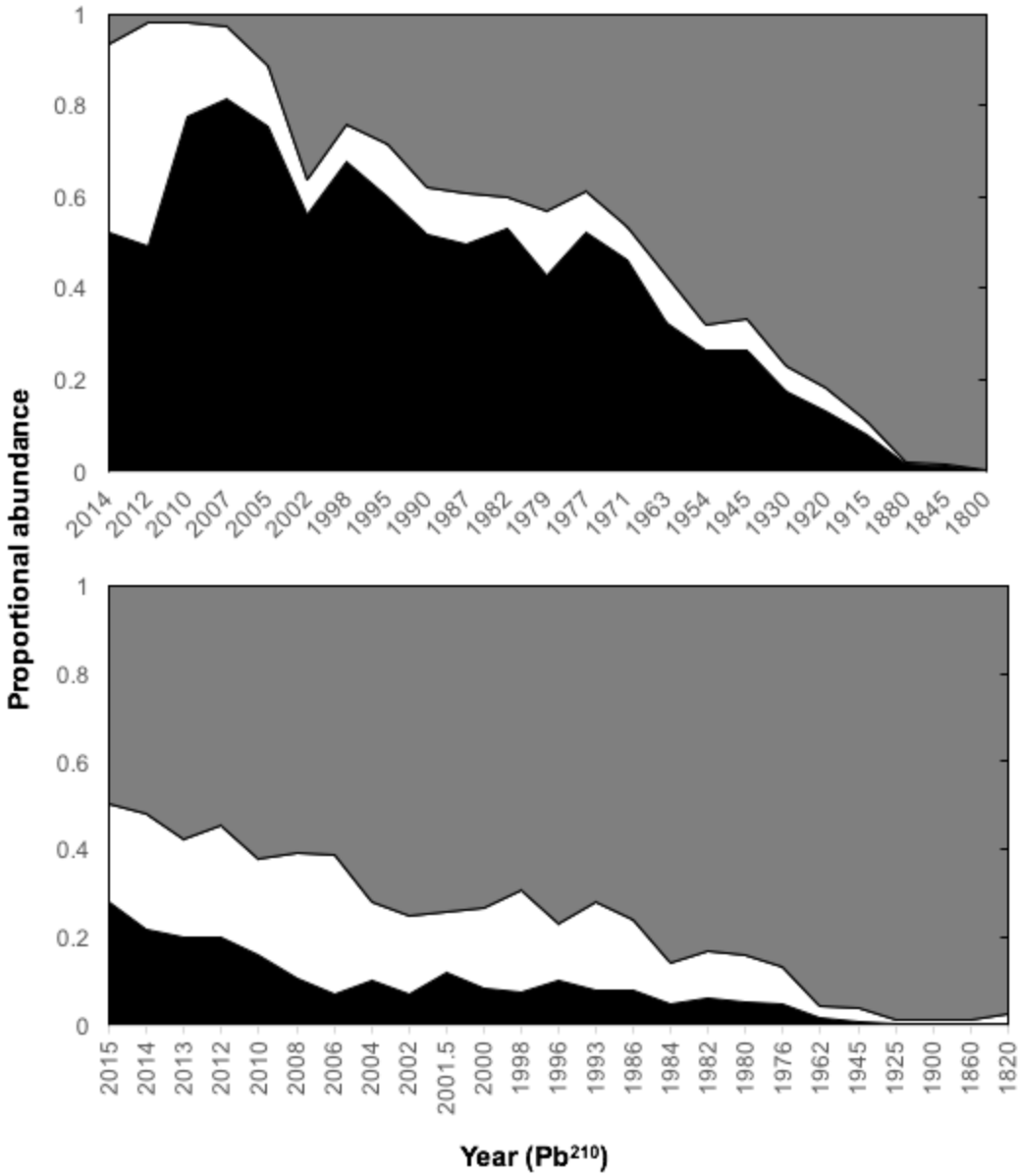
**Figure 9.** Proportion of *Daphnia pulicaria* ehippia relative to all *Daphnia* ehippia over time for Square Lake (top) and Big Carnelian Lake (bottom).



