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FINAL REPORT

A Lake Tahoe *Mysis* Control Plan

Control of the Invasive *Mysis* Shrimp to Recover Lake Clarity and Ecosystem Health

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Table of Contents

List of Figures

List of Tables

A Lake Tahoe *Mysis* Control Plan

Project Summary

Project Name: Control of the Invasive *Mysis* Shrimp to Recover Lake Clarity and Ecosystem Health

California Tahoe Conservancy CTC 17 018L

Nevada Division of Environmental Protection (DEPS 18-011)

Date submitted: January 4, 2021; Prepared by S. Geoffrey Schladow

Objectives of the Project: The essence of the project was to "*plan, test and optimize a strategy to improve water clarity in Lake Tahoe by reducing the abundance of Mysis shrimp*". The work was to focus on Emerald Bay, where an earlier "natural" disappearance of *Mysis* shrimp gave rise to this project. The collection of extensive data sets from both Emerald Bay and Lake Tahoe proper on bioacoustics; *Mysis*, zooplankton and phytoplankton population physiology and dynamics; physical limnology; and trawling efficiency have allowed us to address this fundamental objective. Using the data obtained, and the analyses conducted, we have produced a Plan for the Removal of *Mysis* from Lake Tahoe itself, and provided very specific recommendations based on all the lessons learned from the conduct of this project.

Finding, Conclusions, Data and Recommendations: The study found that Emerald Bay was a suitable analog for Lake Tahoe. Despite size differences, nearly all physical, chemical and biological characteristics of both systems behaved essentially in the same manner, meaning that much of what occurs in Emerald Bay could be expected to play out in Lake Tahoe, just as they had when Mysis were first introduced in the 1960s. Using a combination of traditional limnological measurements together with bioacoustics, we have developed a comprehensive description of *Mysis* behavior in Emerald Bay. Based on this we have developed a strategy through which *Mysis* could be removed efficiently by working in synchrony with the annual stratification cycle and the life cycle of the *Mysis* themselves. Our research vessel and trawling system was found to be too small to remove enough *Mysis* to actually complete this, but on the basis of our work and the results on our catch per unit effort we have developed a plan (including

a net design) for a commercial trawler to remove *Mysis* as part of future phases of this work. Our results also highlighted the challenges of quantifying the density and distribution of *Mysis* in a lake as large as Lake Tahoe, and the challenges of knowing where to trawl. For both of these issues we have advocated the development of autonomous technologies, which will both lower the costs of mapping and help optimize the efficiency of future trawling operations.

A detailed timeline of next steps needed to move forward with the removal of *Mysis* from Emerald Bay and Lake Tahoe is provided, along with a timeline for refining the needed technologies. As part of this plan, financial projections have been made that indicate that such a project could actually be cost neutral or even profitable.

All data collected from this project have been archived.

Financial Analysis: There were no cost overruns for this project. The CTC funding will be fully expended by the time the final report is submitted. For the NDEP funding, all funds have been expended.

Future Public and Private Support: We seek to continue with the future work recommended in this report. As indicated, with the potential for utilizing harvested *Mysis* as a commercial source of high purity Omega-3 fatty acids and their value-added use as high-end dog treats, revenues of $37.7M over a 15-year project are projected. This is in excess of the estimated $32.0M cost of reducing *Mysis* densities to a level at which *Daphnia* and *Bosmina* can co-exist with them, thus allowing clarity improvement to become a self-sustaining operation (with an Internal Rate of Return of 20%). As well as Lake Tahoe's clarity improving, other benefits would include the removal of an invasive species, the lake food web more closely resembling its original state, and a new local industry will be created. While *Mysis* control will need to continue in perpetuity, the fact that it is a profitable undertaking should be viewed as a positive attribute with respect to local employment. This should be contrasted with the current expenditure levels on clarity restoration programs that are on the order of tens of millions of dollars annually, with no avenue for revenue generation and with no end in sight.

We are currently pursuing private support for the commercial aspect. However, it is still necessary to have some level of public funding initially to allow testing to be completed, tools to be developed and the process scaled up to a level where it can become stand-alone in the long-term. Given the large expenditures made on the restoration of Lake Tahoe in the past, we believe the funding we will be seeking will be extremely modest.

Media Coverage: This concept has attracted wide media attention, although we did not specifically produce content for that coverage. Possibly the broadest and most detailed coverage was through Capitol Public Radio's Tahoe Land podcast in 2019. These podcasts and additional web content are available directly through Capital Public Radio. We also featured this project and our previous research on *Mysis* in Emerald Bay as part of our State of the Lake Reports in 2018, 2019 and 2020. They are available online.

Acknowledgements

We are grateful to the opportunities provided through funding from the California Tahoe Conservancy and the Nevada Division of Environmental Protection.

The UC Davis authors wish to acknowledge the field assistance provided by Brandon Berry, Kian Bagheri, Raphael Townsend and Cordielyn Goodrich; Laboratory assistance provided by Anne Liston and Steve Sesma; Education and outreach support provided by Heather Segale, Alison Toy, and two generations of AmeriCorps members; Administrative support provided by Carmen Bedke; Report Compilation by Alison Toy and Heather Segale; and Development support provided by Antonina Myshyakova.

UC Davis also wishes to gratefully acknowledge technical assistance from Damien Guihen (University of Tasmania) for his continued advice on bioacoustics data processing, Biji Kobara (Biosonics), Juliana De Moura Bell and her lab for their expertise and guidance on the lipids contained within *Mysis*, Jackson Grosse for his guidance on the practical aspects of invasive species removal, Morgan Renner for his flume experiments on net design, and Bob Richards for his astuteness in not only documenting the removal of *Daphnia* by *Mysis* in the 1960s, but also recording the emergence of *Cyclotella* as part of the response.

We are particularly indebted to the students of the UC Davis Graduate School of Management (Yuan Cheng, Megann Kerr, Jae Hwan Kim, Michael Ries, Patrick Rosenberg and Tomas Sadliek) who developed and completed their project, "Restoring the Lake Tahoe Ecosystem:

Creating a Product from an Invasive Species." Their findings have helped propel the concept of turning an invasive species into a commodity and using the revenues to finance environmental restoration.

1.0 Introduction

1.1 Project Scope

This planning project was funded by the California Tahoe Conservancy with a Scope of Work as described below. The Nevada Division of Environmental Protection provided additional funding to purchase the Biosonics sonar system, an essential requirement of the project. This report constitutes the "Administrative Draft" final report for both agencies.

The essence of the project was to "plan, test and optimize a strategy to improve water clarity in Lake Tahoe by reducing the abundance of Mysis shrimp". The work was to focus on Emerald Bay, where an earlier "natural" disappearance of Mysis shrimp gave rise to this project. Preliminary estimates suggested that it may be possible for the UC Davis research vessel to accomplish the substantial reduction of Mysis from Emerald Bay. However, as described in the report, actual trawling experiments revealed that a combination of trawl size and vessel speed did not allow for the substantial reduction within a reasonable time. As a result, the proposed performance measures relating to changes in the abundance of Mysis and Daphnia, changes to the clarity of Emerald Bay, and impacts on fish were no longer meaningful. However, the collection of extensive data sets from both Emerald Bay and Lake Tahoe proper on bioacoustics; Mysis, zooplankton and phytoplankton population physiology and dynamics; physical limnology; and trawling efficiency have allowed us to address the fundamental objective stated at the beginning of this paragraph.

The specific tasks that formed the scope of work are as follows:

Task 1 – Stakeholder outreach: carried out through a range of means. A final round of stakeholder meetings will be organized after the submission of the final report. Separate meetings will be organized for Agencies and for the fishing guide industry.

Task 2 – Emerald Bay Echosounder surveys – described in this report (Sec. 4.2)

Task 3 – Lake Tahoe Echosounder surveys – described in this report (Sec. 4.2)

Task 4 – Emerald Bay phytoplankton and zooplankton surveys (Sec. 4.1)

Task 5 – Emerald Bay particle size distributions, Secchi depth and water column profiles (Secs. 4.3-4.6)

Task 6 – Zooplankton diet analysis – described in this report (Sec. 6)

Task 7 – Fish diet analysis – the fish diet work was predicated on removal of *Mysis* and return of *Daphnia*, so was not undertaken

Task 8 – *Mysis* trawling (Sec. 5)

Task 9 – Project team meetings – these were held weekly for a large part of the project period

Task 10 – Report of Emerald Bay Results – this document

Task 11 – Report of Lake Tahoe Plan (Sec. 9)

Task 12 – Progress reports – completed

Task 13 – TSAC-led peer review – to ensure that the results and the recommendations contained within this report are scientifically sound. The peer review comments and responses are included as Appendix 1.

Task 14 – Final Report – this document

1.2 Geographic Setting

Emerald Bay was carved by glaciation during the last ice age and is connected to the main body of Lake Tahoe by a shallow sill (4 m max depth; 400 m long) created by the glacier's submerged terminal moraine. The shallow sill provides a barrier to large scale water movements and a deterrent to immigration and emigration of deep living species (lake trout and *Mysis*), although *Mysis* can cross once Emerald Bay has vertically mixed in the late fall. The bay has a surface area of 1.92 km^2, with the deepest point being 68 m. The 22.98 km^2 watershed of Emerald Bay is largely contained within the Desolation Wilderness Area, protecting it from development and associated anthropogenic impacts. Lake Tahoe is a deep, graben lake (average depth 305 m; maximum depth 501 m) and large (surface area 495 km^2) located at an elevation of 1,897 masl within a watershed with an area of 800 km^2. The lake is monomictic but does not completely mix to the bottom every year.

1.3 Project background

The non-native opossum shrimp, *Mysis diluviana* (formerly *M. relicta*, hereafter referred to simply as *Mysis*), was introduced to Lake Tahoe and Emerald Bay in 1963-65 to provide forage for game fish (Hansen 1966). As reported by Richards et al. (1975), and summarized in Section 2.0 of this report, what followed this introduction were population crashes of the native cladocerans, *Daphnia* and *Bosmina*. They were essentially absent from the lake by mid-1971, although short-term rebounds with low population numbers have been recorded.

In 2011, the UC Davis Tahoe Environmental Research Center (TERC) recommenced the monitoring of Lake Tahoe and Emerald Bay *Mysis* populations. Monitoring had only occurred sporadically in Emerald Bay since the mid-1970s. After approximately five years of monitoring (see Section 3.0) a pattern emerged from the Emerald Bay data. *Mysis* were initially absent from Emerald Bay, an observation not made in Emerald Bay or Lake Tahoe since their introduction. Continued monitoring revealed that in the absence of *Mysis*, large populations of first *Bosmina* and then *Daphnia* re-established themselves in Emerald Bay, and with that there was an unprecedented increase in clarity of as much as 11 m (36 feet) over a two-year period. When *Mysis* abundance in Emerald Bay gradually increased to their previous levels following this period, cladoceran numbers again decreased, and clarity declined to their pre-2011 values.

Can Emerald Bay be considered a surrogate for Lake Tahoe? Climatically and hydrologically they are extremely similar, both being enclosed within the same small watershed, physically connected and at the same elevation. The physical limnology, water chemistry, phytoplankton and zooplankton data collected as part of this study and the earlier five-year Emerald Bay study indicate no substantive differences, as detailed in subsequent sections. The obvious difference in size (both area and depth) mainly manifests itself in some of the horizontal physical processes that occur in each water body. In Lake Tahoe there is a greater range and magnitude of basin-scale internal waves and horizontal gyres. Likewise, the greater depth of Tahoe means that the progression of deep mixing takes longer. However, both water bodies are monomictic with the same seasonality of the vertical mixing dynamics.

Biologically, Emerald Bay and Lake Tahoe have had very similar species composition (Goldman 1981). The work conducted as part of this project and the 2011-2017 measurement period confirms this as well. Primary productivity (Carbon sequestration rate) of Emerald Bay was higher than that of Lake Tahoe, based on measurements taken for several months each year between 1967 and 1971 (Goldman 1974), the period when *Mysis* were becoming established and the entire ecosystem was rapidly changing. Four measurements of summer primary productivity taken in 1978 (Morgan 1979) showed that Emerald Bay primary productivity was higher than for Lake Tahoe at that time. However, Lake Tahoe's annual primary productivity has increased steadily by almost a factor of four since 1978. As primary productivity in Emerald Bay has not been measured since 1978, current differences in rates of primary production between the two systems is unknown.

Given their similarities, the natural experiment within Emerald Bay highlighted the extent to which the introduction of *Mysis* and the elimination of *Daphnia* and *Bosmina* may bear on restoration of clarity in Lake Tahoe itself, something that had not been realized or even hypothesized prior to this 2011-2017 period of monitoring. The prevailing paradigm at Lake Tahoe had been that urban development since the 1960s and its impacts on the introduction of fine particles and nutrients to the lake was the primary cause of clarity decline. Although the observational findings from Emerald Bay did not rule out the roles of fine particles or nutrients on historic declines in water clarity, the data suggested the importance of other mechanisms, specifically, the role of cladocerans as a mechanism for particle loss from the water column. A key finding, from that 2011-2017 period of monitoring, highlights the critical ecosystem service provided by *Daphnia* and *Bosmina*. Data produced through this project have shown that *Daphnia spp.* in Lake Tahoe can feed in the 5-30 µm range and *Bosmina* can feed on particles in the 1-3 µm range and sometimes larger. Thus, these two native taxa could act as controls on the particle sizes remaining in Lake Tahoe and Emerald Bay. Data by others from other systems have shown the potential for an even broader range of fine particle removal. In the absence of fine particle clearing, the effects of watershed inputs, and in particular, urban runoff would cause water clarity to decline.