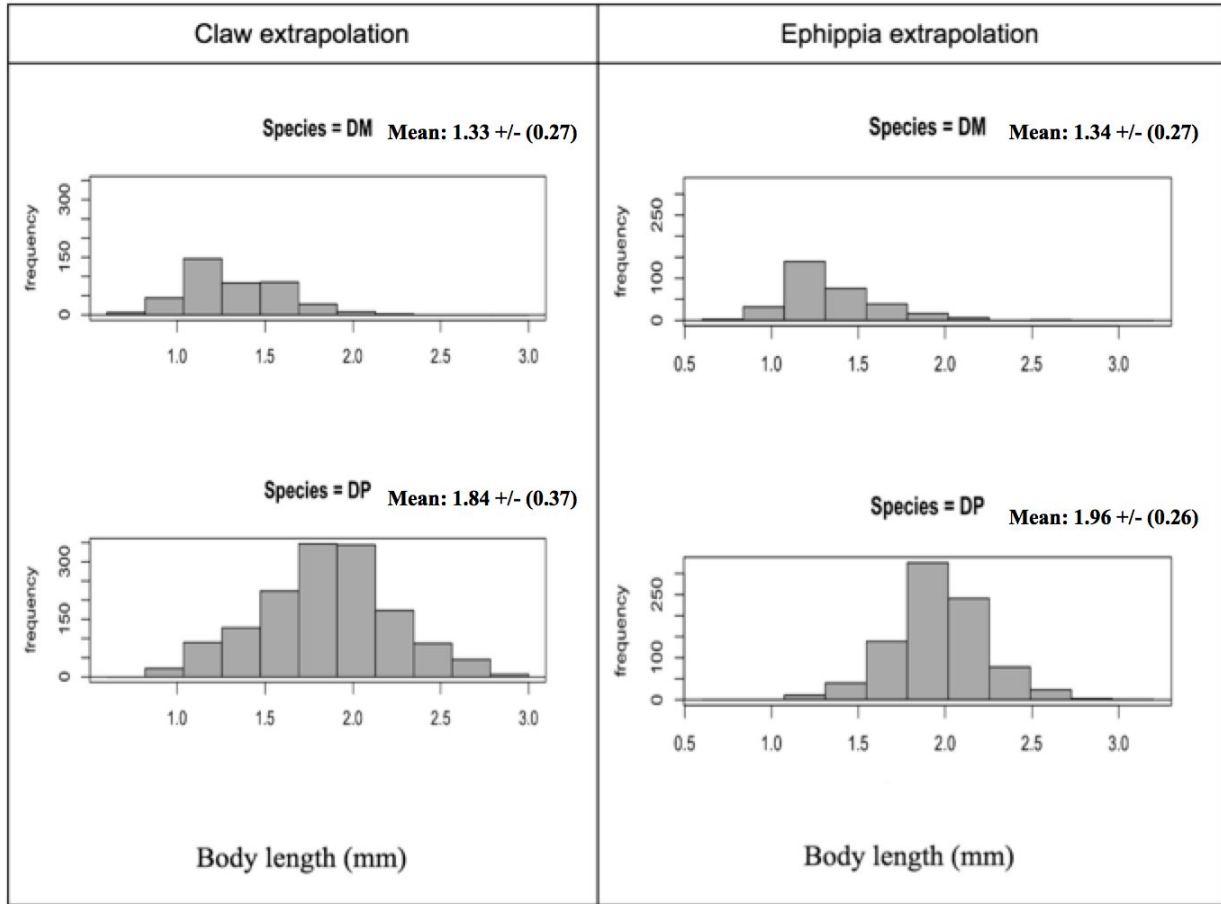
**Figure 10.** Influx rates for *Daphnia pulex* (black circles) and *D. mendotae* (white circles) post-abdominal claws and *Bosmina* head shields (gray circles) over time for Square Lake (top) and Big Carnelian Lake (bottom).



**Figure 11.** Proportions of exoskeletal remains of *D. pulicaria* (black), *D. mendotae* (white) and *Bosmina* (gray) over time in Square Lake (top) and Big Carnelian Lake (bottom).

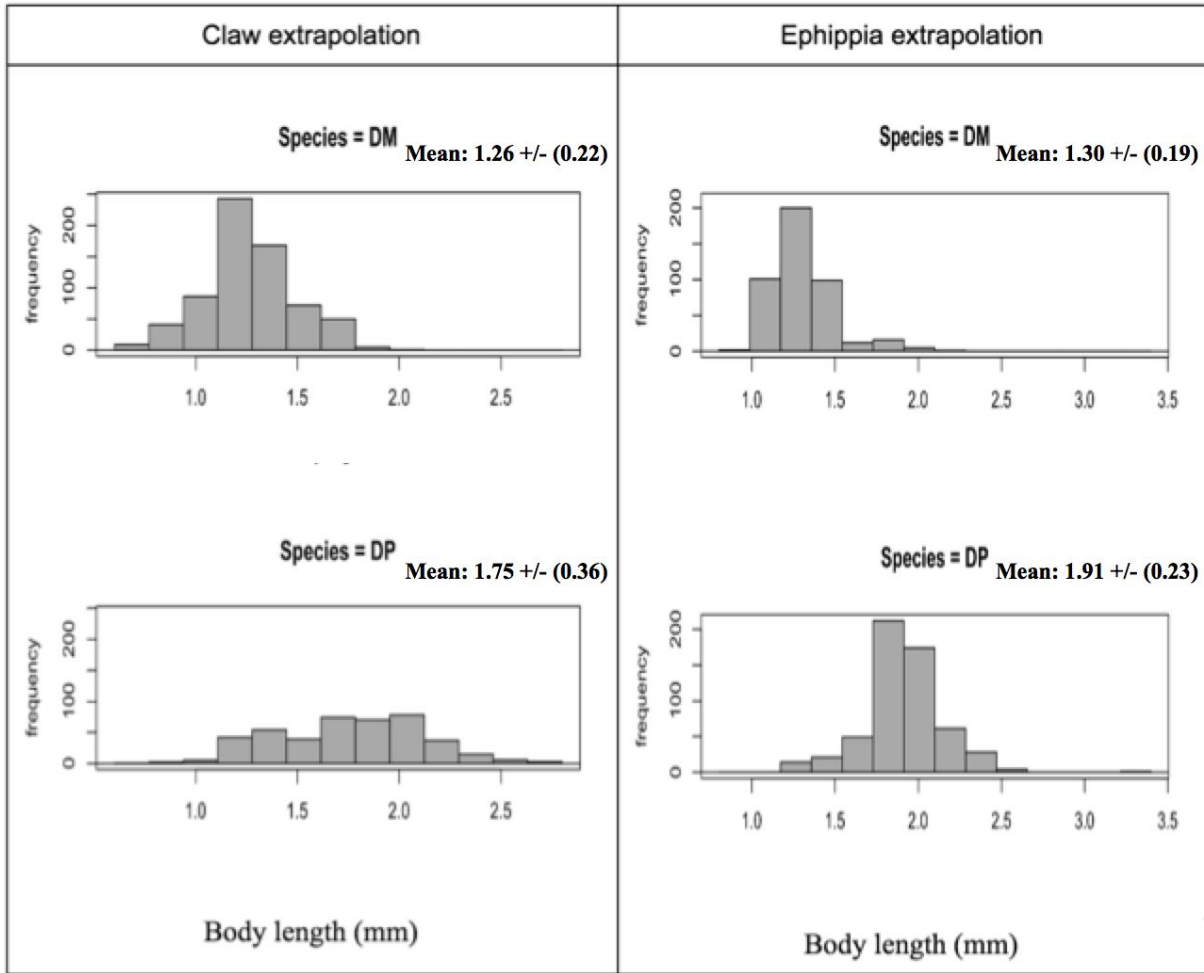


**Figure 12.** Histogram figure for *Daphnia* body lengths extrapolated from post-abdominal claws and ephippia for Square Lake. Independent t-tests were run for both species. *D.pulicaria* (DP):  $t = -8.55$ ,  $df = 2254.9$ ,  $p < 0.001$ . *D.mendotae* (DM):  $t = -0.46$ ,  $df = 669.02$ ,  $p = 0.64$ .