Historically, the approach to addressing clarity decline since the 1960s had been to attempt to reduce watershed and urban loads of fine particles and nutrients before they reached the lake, culminating in the development of the current bi-State (TMDL) Total Maximum Daily Load program. This type of approach had been widely adopted across the US and elsewhere, although the Lake Tahoe system is somewhat unique in its exceptionally high clarity. The 2011-2017 data suggest that if *Mysis* could be controlled to an extent where *Daphnia* and *Bosmina* could co-exist with them, then clarity in both Emerald Bay and Lake Tahoe could be greatly improved at current levels of watershed load management. In other words, the data pointed to the potential for "top-down control" to help restore clarity, separate from the "bottom-up approach" implicit in the TMDL.

Past research has shown that the micron-scale algal cells as well as inorganic particles are increasingly present near the surface of Lake Tahoe (where they can impact Secchi depth) due to the impact of climate change on lake stratification (Winder et al. 2008; Sahoo et al. 2015; Naranjo et al. 2020). This opens the possibility of *Mysis* control (aka *Daphnia* return) as effectively being a viable option to restore the clarity of Lake Tahoe, and at the same time mitigating the impacts of water temperature and stratification increases on clarity.

It was against this backdrop of declines in clarity during some summer months and the absence of improving annual clarity, that TERC proposed to the California Tahoe Conservancy (CTC) and the Nevada Division of Environmental Protection (NDEP) to plan, test and optimize a strategy to improve water clarity in Lake Tahoe by reducing the abundance of *Mysis* shrimp, one of Lake Tahoe's most ubiquitous aquatic invasive species. Based on the findings, this could be an initial step in the commencement of a two-pronged approach to Tahoe's clarity and invasive species control as follows:

- Maintain the current TMDL-based approach to limit fine particles and nutrients (bottom-up control). This is needed both for clarity improvement and for limiting the lake's growing oxygen demand, a factor expected to be increasingly more important with climate change.

- Commence the harvesting of the invasive *Mysis* shrimp throughout Lake Tahoe (including Emerald Bay and other regional lakes where *Mysis* had been introduced in the past). As well as allowing for the return of the native zooplankton, this would also have benefits in countering the increase of smaller diatoms that the TMDL may not be effective in countering.

1.4 Report Organization

This report is organized as follows:

Section 2.0 presents a brief literature review of *Mysis* at Lake Tahoe and other lakes. A more complete review is provided in the manuscript included as Appendix 1. This Section was written by UC Davis.

Section 3.0 summarizes TERC's data for the period 2011-2017 in Emerald Bay that led to the proposal and this report. A full description of the data from that period also forms part of Appendix 1. This Section was written by UC Davis.

Section 4.0 describes the field work pertaining to characterization of the physical and biological conditions in Emerald Bay and Lake Tahoe. This specifically covers Biosonics (echosounding) data, physical profile and nutrient data, thermistor chain data, *Mysis* and zooplankton sampling, and phytoplankton sampling. The results and the conclusions of this work are presented here. This Section was written by UC Davis.

Section 5.0 describes the *Mysis* trawling operations that were undertaken. This includes the design and modifications to the trawling system, the results of trawling tests in Emerald Bay and the conclusions that can be drawn about future *Mysis* harvesting operations. This Section was written by UC Davis.

Section 6.0 describes the results of experiments with native zooplankton and mysids and their influence on pelagic processes. This Section was written by University of Nevada, Reno.

Section 7.0 quantifies the diet and feeding behavior of *Mysis* from Emerald Bay and Lake Tahoe. This Section was written by University of Nevada, Reno.

Section 8.0 summarizes the lessons learned through the conduct of this project and provides suggestions for future actions. This Section was written by UC Davis.

Section 9.0 presents the Plan for the control of *Mysis* in Emerald Bay and Lake Tahoe, together with a detailed timeline and financial projections over a 15-year period. This Section was written by UC Davis.

2.0 Literature Review

The exceptional clarity of Lake Tahoe, a deep, oligotrophic lake in the Sierra Nevada, the major mountain range dividing California and Nevada, has been declining for over five decades (Schladow, 2019). The cause of this decline has been attributed to land use change spurred by rapid development and population growth in the Lake Tahoe basin starting in the 1950s. The population in the Tahoe basin increased from a handful of residents in 1950 to near 100,000 by 1980 (Goldman 1988). The underlying hypothesis was that clarity was declining as a result of mild eutrophication, controlled by runoff from the watershed (Goldman 1988), as well as by enhanced atmospheric deposition of nitrogen from vehicle emissions (Jassby et al. 1994), both of which contribute to algal productivity. Although it was initially thought that enhanced nutrient inputs were the largest cause of clarity decline (Goldman 1988), it was subsequently found that fine particulates had a larger impact on lake clarity (Jassby et al. 2003; Swift et al. 2006). Shifts in phytoplankton community structure were also attributed to increased nutrient inputs (Hunter et al. 1990). As a result of these considerations, the majority of restoration efforts in the Lake Tahoe basin over the last two decades have been targeted at remediating or mitigating legacy development projects and their impacts on fine particle and nutrient additions (EIP 2018).

From 1963 to 1965, coincident with the period of rapid urban development and population growth, the non-native opossum shrimp, *Mysis* (from Upper Waterton Lake, Alberta, Canada) were introduced to Lake Tahoe (including Emerald Bay) by U.S. Fish and Game. The motivation was to provide an additional food source for recreationally important game fish lake trout (*Salvelinus namaycush*) and kokanee salmon (*Oncorhynchus nerka*)). Two introductions totaling 165,300 individuals were made to Emerald Bay and Lake Tahoe (Linn and Frantz 1965; Hansen 1966). The diffuse population of newly introduced mysids took several years to establish lake wide. It wasn't until 1970, that rapid expansion of the mysid population became evident through diet analysis of deep living lake trout (Richards et al. 1975).

Once established, *Mysis* quickly altered the aquatic food web. By selectively feeding on native cladoceran species *Bosmina longirostris*, *Daphnia rosea* and *D. pulex* (Cooper and Goldman 1980; Threlkeld 1981), they effectively depressed the abundances of all three species within Lake Tahoe by 1973 (see **Fig. 2.1**). The resulting pelagic zooplankton assemblage became dominated by copepods (*Epischura nevadensis*, *Diaptomus tyrrelli*) and the rotifer *Kellicottia longispina*. Examination of *Mysis* stomach contents collected from the lake revealed remains of all of the non-cladoceran zooplankton (Threlkeld et al. 1980) pointing to the importance of this organism as a driver for change in the native ecosystem. Copepods have been shown to be less efficient phytoplankton grazers than cladocerans (Sommer and Sommer 2006) and can release pressure on smaller algae species by grazing on larger forms of phytoplankton, enhancing the pelagic biomass of small sized algae. Richards et al. (1975) also noted that there had been a concomitant increase in the occurrence of the small diatom genus, *Cyclotella spp*. *Cyclotella* is currently considered to be a genus that is having a large negative impact on clarity at Lake Tahoe (Winder et al. 2008; Naranjo et al. 2020).

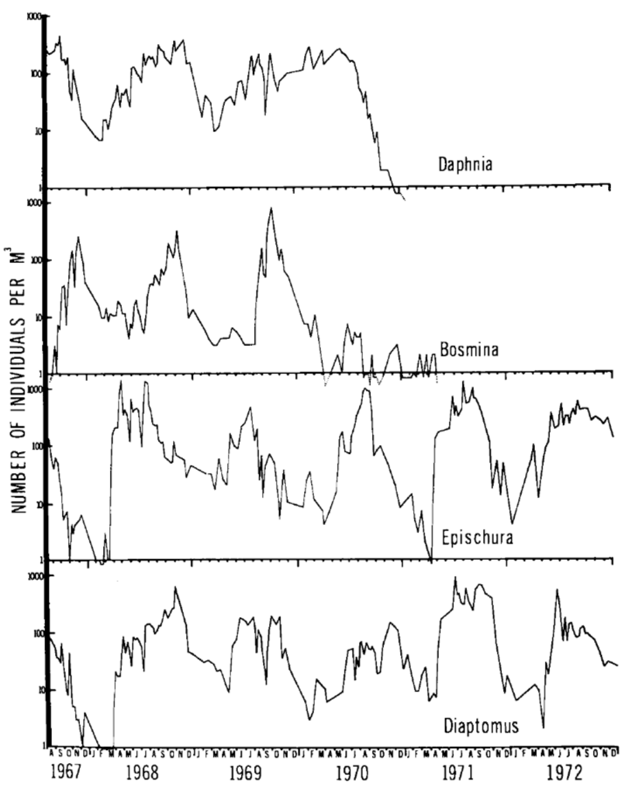

Fig. 2.1: Time series of population counts (ind./m^2) *for Daphnia*, *Bosmina*, *Epischura* and *Diaptomus* from 1967-1973 following the introduction of *Mysis* (from Richards et al. 1975).

Another important issue is whether *Daphnia* could also remove fine inorganic particles, in addition to small *Cyclotella* cells. Fine inorganic particles have been identified as a major cause of declining clarity in Lake Tahoe. Most experiments to determine *Daphnia* filtering rates use inorganic particles. These have included glass beads (Burns 1968) and polystyrene beads (Gophen and Geller 1984). The latter found that *Daphnia* would solely exclude polystyrene particles larger than their filter meshes (0.4-0.7 microns). They used particle concentrations of order (104-108 microns)/ml for the size range 0.5-5 microns. This is the particle size range most important for light scattering and clarity loss (van de Hulst 1957; Davis-Colley and Smith 2001). The conclusion is that *Daphnia* could readily clear inorganic particles, which exist at concentrations of 10^3 to 10^5 particles/ml in Lake Tahoe (Schladow et al. 2020). Has the return of *Daphnia* been linked to the return of clarity before? In one of the most famous limnological examples, the clarity of Lake Washington doubled with the return of *Daphnia* (Edmonson and Litt 1982).

Algal primary productivity, as measured by uptake of C-14, has also increased at Lake Tahoe (Schladow 2020), rising near-monotonically from less than 50 mg C m-2 yr-1 in the 1960s to values in excess of 250 mg C m-2 yr-1 observed today (**Fig. 2.2**). As evident in **Fig. 2.2**, the commencement of the rising primary productivity coincided with the introduction of *Mysis*. The precise causes of this increase are unknown and it may be related to both development impacts and changes wrought by *Mysis*. The higher intrinsic growth rate of smaller phytoplankton (Sommer and Sommer 2006) that *Mysis* predation patterns have led to would be consistent with this observation.

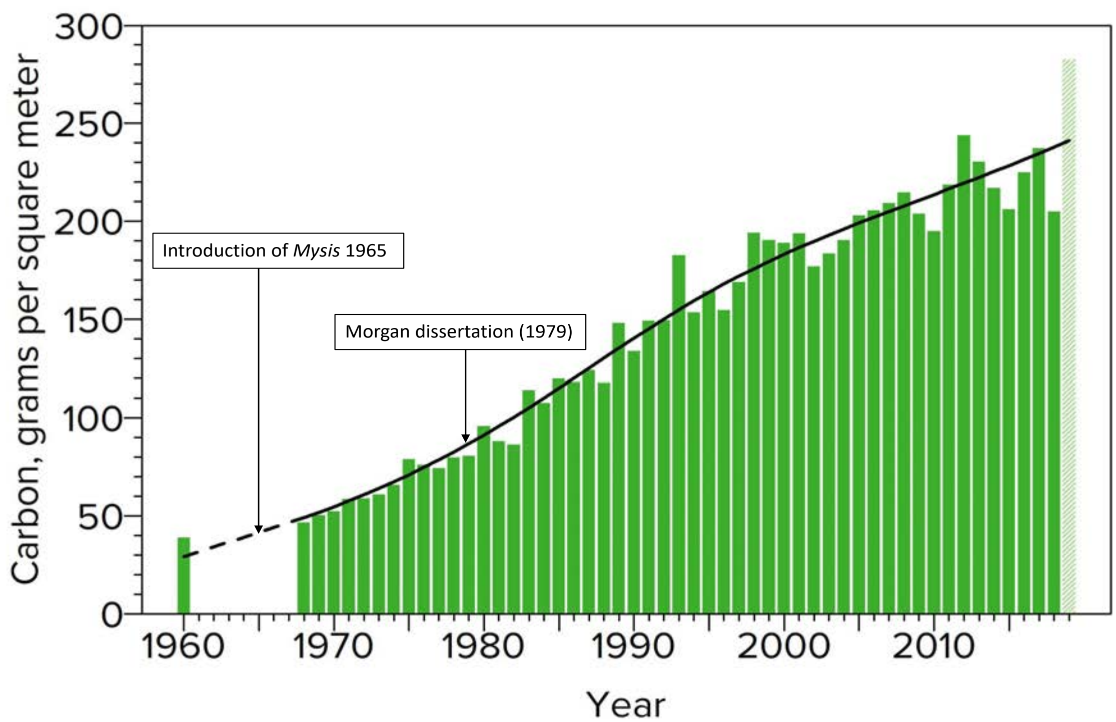

Fig. 2.2: Primary productivity in Lake Tahoe. The dates of *Mysis* introduction and the last comprehensive study of *Mysis* in Emerald Bay (Morgan 1979) are shown. 2019 data are considered provisional.

The changes to clarity at Lake Tahoe over the same period are summarized through the annual average Secchi depth (Schladow 2020), typically based on 25 measurements throughout the year (**Fig. 2.3**). As both **Fig. 2.2** and **Fig. 2.3** highlight, the major period of alteration at Lake Tahoe was coincident with the introduction of *Mysis*.

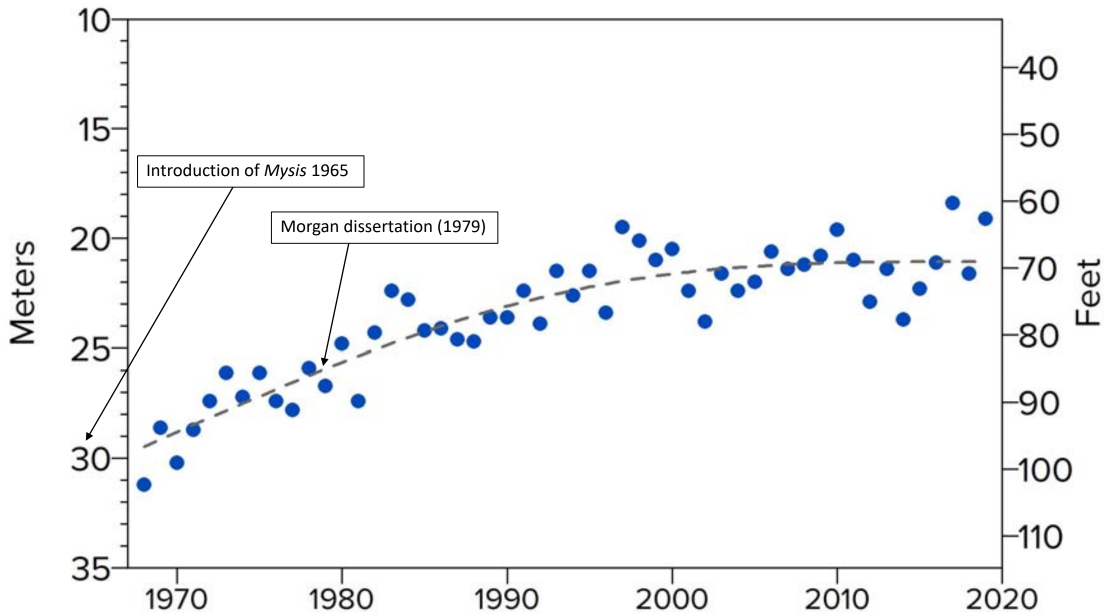

Fig. 2.3: Annual Secchi depth in Lake Tahoe. The dates of *Mysis* introduction and the last comprehensive study of *Mysis* in Emerald Bay (Morgan 1979) are shown.

System-wide changes following the introduction of *Mysis* are not unique to Lake Tahoe (Rieman and Falter 1981; Chipps and Bennett 2000; Branstrator et al. 2000). At Flathead Lake, Montana, *Mysis* have caused major, long-term effects throughout the food web. *Mysis* introduction was found to be responsible for changing the dominant fish species from kokanee salmon to lake trout, for greatly reducing cladocerans populations, and for a downward shift in the size of phytoplankton (Ellis et al. 2011; Stafford et al. 2002). Primary productivity was also impacted, with the highest rates of primary productivity measured in the year when *Mysis* numbers peaked with an overall 27% increase in primary production over prior rates. It is noteworthy that *Mysis* populations at Flathead Lake have fluctuated between approximately 20 to 80 individuals/m^2 between 1988 and 2005. By contrast, *Mysis* concentrations in Lake Tahoe and Emerald Bay have generally been in the 100 to 250 ind./m^2 range since their introduction, although monitoring has been far from continuous.

Despite the early warning of large, system-wide changes to both the zooplankton and phytoplankton communities by Richards et al. (1975), the potential impact of *Mysis* was largely unexplored in subsequent decades. For example, Abbott et al. (1982) did not mention grazing as a possible cause of the observed phytoplankton spatial distribution and instead focused on stream-borne nutrient sources; Hunter et al. (1990) viewed the cause of change in the phytoplankton community structure as being solely nutrient-driven; Carney and Elser (1990) and Elser and Goldman (1991) specifically discounted the impact of grazing in Lake Tahoe on the phytoplankton population; and Jassby et al. (1992) only considered the impact of *Mysis* to be their potential to transport nutrients vertically. Over the next 40 years, research on *Mysis* was limited (Rybock 1978; Morgan 1979; Goldman et al. 1979; Morgan 1981; Threlkeld 1981; McCoy 2015) but confirmed the ubiquitous distribution of *Mysis* throughout Lake Tahoe and Emerald Bay and its dominance in the food web. Despite intermittent increases in *Daphnia* or *Bosmina* populations for a season or two (Byron et al. 1986), the populations were extremely low and there has been no evidence of change to the *Mysis* domination of the ecosystem and a return of cladocerans playing a significant role in the food web. Hypotheses indicating that increasing primary productivity and eutrophication levels would lead to the return of *Daphnia* and *Bosmina* by accelerating their fecundity (Byron et al. 1986) have not been borne out over the last 30-40 years.

3.0 The 2011-2017 Emerald Bay Natural Perturbation

TERC commenced a program to monitor *Mysis* in Emerald Bay in November 2011, with the intention of sampling the bay via vertical trawls at 3-month intervals. The protocols adopted closely followed those developed in previous Emerald Bay and Lake Tahoe monitoring of the 1970s. The measurements were taken at night on account of the vertical migration of *Mysis* away from the lake surface during the daylight (they are negatively phototactic). As shown in **Fig. 3.1**, on the first evening of sampling the *Mysis* density was 1 ind./m^2. An earlier sampled period, July 1979 – June 1985, *Mysis* densities ranged from 21-292 ind./m^2 with an average of 120 ind./m^2.

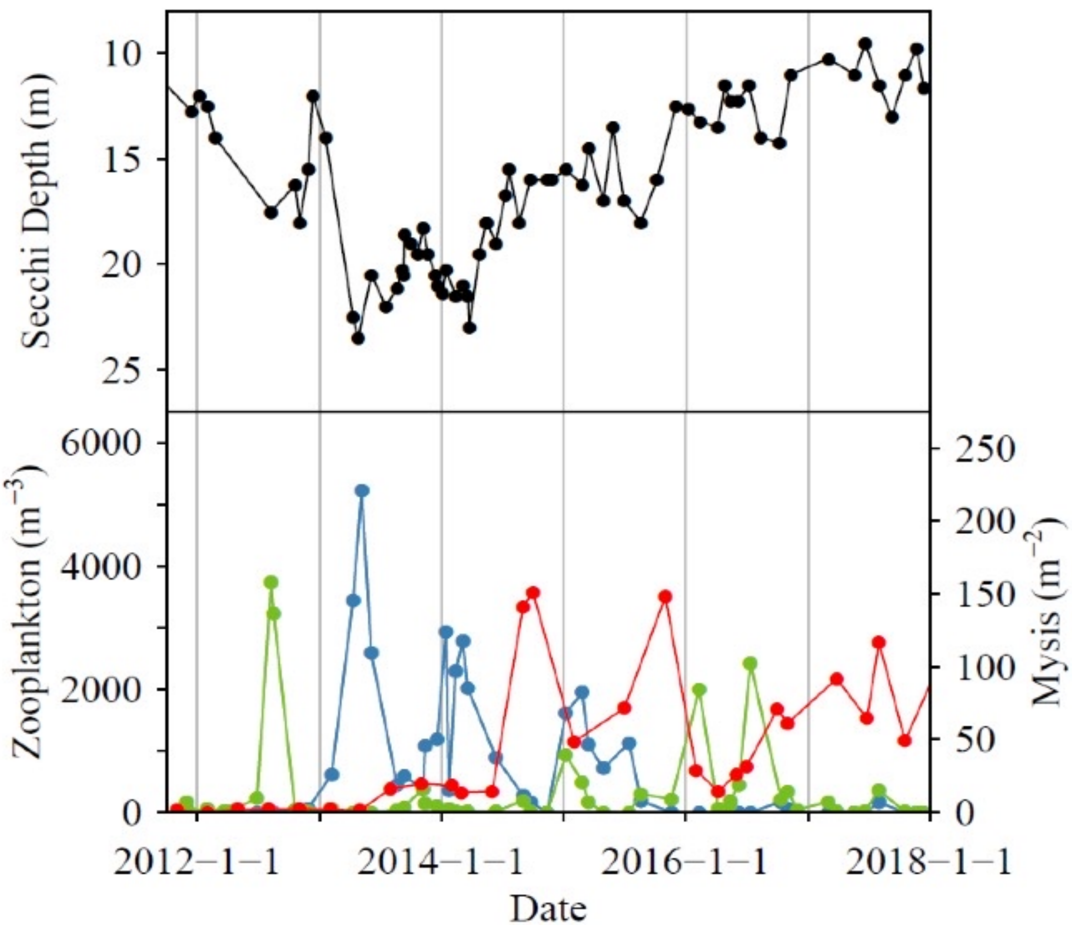

Fig. 3.1: Secchi depth and *Mysis* shrimp and Zooplankton abundance in Emerald Bay. **Top:** Secchi depth (m) in Emerald Bay, **Bottom:** *Mysis* shrimp abundance in number of ind./m^2 shown as red circle, *Daphnia* spp. and *Bosmina* spp. abundance in number of ind./m^3 are shown in blue and green circles, respectively. *Mysis* abundance can be converted to a volumetric basis by dividing by the sampling depth of 60 m.

The depressed *Mysis* values lasted until roughly August 2014, when values jumped to approximately 150 ind./m^2, and for the next 40 months averaged approximately 100 ind./m^2. The causes of the *Mysis* crash are unknown. There were no unusual conditions prior to this happening (extremely warm water temperatures, unusually large inflows of contaminants, for example). An epizootic episode has been suggested, but no data were collected.

Fig. 3.1 also shows the cladocerans in Emerald Bay during this period. *Bosmina* numbers rose first, with an initial peak exceeding 3,000 ind./m^3 occurring in August 2012. By May 2013, *Bosmina* concentrations were overtaken by *Daphnia* that remained the dominant cladoceran until July 2015. The cladoceran populations fluctuated (usually 180 degrees out of phase with *Mysis* values), but for the period from May 2013 through July 2015 the mean *Daphnia* population was 1,289 ind./m^3. The 2015 peak of *Daphnia* occurred exactly when *Mysis* decreased, but in 2016, it was the concentration of *Bosmina* that peaked. This suggests that there may be cofactors involved in the abundance of *Daphnia* that are not only related to the decrease in *Mysis*. Cladoceran numbers have been near-zero in both Emerald Bay and Lake Tahoe over most of the previous 40 years (Bürgi et al. 1993; Allen, unpublished data), with the dominant zooplankton being rotifers and copepods. The dominant zooplankton throughout the entire measured period in Emerald Bay were rotifers and copepods, with numbers an order of magnitude greater than cladocerans. Subsequent measurements in Emerald Bay (see Section 4.1) show that cladoceran populations continued to be at the low values displayed in 2017.

Secchi depth was also measured within a few days of each *Mysis* trawl. As seen in **Fig. 3.1**, as *Bosmina* and *Daphnia* numbers increased, the Secchi depth increased. Values of Secchi depth increased over the typical Emerald Bay values by over 11 m (36 feet) during this period. This is particularly noteworthy, as a change of clarity within either Emerald Bay or Lake Tahoe of this magnitude has never been recorded, except for the response to very brief (days) upwelling events (for example, Schladow et al. 2004) and after exceptionally rare high storm flow events (Hackley, pers. comm.).

4.0 Field Measurements Associated with this Study

The primary focus of this study was Emerald Bay in the south-west corner of Lake Tahoe (**Fig. 4.1**). The locations of *Mysis* monitoring sites, the meteorological station (TB-3) and the thermistor chain used to identify the vertical temperature structure of Emerald Bay are shown on the figure. While Emerald Bay was the focus, one of the broader goals of the project was to establish whether the deliberate removal of *Mysis* shrimp from the entire Lake Tahoe system was both feasible and would produce the desired results on lake clarity, so measurements in Lake Tahoe also took place.

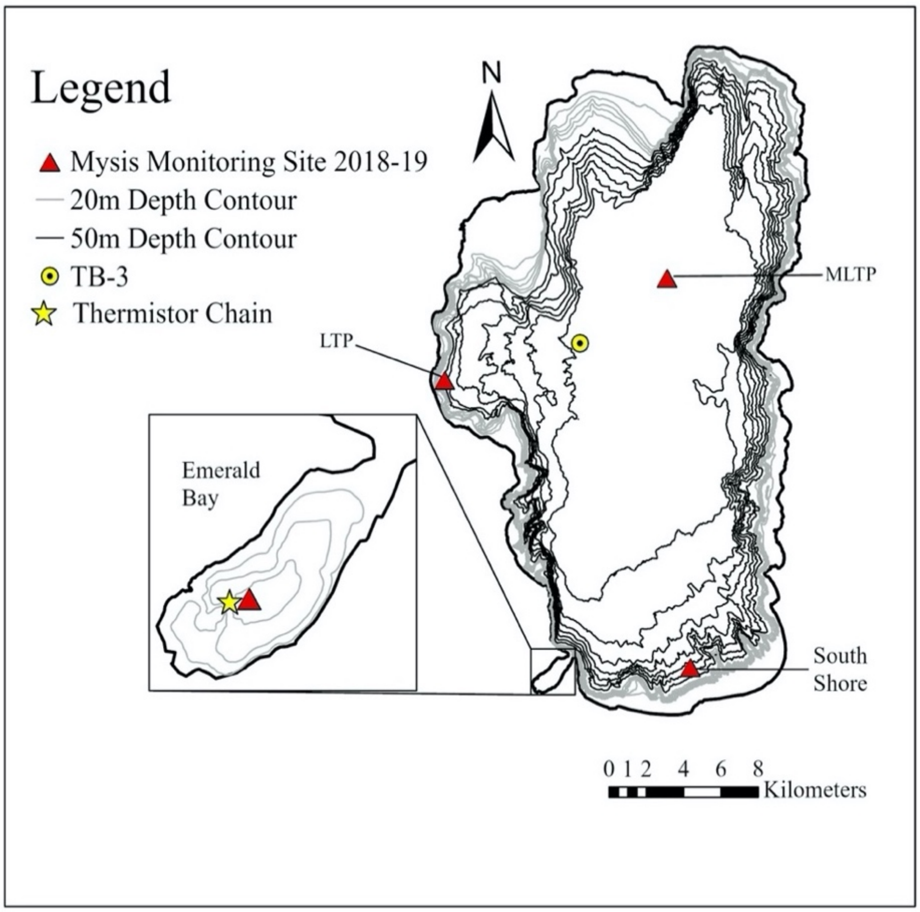

Fig. 4.1: *Mysis* monitoring sites maintained from 2018-2019 (red triangles). Yellow star is the location of a thermistor chain station installed in Emerald Bay. Yellow circle is Buoy TB3 where overnight Biosonics surveys were conducted. Depth contours shown at 20 m intervals (gray) to 120 m depth, and at 50 m intervals (black) from 150 m to 450 m. MLTP (Mid-Lake Tahoe Productivity) and LTP (Lake Tahoe Productivity) are the two long-term monitoring stations maintained by UC Davis.