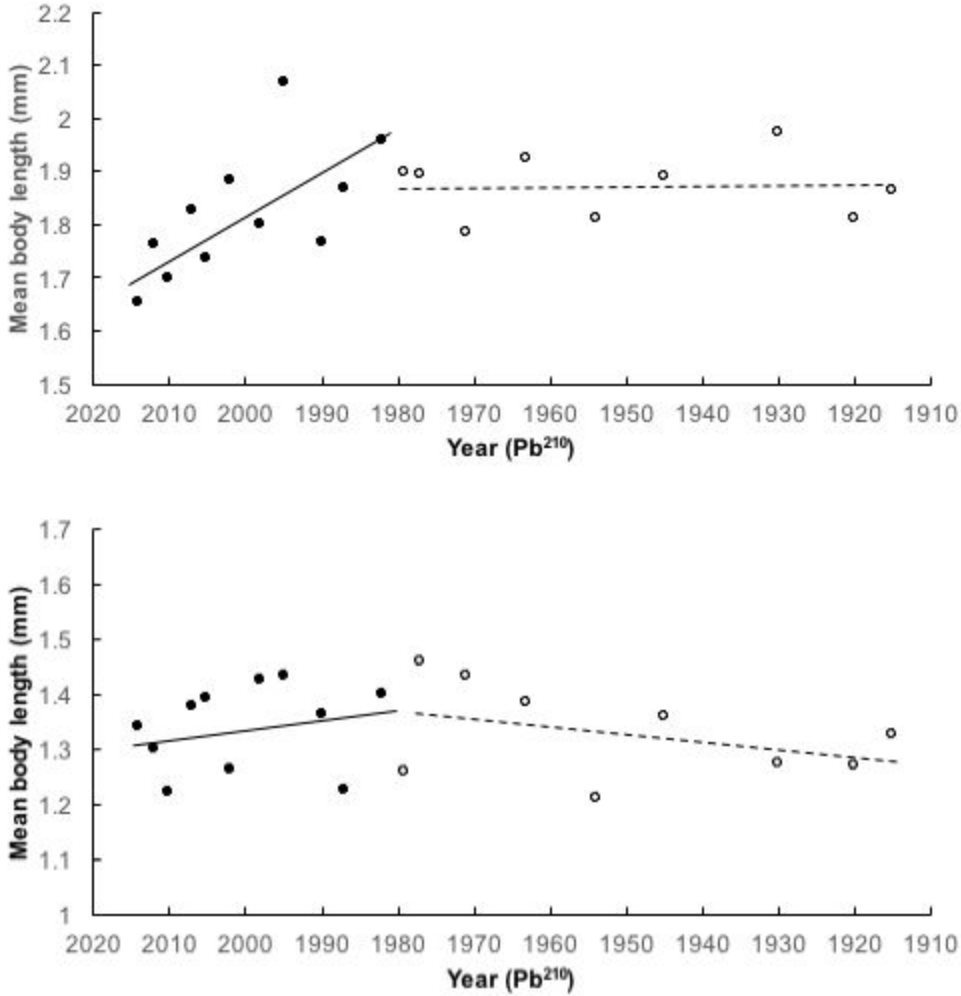




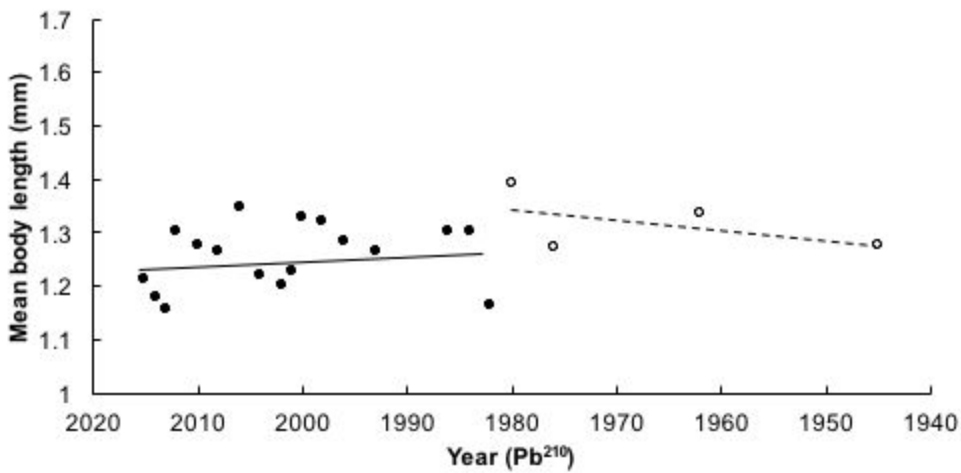
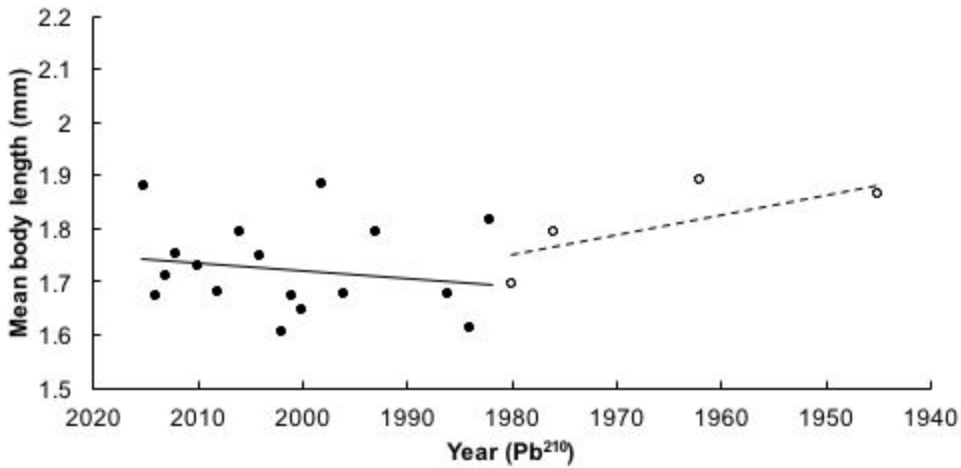
**Figure 13.** Histogram figure for *Daphnia* body lengths extrapolated from post-abdominal claws and ephippia for Big Carnelian Lake. Independent t-tests were run for both species. *D.pulicaria* (DP):  $t = -7.76$ ,  $df = 686.8$ ,  $p < 0.001$ . *D.mendotae* (DM):  $t = -3.33$ ,  $df = 1020.9$ ,  $p < 0.001$ .



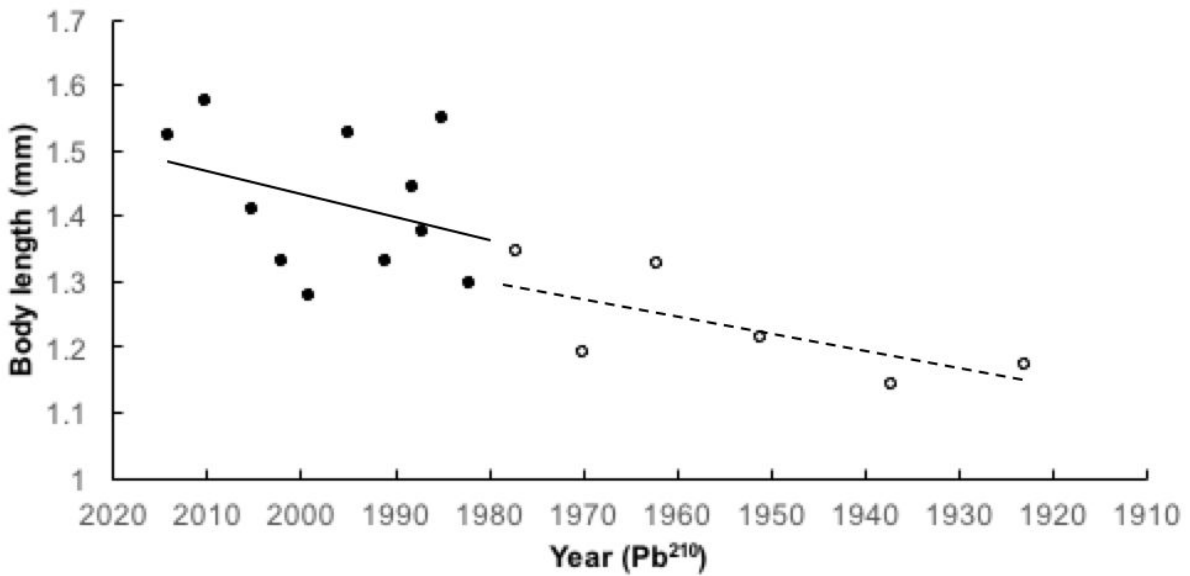
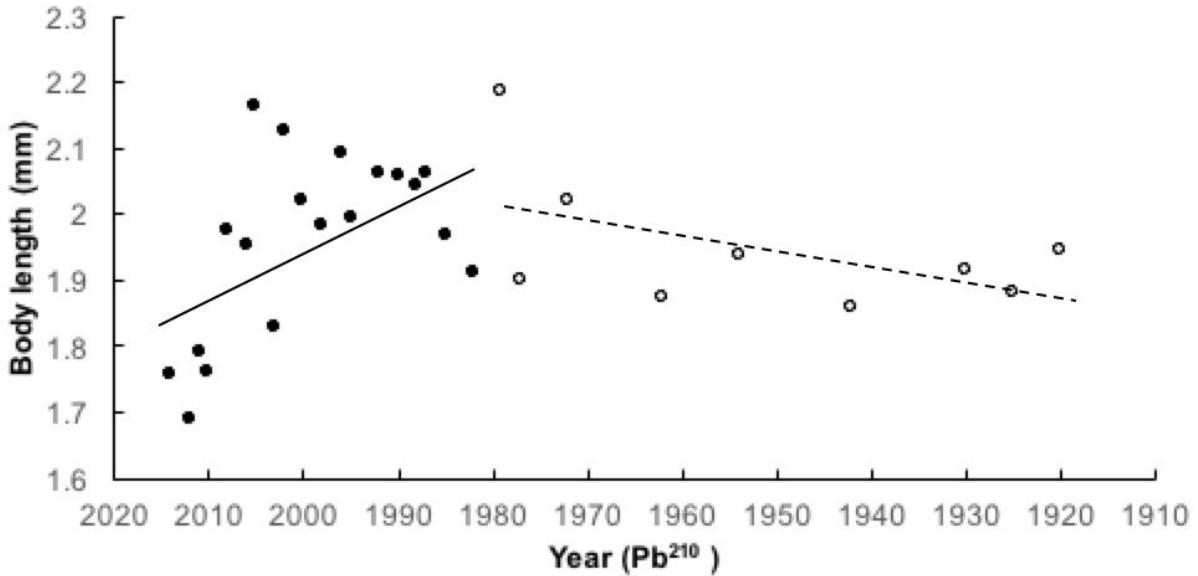
**Figure 14.** *Daphnia pulicaria* (top) and *D. mendotae* (bottom) mean body size (extrapolated from claw lengths) over time for Square Lake. Black circles are for years after 1981 when rainbow trout stocking program was initiated and white circles for years prior to 1981. Best fit linear regression lines are shown for