The measurement program included (summarized in **Table 4.1**):

Table 4.1: Field work schedule for the *Mysis* project.

	2017			2018												2019												2020		
TASK	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar
Synoptic - EB	🦐			🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐			🦐	🦐		🦐	🦐	🦐			🦐		🦐		🦐			
Synoptic - LT	🦐			🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐		🦐	🦐		🦐	🦐	🦐			🦐		🦐		🦐			
Biosonics - EB				🦐	🦐	🦐			🦐	🦐	🦐	🦐						🦐			🦐		🦐				🦐			
Biosonics - LT									🦐	🦐		🦐	🦐	🦐				🦐			🦐		🦐		🦐		🦐			🦐
Full WQ - EB				🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐		🦐	🦐	🦐		🦐	🦐	🦐	🦐	🦐	🦐	🦐		
Dry EB (Seabird & Secchi)				🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐		
Phytoplankton	🦐		🦐	🦐	🦐	🦐		🦐		🦐	🦐	🦐	🦐	🦐	🦐	🦐		🦐	🦐	🦐		🦐	🦐	🦐	🦐	🦐	🦐			
Trawling - EB				🦐	🦐	🦐						🦐	🦐	🦐	🦐					🦐	🦐			🦐						
Thermistor chain					🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐	🦐		

- Synoptic measurements in both Emerald Bay and Lake Tahoe at the *Mysis* monitoring sites (**Fig. 4.1** – red triangles). These comprise vertical net tows for both *Mysis* and zooplankton in order to identify and enumerate them.

- Biosonics Surveys – these were echosounding surveys to measure the distribution of *Mysis* in both the vertical and horizontal directions (multiple locations). By linking these surveys with the synoptic measurements, it is possible to calibrate the Biosonics data and obtain a far greater data coverage for assessing the *Mysis* numbers and locations. Surveys were also conducted in Lake Tahoe to better understand the distribution in the much larger and deeper water body. Most surveys were boat mounted transects (the instrument was mounted to the research vessel and pre-set transects were run) or point measurements in conjunction with vertical net tows to provide calibration data. There were also several fixed deployments where the instrument was mounted on a platform overnight in order to better track the change in *Mysis* distribution over a longer time span.

- Trawling Operations – trawling operations were experimental and aimed to quantify an appropriate net design, the effort required to harvest the yield of specific trawling operations and the impacts of time of year on trawling operations.

- Full Water Quality Surveys – conducted monthly throughout Emerald Bay. This comprised water sampling at several depths for subsequent nutrient analysis and particle size distribution analysis. These results are comparable to similar long-term measurements taken in Lake Tahoe (under separate funding).

- “Dry” Emerald Bay Water Quality Surveys – comprised of physical sampling of the water column using instruments (and not taking water samples). The instruments included a Seabird multi-parameter profiler, LISST particle size analyzer, a Biospherical UV profiler and a Secchi disk. These results are comparable to similar long-term measurements taken in Lake Tahoe (under separate funding).

- Phytoplankton Identification and Enumeration – conducted with phytoplankton samples collected at the pelagic site Station 4 in Emerald Bay during 2013-2014 and 2017-2019. Sampling depth and water-integration procedure varied somewhat between the years. In 2013 and 2014, discrete water samples were collected from 2, 5, 10, 20, 35 and 50 m, or 5, 10, 20 and 40 m depths. From 2017 to 2019, depth-integrated water samples were prepared by combining water from 5, 10 and 20 m, except for June and October 2017, when water from 10, 20 and 30 m was pooled. Phytoplankton samples from Lake Tahoe were collected at monthly (or shorter) intervals at the offshore Index station in the main basin (**Fig. 4.1** – LTP sampling site). These collections consisted of discrete samples taken from 6 to 13 depths between the surface and 105 m. The complete data set is available and includes collections for the period between 1967-1989 and 2002-2019. All phytoplankton samples were collected with a Van Dorn sample bottle and preserved with Lugol’s solution. Phytoplankton analysis was conducted according to the Utermohl method.

- Thermistor Chain – the nature of the thermal stratification is believed to be an important element influencing the vertical distribution of *Mysis* and their eventual control. It is only in the presence of a thermal stratification that *Mysis* appear to be able to form a distinct, focused stratum. The development of such strata is critical to efficient *Mysis* removal as it effectively concentrates them vertically. A thermistor chain was maintained in Emerald Bay in order to provide a continuous measure of the change in temperature stratification over time.

4.1 Synoptic Surveys of *Mysis* and Zooplankton

4.1.1 Methods

Mysis were sampled at night using a conical net (0.75 m diameter, 0.5 mm mesh) while zooplankton were sampled during daylight hours using a net with the same diameter but a 0.08 mm mesh. The zooplankton net was equipped with a TSK flowmeter for net tow calibration as the finer mesh produces significant resistance sacrificing net efficiency. Samples were preserved in 10% sucrose buffered formalin. Replicate vertical tows were collected at each site. The sampling station at Emerald Bay sampled the entire water column from 60 m to the surface whereas in Lake Tahoe vertical net tows were made from 100 m to the surface at the LTP site and from 200 m to the surface at MLTP and South Shore sites. *Mysis* were collected between one hour after dusk when they enter the upper water column to feed and one hour before dawn as they migrate down to depth. Samples were collected monthly in 2018 but due to weather and operational issues, only seven monthly *Mysis* and zooplankton sampling events occurred in 2019 (water quality, phytoplankton and physical sampling was able to largely adhere to the complete schedule).

Part of those operational issues was the necessity to establish nine additional sampling sites in Emerald Bay across various depths in November 2018 to provide ground truthing data for Biosonics survey validation, data on seasonal distribution and movement of *Mysis* throughout the bay and as monitoring stations to show potential impacts of trawling efforts. A total of 12 samplings at all of the 10 sites was conducted.

Mysis densities were expressed on a per square-meter basis by aggregating the total number of individuals and dividing by the net area (this was the method historically used at Lake Tahoe and many other lakes). *Mysis* body length was determined by measuring from the tip of the rostrum to the cleft in the telson under a dissecting microscope fitted with a calibrated ocular micrometer. Individuals were identified as male if an extra-long fourth pleopod was present (Morgan and Beeton 1978). All *Mysis* in both replicate samples were enumerated and measured. *Mysis* lengths were used to create size frequency distribution for analysis of growth patterns and cohort size of the *Mysis* population over time. Zooplankton densities were expressed on a per cubic-meter basis after all collections have been standardized for calibrated filtration efficiency, net size and tow depth. The filtrated water volume was calculated by the coefficient between difference flow-meter readings and revolutions.

4.1.2 Emerald Bay Results

Mysis were present in Emerald Bay all year round (red circles, **Fig. 4.2**) and despite the concurrent removal effort *Daphnia* and *Bosmina* were absent or very low throughout the period (mean 25 and 36 ind./m^3, respectively). While there were increases of these species, abundance was much lower than those observed in 2012-2014 (mean 946 and 277 ind./m^3). It is particularly

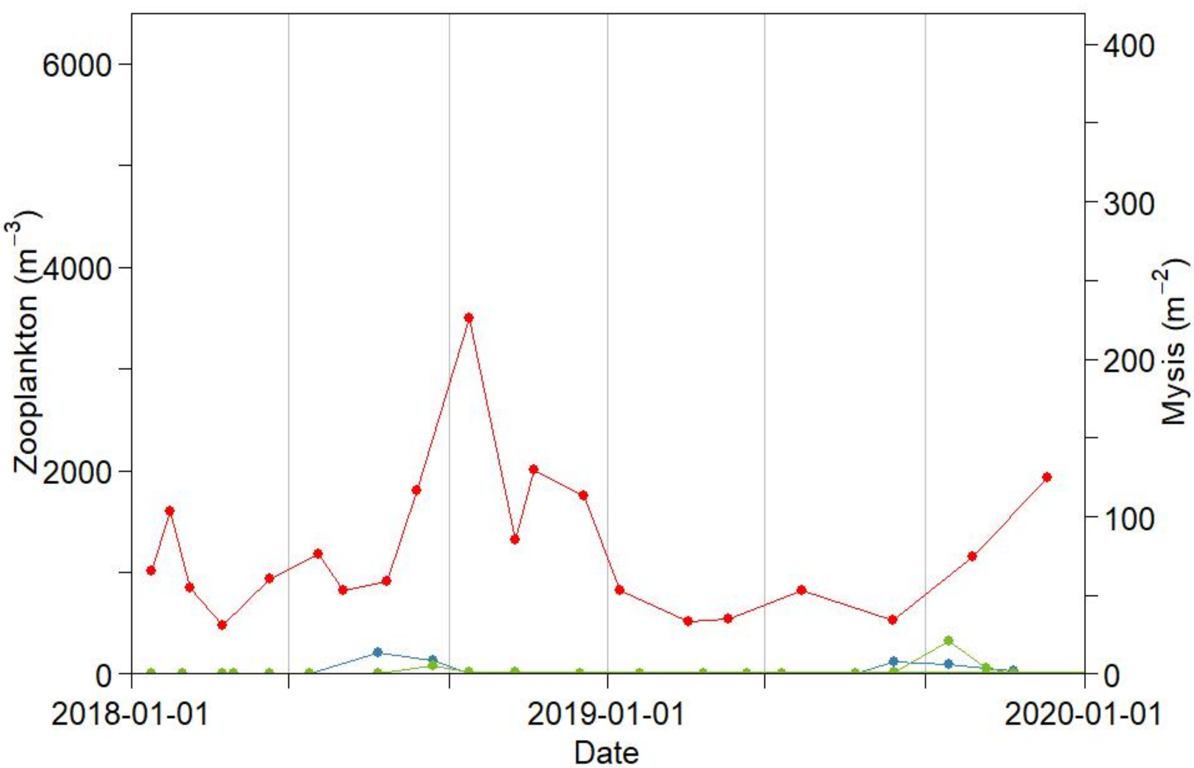

Fig. 4.2: *Mysis* shrimp and Zooplankton abundance in Emerald Bay. *Mysis* shrimp abundance in number of individuals per square meter shown as red circle, *Daphnia spp.* and *Bosmina spp.* abundance in number of individuals per cubic meter are shown in blue and green circles, respectively. *Mysis* abundance can be converted to a volumetric basis by dividing by the sampling depth of 60 m.

noteworthy that with the relatively low *Mysis* abundance in summer 2018, there was an increase in both *Daphnia* and *Bosmina* abundances. Their abundance declined again, however, when *Mysis* numbers exceeded 200 ind./m^2. More significant for this project, in 2019 while the limited trawling efforts were underway, *Mysis* abundance fell to its lowest level (below 40 ind./m^2, but above the target of 27 ind./m^2), and *Daphnia* and *Bosmina* abundances again increased. As shown later in the report (**Fig. 4.36**), both these periods of *Daphnia* increase were associated with increases of Secchi depth.

The percent of female *Mysis* that are reproductive in both Emerald Bay and in Lake Tahoe are shown in **Fig. 4.3**. In the fall and winter, almost 80% of female *Mysis* in Emerald Bay are reproductive as compared to only 29% at the LTP station in Lake Tahoe. This is believed to be

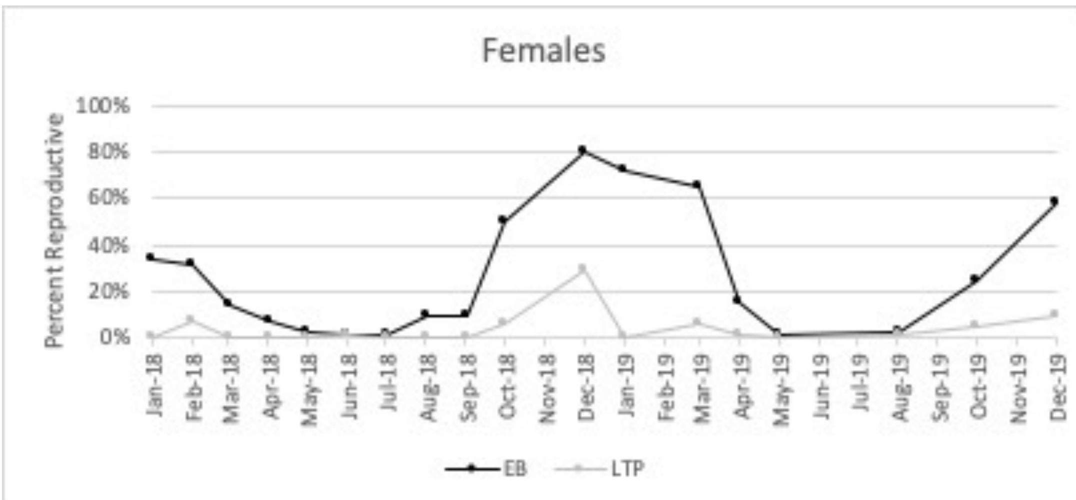

Fig. 4.3: Percentage of female *Mysis* population that is reproductive.

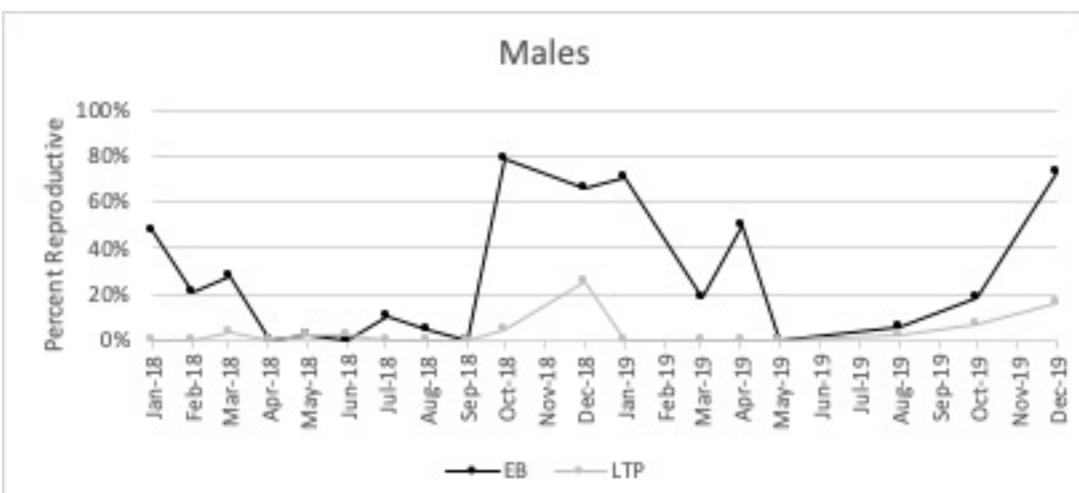

Fig. 4.4: Percentage of male *Mysis* population that is reproductive.

due to the 1-2 year *Mysis* life cycle in Emerald Bay compared to the 3-4 year life cycle within Tahoe (Morgan 1981). This may be advantageous to trawling in Emerald Bay, as many reproductive females may be removed in the fall before they release their broods in the March to April time period. The percent of males that are reproductive is shown in **Fig. 4.4**. Similar to females, almost 80% of males are reproductive in Emerald Bay in the fall as compared to only 25% of males at the LTP station in Lake Tahoe.

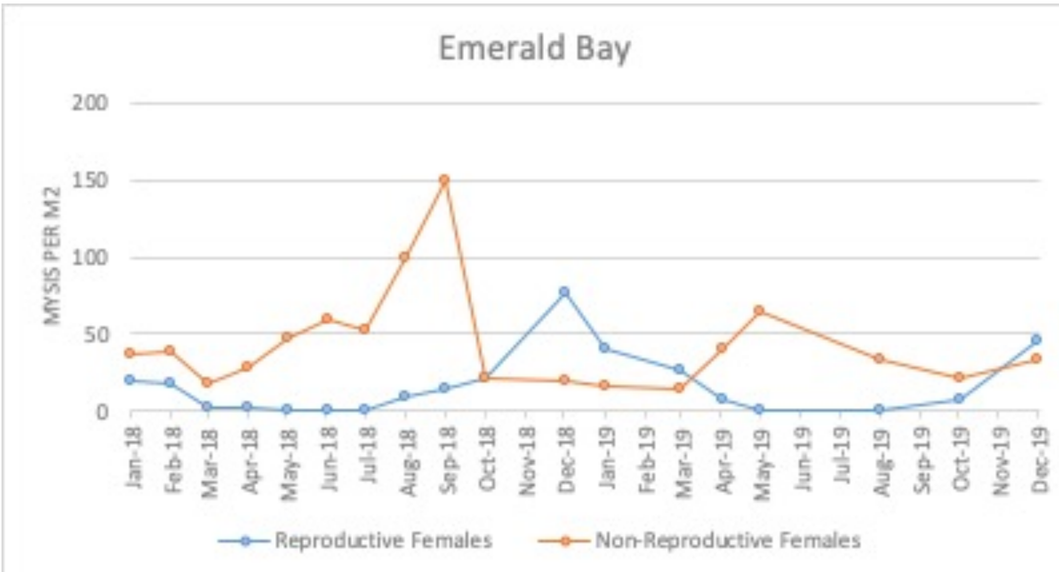

Fig. 4.5: Reproductive vs non-reproductive *Mysis* females in Emerald Bay.

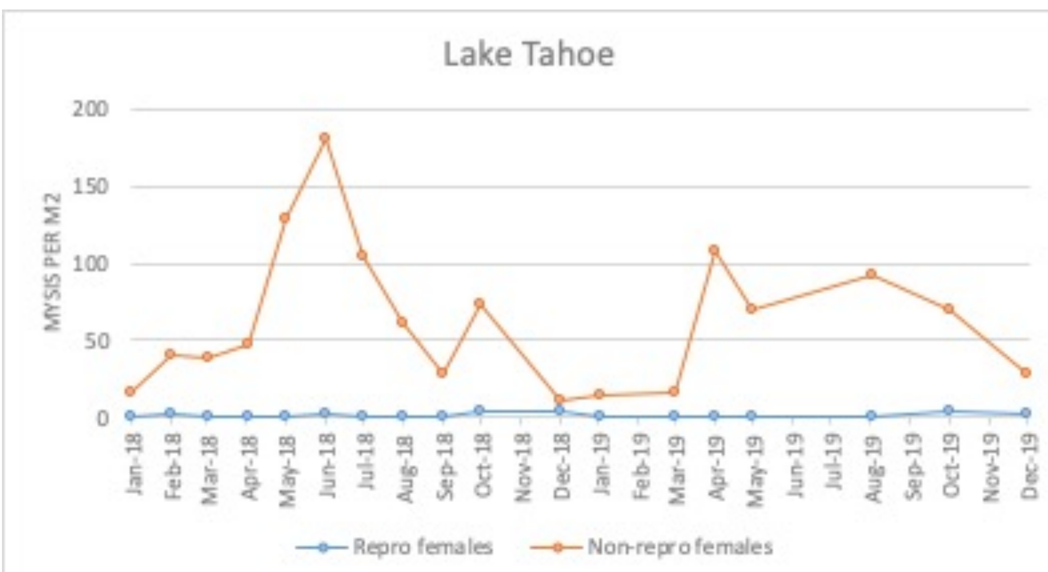

Fig. 4.6: Reproductive vs non-reproductive *Mysis* females in Lake Tahoe.

The density of reproductive and non-reproductive *Mysis* females in Emerald Bay is shown in **Fig. 4.5**. Most females become reproductive in the fall. The equivalent plot for Lake Tahoe is shown in **Fig. 4.6**. The patterns for reproductive males in both Emerald Bay and Lake Tahoe show similar distributions.

The fecundity of *Mysis* is an important measure of population stability and its ability to rebound following perturbation. This is especially true in water bodies like Emerald Bay where the life cycle is only one year for the majority of the population. A year of poor recruitment can dramatically alter overall *Mysis* abundance while high survivorship through egg development can replenish a depleted population with juvenile life stages.

Mature females begin carrying eggs in their brood pouch shortly after breeding. The first appearance of egg carrying females occurs in late September with both Emerald Bay and Tahoe *Mysis* having an average of 12.6-13.2 eggs per female in 2018 and 2019 (**Tables 4.2 and 4.3**). Brood size has been strongly correlated to *Mysis* length (Morgan 1981, Johannsson 2009). Both

Table 4.2: *Mysis* brood size data for Emerald Bay and Lake Tahoe in 2018.

Emerald Bay 2018						
Brood Stage	Average Brood Size	N =	Min	Max	Std. Dev. Sample	Std. Err
Eggs	12.57	63	9	17	1.87	0.24
S1	11.91	35	7	17	2.12	0.36
S2	14.11	9	8	18	3.18	1.06
S3	12.33	3	8	16	4.04	2.33
S5	12.86	14	8	17	2.80	0.75
S6	14	1	14	14	-	-

LTP 2018						
Brood Stage	Average Brood Size	N =	Min	Max	Std. Dev. Sample	Std. Err
Eggs	12.60	5	8	18	3.65	1.63
S1		0				
S2	7	1	7	7	-	-
S3		0				
S5		0				
S6	13	1	13	13	-	-

Emerald Bay and Lake Tahoe females carry the same number of eggs, although in Emerald bay this happens for all females on an annual basis.

Emerald Berrill (1969) described the six observable stages of development within the brood pouch before release of juvenile *Mysis* to the water column in spring. We tracked the development from eggs through to brood pouch juveniles to establish if mortality prior to release was a limitation to free swimming juvenile recruitment (**Figs. 4.7 – 4.12**). We were not able to image stage 4 of development as the transition from stage 3 to stage 5 happened more quickly than our sampling schedule allowed. This was also experienced by Morgan (1980) who carried out a biweekly sampling regime. The low number of egg-carrying females captured at the LTP site in Tahoe (as only one quarter of the females are carrying eggs each year) limited our ability to observe all developmental stages. It is possible ripe females move further offshore and thus were not captured in our sample.

Table 4.3: *Mysis* brood size data for Emerald Bay and Lake Tahoe in 2019.

Emerald Bay 2019						
Brood Stage	Average Brood Size	N =	Min	Max	Std. Dev. Sample	Std. Err
Eggs	13.19	37	7	21	3.05	0.50
S1	12.73	37	8	19	2.50	0.41
S2	11.29	17	9	14	1.49	0.36
S3	15.00	2	12	18	4.24	3.00
S5	11.42	12	8	16	2.19	0.63
S6	12.00	4	9	17	3.56	1.78

LTP 2019						
Brood Stage	Average Brood Size	N =	Min	Max	Std. Dev. Sample	Std. Err
Eggs	12.6	5	8	20	4.56	2.04
S1		0				
S2		0				
S3		0				
S5		0				
S6		0				

In Emerald Bay, there was no statistically significant mortality recorded from the onset of egg formation to juvenile release from the brood pouch. This has implications for population management as next year's class of *Mysis* can be severely depleted by harvesting mature females up until the time they release their young which is completed by the end of April.

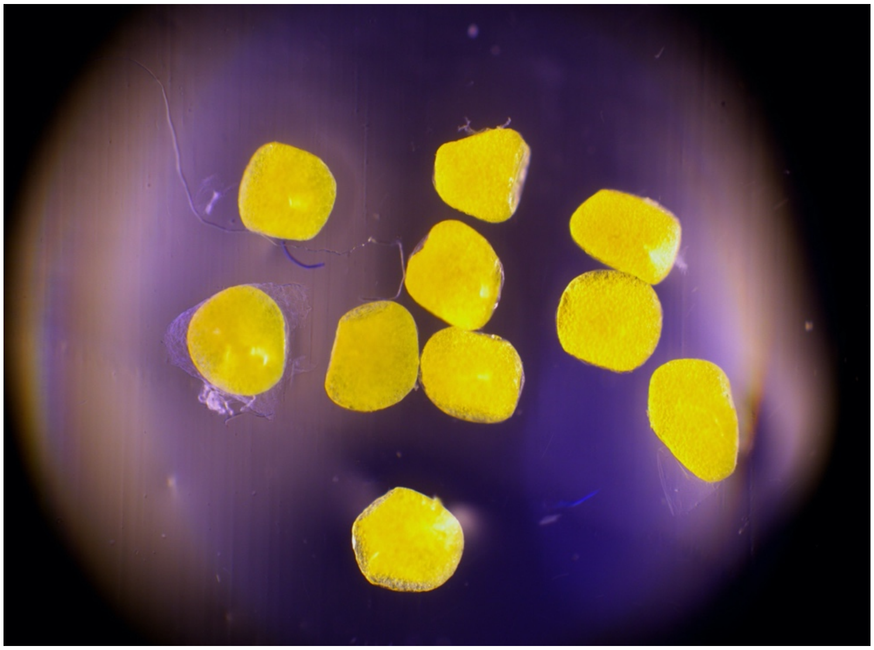

Fig. 4.7: *Mysis* eggs just after appearance in brood pouch.

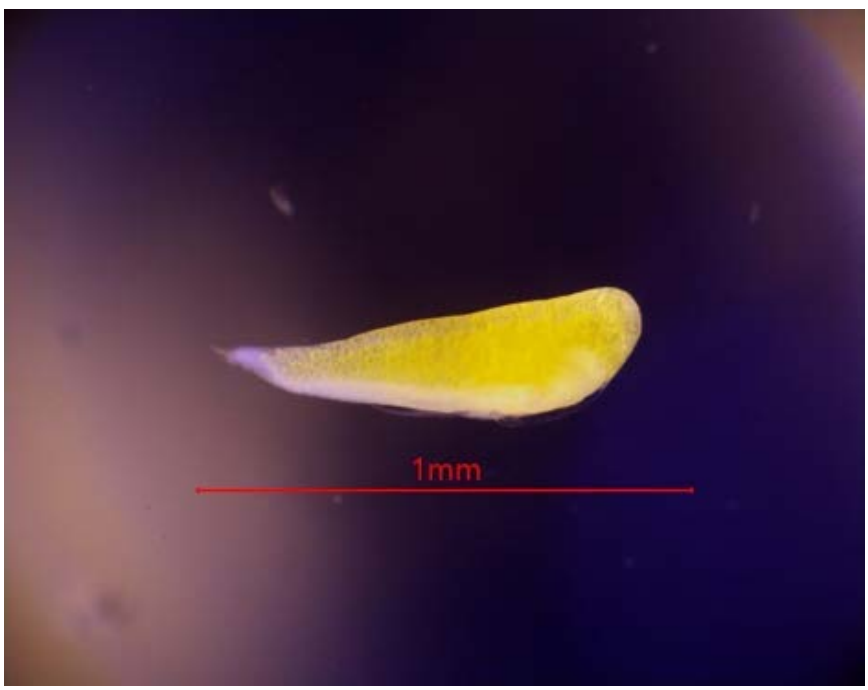

Fig. 4.8: Stage 1 of *Mysis* development within brood pouch.