both time periods. *D. pulicaria* body size decreases significantly after 1981 ( $p = 0.032$ ). Linear regressions for pre-1981 *D. pulicaria* data and for *D. mendotae* are not statistically significant.



**Figure 15.** *Daphnia pulicaria* (top) and *D. mendotae* (bottom) mean body size (extrapolated from claw lengths) over time for Big Carnelian Lake. Black circles are for years after 1981 when rainbow trout stocking program was initiated and white circles for years prior to 1981. Best fit linear regression lines are shown for both time periods. None of the linear regressions are statistically significant.



**Figure 16.** *Daphnia pulicaria* (top) and *D. mendotae* (bottom) mean body size (extrapolated from ephippia lengths) over time for Square Lake. Black circles are for years after 1981 when rainbow trout stocking program was initiated and white circles for years prior to 1981. Best fit linear regression lines are shown for both time periods. *D. pulicaria* body size decreases significantly after 1981 ( $p = 0.017$ ). Linear regressions for pre-1981 *D. pulicaria* data and for *D. mendotae* are not statistically significant.



**Figure 17.** *Daphnia pulicaria* (top) and *D. mendotae* (bottom) mean body size (extrapolated from ephippia lengths) over time for Big Carnelian Lake. Black circles are for years after 1981 when rainbow trout stocking program was initiated and white circles for years prior to 1981. Best fit linear regression lines are shown for both time periods. For *D. pulicaria*, body size has increased significantly ( $p= 0.008$ ) since 1981. Linear regressions for pre-1981 *D. pulicaria* data and for *D. mendotae* are not statistically significant.