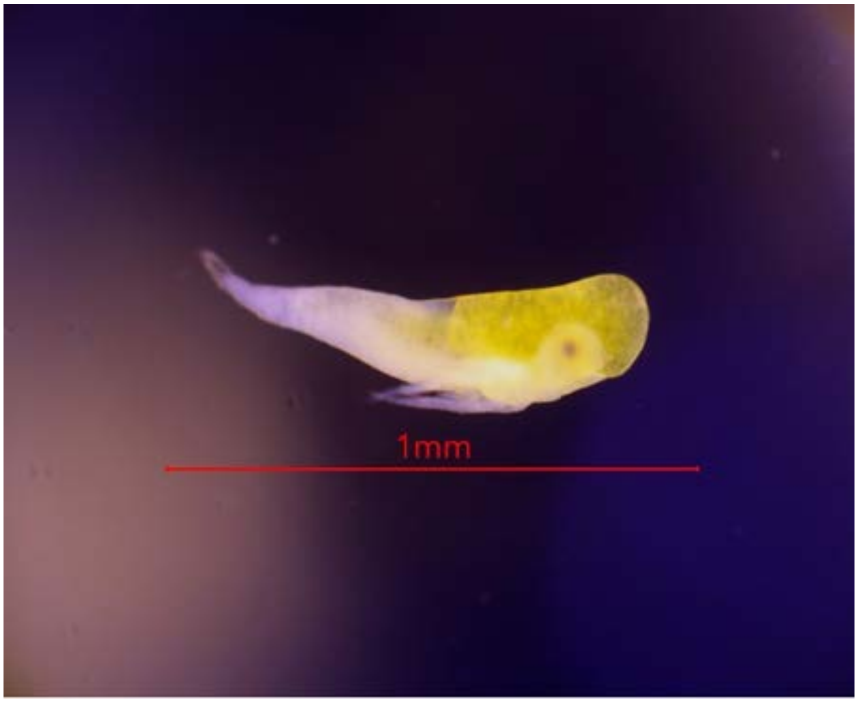

Fig. 4.9: Stage 2 of *Mysis* development within brood pouch.

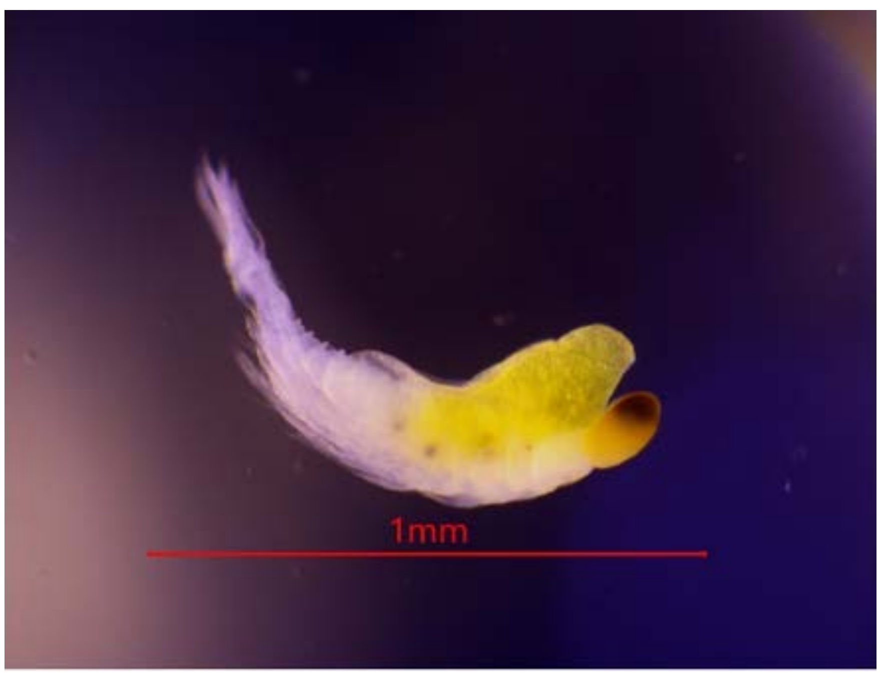

Fig. 4.10: Stage 3 of *Mysis* development within brood pouch.

Fig. 4.11: Stage 5 of *Mysis* development within brood pouch.

Fig. 4.12: Stage 6 of *Mysis* development within brood pouch.

The high success rate of egg development to free swimming juvenile *Mysis* implies there is significant natural mortality during the first four months of life. Otherwise, the Emerald Bay population would be expected to swell six-fold year over year based on 2018 reproduction. As a point of comparison, McCoy (2015) estimated the annual survival rate of age-0 *Mysis* was

17.7%, essentially the same result. This study did not evaluate juvenile survival outside the brood pouch, but adult *Mysis* have been known to cannibalize their young (Nordin 2008). Perhaps the rapid rebound of Emerald Bay *Mysis* in 2014 was partially the result of high juvenile survivorship to adulthood in the absence of 1+ year old adults in the water body.

Nine additional *Mysis* monitoring sites were added in Emerald Bay across various depths in November 2018 (**Fig. 4.13**) to provide ground truth data for the Biosonics survey validation, data on seasonal distribution and movement of *Mysis* throughout the bay, and as monitoring stations to show potential impacts of trawling efforts.

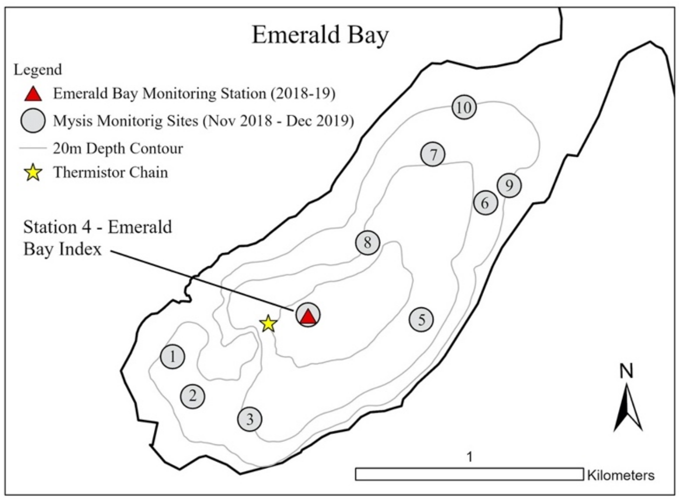

Fig. 4.13: Nine additional sampling stations established in Emerald Bay in late 2018 to monitor seasonal movement of *Mysis* at various life stages throughout the bay. Station 4 is the long-term monitoring station for Emerald Bay maintained since 2011.

The data showed that *Mysis* abundance peaks at shallow sites in the summer when young-of-the-year (YOY) move to the shallows to escape predation. Abundance peaks at deep sites in the fall when adults move to center of the bay to reproduce (**Fig. 4.14**). This confirms Morgan and Threlkeld (1982) findings for size dependent horizontal migration within the bay. It also suggests the trawling strategy within the bay could be modified for seasonal changes in order to get greatest Catch-Per-Unit-Effort (CPE), as discussed below.

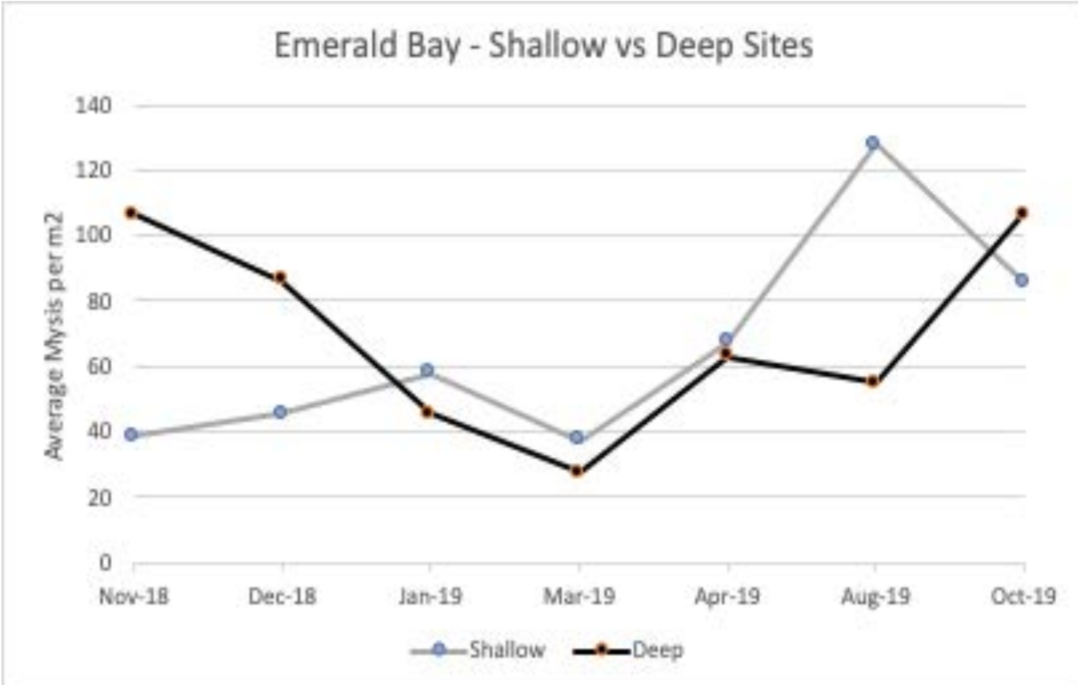

Fig. 4.14: Average *Mysis* density at shallow sites (depth <30m, n = 4) and deep sites (depth >30m, n = 6).

A significant shift in the zooplankton community in Emerald Bay was observed during this period. It is possible that the modest efforts in *Mysis* removal in Emerald Bay since 2018 impacted the zooplankton distributions, however, its context in the longer term is uncertain as the measurement time frame was so short, and it followed a period of major perturbation. The zooplankton in Emerald Bay used to be rotifer-dominated during the winter and copepod-dominated in summer, but since 2017 the rotifer community has abruptly decreased to a level of 17% of total zooplankton (**Fig. 4.15**). The abundance of *Daphnia* was typically less than *Bosmina*, but in September 2019 the *Daphnia* peak was almost the same as *Bosmina* had been in 2017. Note, however, these peaks are all far smaller (by a factor of 20 or more) than the peaks recorded during 2013-15 (**Fig. 3.1**).

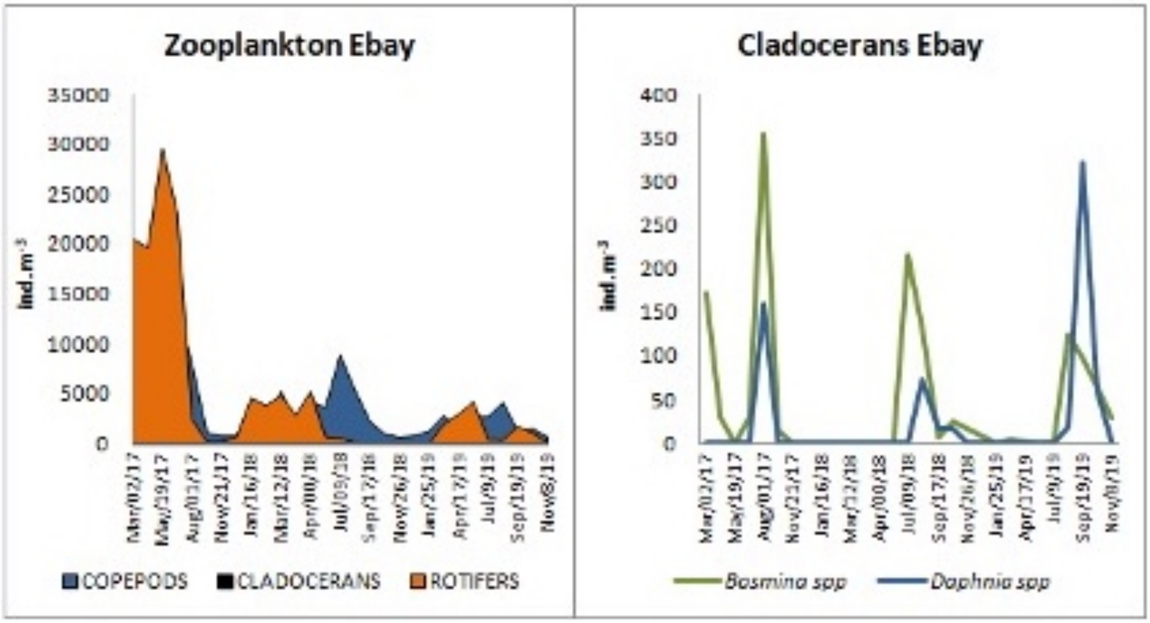

Fig. 4.15: Zooplankton density in Emerald Bay, 2017-2019.

4.1.3 Lake Tahoe Results

Similar to the result of Emerald Bay in the previous section, *Mysis* were present year-round at the LTP site of Lake Tahoe, and *Daphnia* and *Bosmina* were largely absent (mean 0.3 and 70 ind./m^3, respectively; **Fig. 4.16**). *Bosmina* abundance in Emerald Bay and the Lake Tahoe were similar in the same period (**Fig. 4.17**), but most noticeable was that the population peaked earlier in Emerald Bay (July – September) than in Lake Tahoe (September – November). Note that the zooplankton abundance numbers for 2017 were not available at the time of Report preparation. Samples were collected and archived for future identification and enumeration.

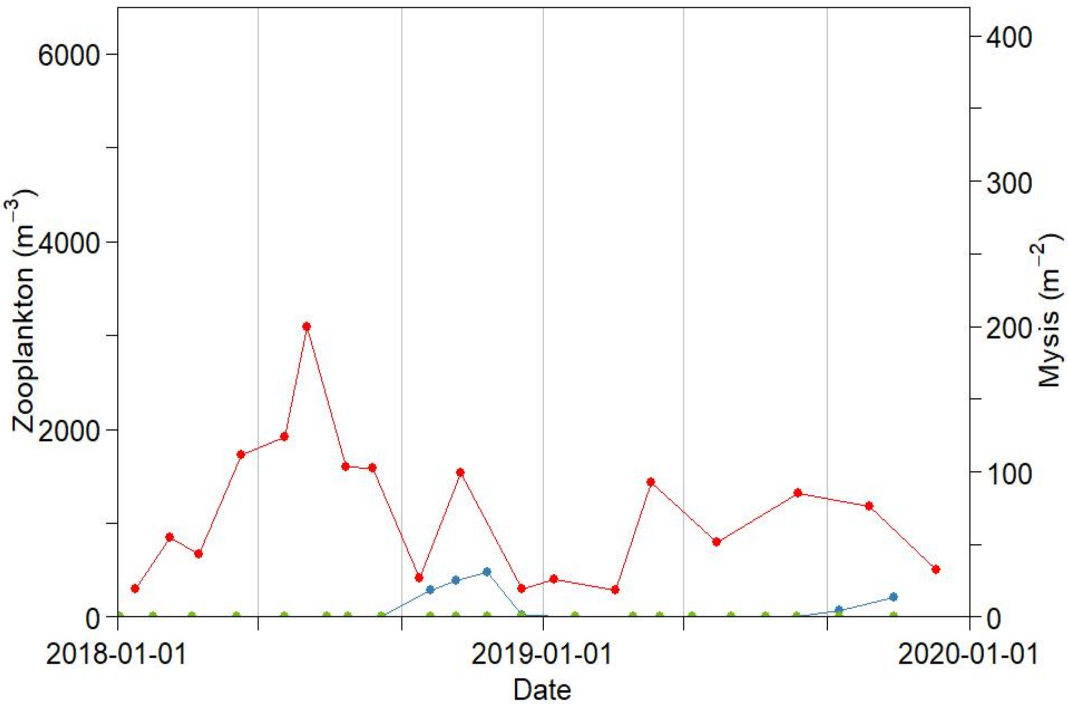

Fig. 4.16: *Mysis* shrimp and zooplankton abundance at Index station (LTP) in Lake Tahoe. *Mysis* shrimp abundance in number of individuals per square meter shown by red circles, *Daphnia* and *Bosmina* abundance in number of individuals per cubic meter are shown in blue and green circles, respectively. *Mysis* abundance can be converted to a volumetric basis by dividing by the sampling depth of 100 m.

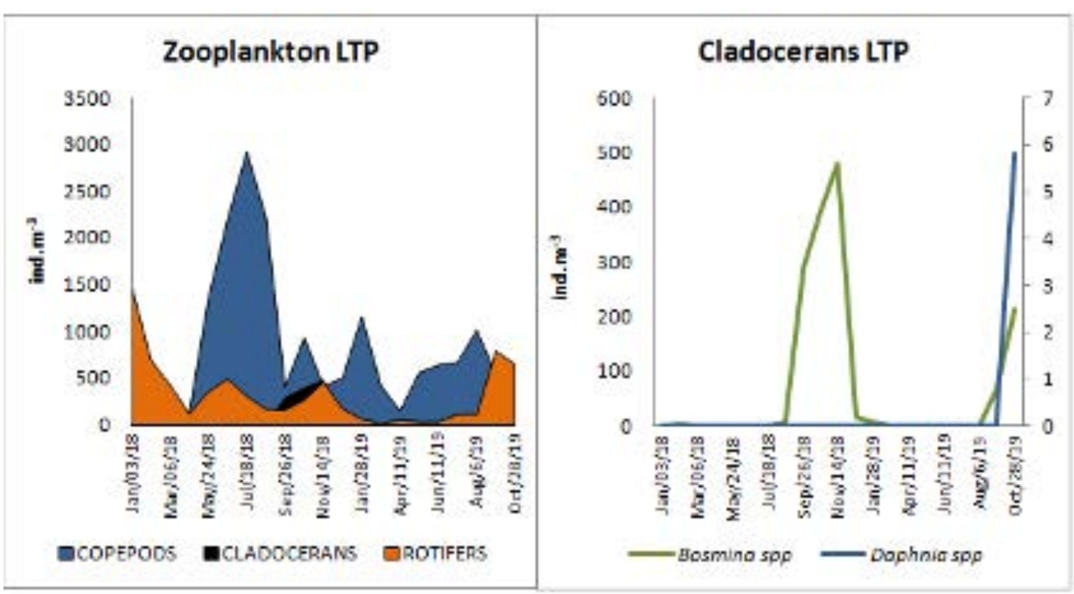

Fig. 4.17: Zooplankton abundance at the Index Station (LTP), 2018-2019.

4.2 Biosonics Echosounder Surveys

4.2.1 Methods

The Biosonics DT-X Extreme Split-beam Echosounder was attached to the research vessel using a detachable pole mounted configuration. The sonar head was lowered beneath the depth of the hull to ensure minimal wake interference (e.g. bubble generation). The Echosounder transmits/receives acoustic signals at 75 and 200 kHz (**Fig. 4.18**). Using the default configuration for the pulse duration (0.4 ms) and sampling rate (41,667 Hz), the instrument was able to resolve 1.72 cm vertical bins directly underneath the sampling vessel. A known limitation of Echosounders is their narrow cone angle (approx. 8 degrees), which means they require a great many passes to achieve complete spatial coverage. In light of this, they are generally used along specific survey lines and a map is produced by interpolation between survey lines. The acoustic "returns" from the instrument were calibrated against individual *Mysis* net tows in order to generate an acoustic algorithm that related *Mysis* density measurements (from tows) to the acoustic return being measured by this instrument. In this way, data can be extracted from the

Fig. 4.18: Biosonics DT-X Extreme Split-beam Echosounder being attached to the R/V Bob Richards (February 2018).

Biosonics equipment to estimate the areal density of *Mysis* with far greater resolution and confidence than is possible with tow data alone.

Bioacoustic surveys were predominantly conducted at night (in coordination with net sampling or trawling activities). Additional continuous measurements were collected for 1-2 days (attached to a surface buoy). Duplicate testing was also conducted during the day to ensure we weren't acquiring false returns from below the thermocline. The DT-X Autonomous Portable Scientific Echosounder operated with a 70 / 200 kHz split frequency. The detection of the *Mysis* shrimp was conducted with the 200 kHz system. The 70 kHz system was used to mask the fish returns. All of the settings that were used were the default settings.

Biosonics, the instrument manufacturer, has software packages for both acquiring (*Visual Acquisition 6*) and processing (*Visual Analyzer 6*) the data being generated. *Visual Acquisition 6* was used to collect the data and merge it with the data from DGPS (Digital GPS) positioning system that was also attached to the boat. *Visual Analyzer 6* was not used as it was tuned to the detection of larger organisms (e.g. fish) rather than *Mysis*. Instead, the merged acoustic and GPS data were exported to Matlab where new processing scripts were generated following the methodologies of Gal et al. (1999a), Gal et al. (1999b) and Rudstam et al. (2008). A flow chart of the process involved is provided in **Fig. 4.19**.

There are two different flow paths to the analysis with both cases being initiated by the acquisition of the raw data using the Biosonics equipment: (1) the calibration of the instrument (left hand path); and (2) the analysis of the data (right hand path). As conditions change regularly, it is important to undertake calibrations as frequently as possible. Once a calibration has been obtained, it is input into the *Mysis* density calculations to process the survey data in turn.

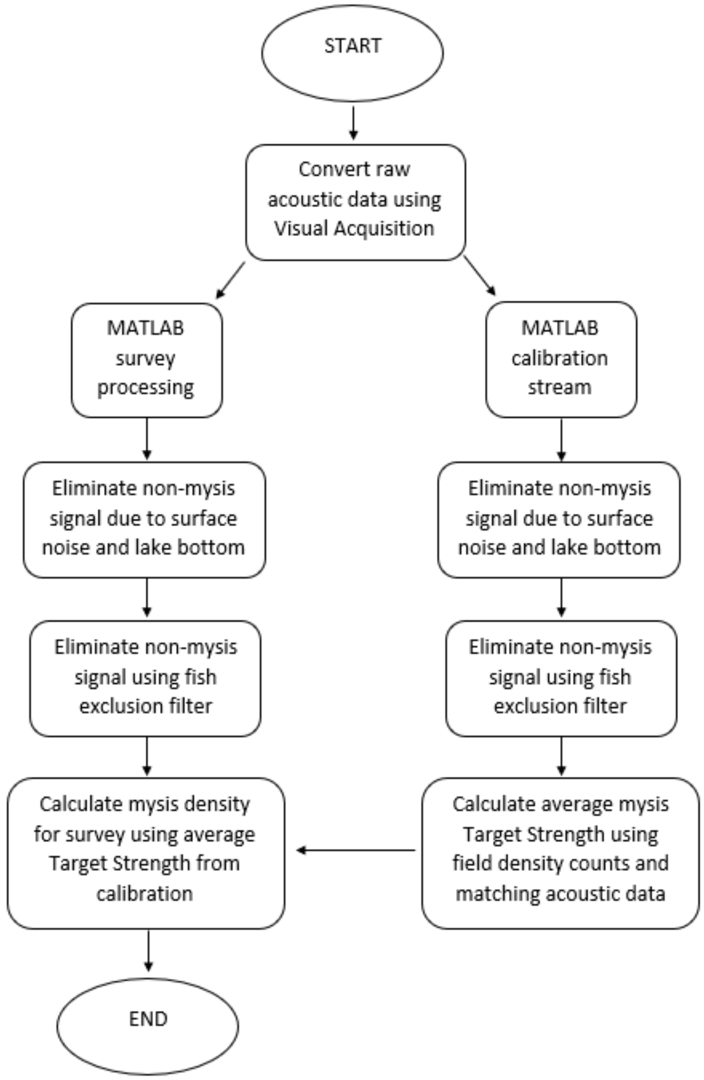

Fig. 4.19: Processing flow chart for analysis of the raw datafiles collected using the Biosonics sonar.

The two biggest challenges in the data pre-processing were: (1) to limit the non-*Mysis* return signals (i.e. waves or bottom returns) and (2) to eliminate single echo detections of large species (i.e. fish). To address this first challenge for Emerald Bay, the thermocline depth (defined in this study as the maximum gradient in the vertical temperature profile) and the lake bottom were defined as the upper and lower boundaries of the *Mysis* location, respectively. To address the second challenge, single echo detections of large species were used to build a masking filter to remove returns from fish and from the surrounding pixels. These spurious signals are quite evident in the data, as fish air bladders have a much stronger acoustic return signal (e.g. < -45 dB in acoustic amplitude) than the *Mysis* which have a typical amplitude return of -82 to -90 dB. While it's accepted that we might have mistook larval stages of fish for *Mysis*, we tried to eliminate the larger fish that were very noticeable in the echograms in the mid-water column. For this, -60 dB worked very well and was a value that came from other work (Rudstam et al. 2008).

Calibration consisted of deploying a tungsten carbide sphere for acoustic collection to compare collected signals with those expected according to specifications from the manufacturer. A calibration was conducted in September 2019 which found a slight correction factor of 0.3 dB for the 200 kHz transducer. This factor was incorporated into future sonar collections and was used to correct previously collected data.

The principal processing of the data involved the generation of an average target strength for *Mysis*. This was accomplished by conducting vertical net trawls with matched echo sounding data. The calibration points that were used for this portion of the study were collected on April 3, 2019 and October 7, 2019 (the September calibration was discounted because only one transducer was able to be corrected). The results of these regressions of field density (density of individuals per square meter – **Fig. 4.20a**) versus prediction density (density of individuals predicted from this model – **Fig. 4.20b**). This is more difficult that it would seem, as *Mysis*

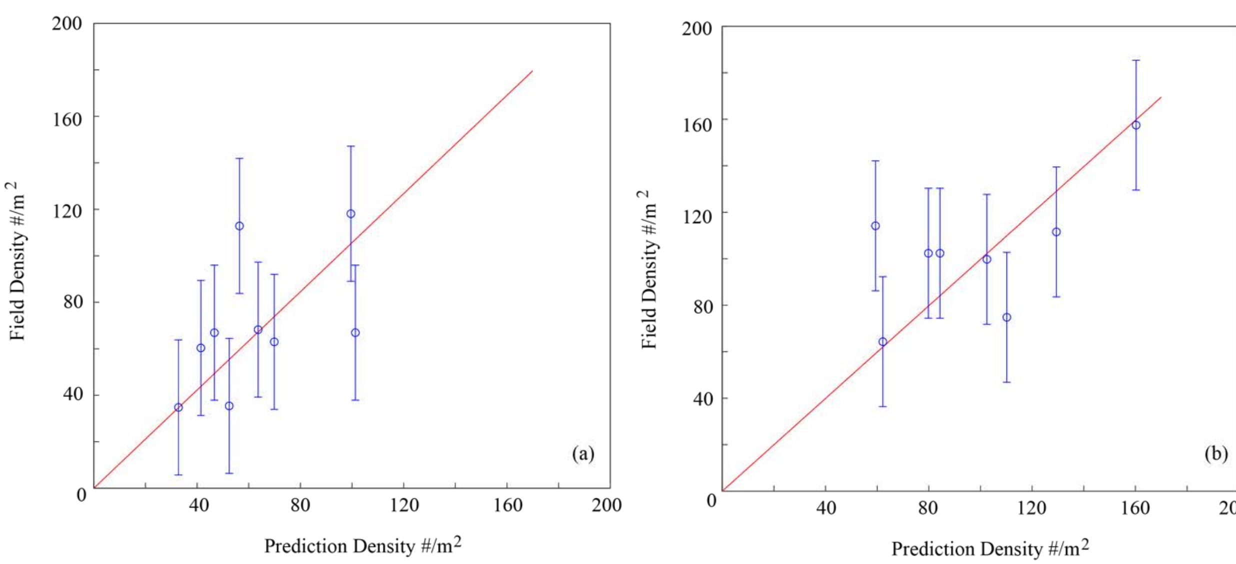

Fig. 4.20: Regression of observed Field Density vs. Prediction Density for: (a) April 3, 2019 and (b) October 7, 2019. Units for both axes are # of individuals per square meter.

populations have significant seasonality in both size class and location. Therefore, it was found necessary to estimate the average target strength as often as the data are available. In the future, it is strongly recommended that paired acoustic and net sampling should be conducted quarterly at a minimum.

Once the average target strength for *Mysis* has been estimated, *Mysis* population volume density for transects can be calculated using **Eq. 4.1**:

$$\rho_v = \frac{s_v}{\sigma_{bs}} \qquad \textbf{Eq. 4.1}$$

Here s_v is volume backscattering coefficient (collected with the Echosounder), σ_{bs} is the backscattering cross-section derived from target strength, and ρ_v is the population density in units of # of individuals/m^3. Areal density in units of # of individuals m^{-2} (the traditional manner in which *Mysis* numbers are reported) is calculated from the depth of *Mysis* layer multiplied by volume density and summed over the depth of the water column.

Mysis density processing was conducted as a two-stage process. In the first stage, acoustic algorithm calibration is conducted to determine average target strength of *Mysis*. This is necessary to use collected acoustic data to determine population density of *Mysis* in the survey region. Calibration is conducted using short sonar ‘snapshots’ and matched field collections to calculate the back scattering cross section σ_{bs} which is related to target strength. Back scattering cross section can be calculated by rearranging $\sigma_{bs} = \frac{s_v}{\rho_v}$ using the field collection density. Back scatter cross section was calculated for each site surveyed and then averaged to determine a mean back scatter. This average *Mysis* target strength value was then used to determine population density and position on full scale surveys. This method was used in previous acoustic survey studies (Rudstam et al. 2008). The calibration found *Mysis* target strengths of -84.16 dB for October 2019 and -89.55 dB for April 2019 both for Emerald Bay. A value of -88.97 dB was found for December 2019 in greater Lake Tahoe. These slight differences point to the importance of performing period calibrations although they are a relatively constant.

Each data set had to be filtered in several ways to eliminate non-*Mysis* sonar returns. This was done by removing non-*Mysis* signals from the bottom and top of the lake, by removing signals beyond a maximum range of 150 m after which interference effects are increased and the coherence of the returned acoustic signal diminished in quality, and by removing signals too large to be *Mysis* using a filter threshold of -60 dB (Rudstam et al. 2008). After this filtering was conducted a beam angle correction was applied to account for the effect of the spreading acoustic wave. The remaining signal was processed via echo integration using the average *Mysis* target strength calculated previously to determine *Mysis* aerial density and position.

Data collected from August 12, 2019 until the end of the study were processed with the use of a fish exclusion filter which removed back scatter returns of individual aquatic objects above a threshold of -60 dB. This approach was used by others in previous sonar survey analysis to remove fish (e.g. Rudstam 2008). Data prior to August 12, 2019 was processed by removal of all backscatter returns above a signal strength of -60 dB. Single object detections were not available for the earlier data as a result of the software settings used at that time. This was estimated to be suitable as test datasets from later in 2019 using both approaches produced similar results in agreement within 10-15%.

4.2.2 Emerald Bay Results

Mysis undergo substantial vertical migration on a daily cycle. In order to understand how quickly this was taking place (i.e., understanding when surveys could be conducted), a series of short stationary soundings were collected on different nights in both Emerald Bay and Lake Tahoe. An example of one of these surveys (from Lake Tahoe) is shown in **Fig. 4.21.** These data were acquired on March 28, 2018. The typical measured ascent rate at the beginning of the evening was 0.9 m/min (2.9 ft/min) and a typical descent rate of -0.8 m/min (-2.7 ft/min). Using a depth of 50 m (165 ft) for scaling purposes, it would take roughly one hour for the full migration to the upper waters to happen in Emerald Bay (maximum depth 68 m). Therefore, for survey purposes, surveys with the Biosonics were conducted at a minimum of one hour after sunset and before sunrise.

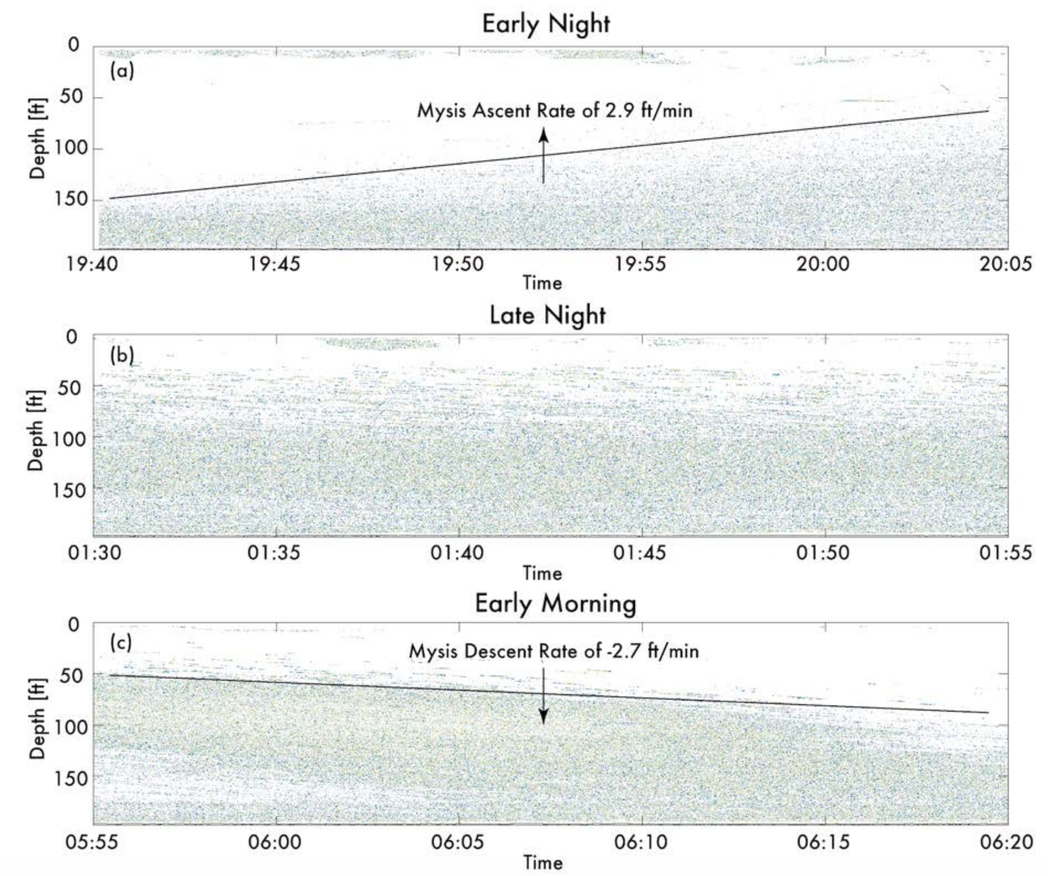

Fig. 4.21: Measured vertical migration rates of *Mysis* for a typical night (March 28, 2018) at (a) the beginning of the night, (b) late night, and (c) early morning as they start undergoing their descent. Data are from Lake Tahoe.

Throughout the study, this nightly migration exhibited different behavior in both the ascent and descent phase. Looking closely at **Fig. 4.21a**, it is noteworthy how the individual *Mysis* appear to be rising in a relatively diffuse cloud. Later in the night (**Fig. 4.21b**), they tend to congregate in a discrete layer within the water column. As this was in March, with no strong thermocline present, this layering is still relatively diffuse. As will be shown in subsequent sections, stronger thermoclines will result in this layering becoming more distinct. During the descent phase, this layer remained relatively intact (**Fig. 4.21c**). In terms of planning harvest techniques, it is important to factor in both the daily and seasonal cycles. The discrete layer that they congregate in appears to be just below the thermocline when present and was a relative constant behavior even as the thermocline varied in depth through the seasons. At those times of the year when the thermocline isn't present (e.g. mid-winter/early spring) this thermal control won't exert any influence on *Mysis* behavior.

Evidence of this from Emerald Bay can be seen in **Fig. 4.22** collected on August 13, 2018. One thing to note here and in subsequent 2D curtain plots is that the data are being displayed as a function of 'Ping' number. As the sonar system samples once per second (1 Hz), and the vessel was traveling at 3-4 knots while surveying, each ping occurs at approximately 1.5-2 m intervals along the transect. As the speed of the vessel varied, the ping distance was likewise variable. As also evident in **Fig. 4.22**, there are still large numbers of individuals below the depth of the thermocline. This may point to only part of the population migrating vertically each night, or a staggered time of migration. In addition, unlike the main body of Lake Tahoe (McCoy 2015), measured returns showed a band to be shallower in Emerald Bay. It is hypothesized that this shallow band in Emerald Bay results from shallower depths and a sharper thermocline. In the larger lake body, the greater migration length would simply mean the *Mysis* would have more room to roam. In order words, the vertical migration distance in Tahoe would likely create a more diffuse and wider band of *Mysis*. This strongly reinforces the need for "operational" Echosounder surveys as part of any future commercial trawling effort, whereby real-time echosounding data are used to guide trawl operations.

Operational Emerald Bay Echosounder Surveys were conducted throughout the study period concurrent with trawling (this occurred for 30 sampling events in the project period). While these data were critical for helping to refine the trawling methods (e.g., choosing the appropriate depths to trawl) they were not ideal to provide a synoptic view of *Mysis* distribution in Emerald

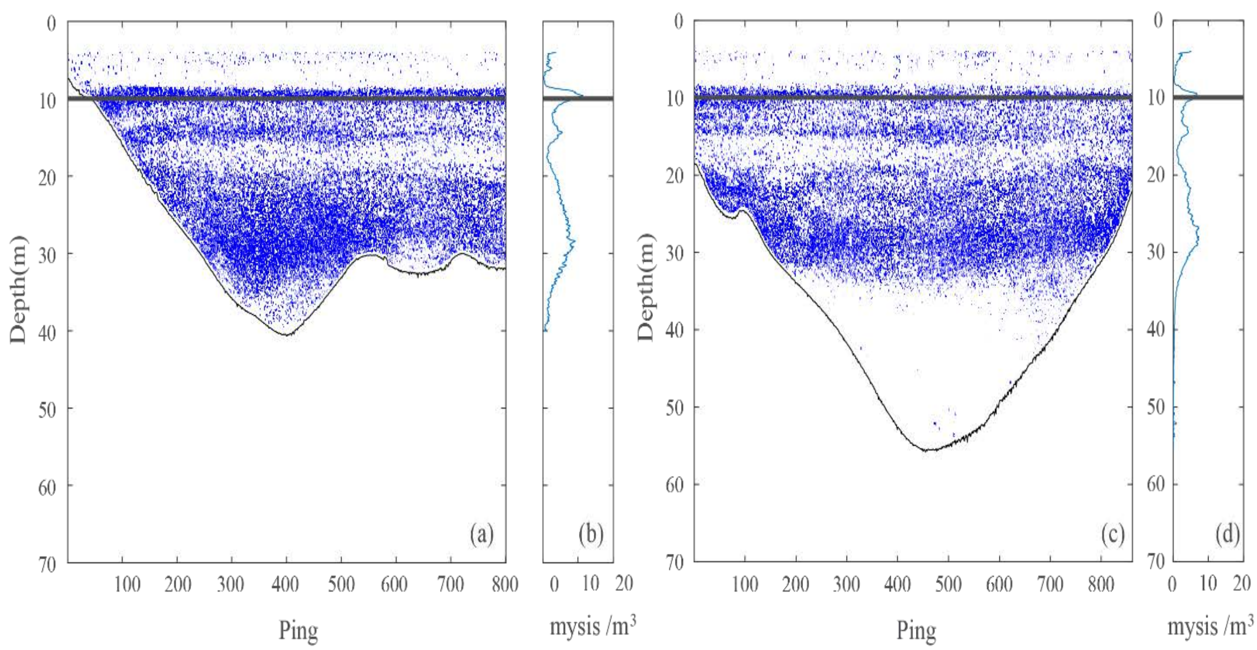

Fig. 4.22: (a) and (c) show two representative transects collected on August 13, 2018 with the depth of the thermocline shown with a solid horizontal line ~10 m depth. (b) and (d) show the vertical profiles of concentration through the deepest section of each transect. While the individuals are bounded at the surface, they spread in a relatively diffuse cloud in the region beneath.

Bay. This was mainly because of the operational constraints of trawling controlled boat operations (boat location, speed and direction). In addition to these sampling events, there were eight days where full synoptic mapping of Emerald Bay occurred: March 19, 2018; June 6, 2018; August 13, 2018; October 23, 2018; March 18, 2019; June 10, 2019; August 12, 2019; and December 9, 2019. For the purposes of this study, synoptic mapping entailed Echosounder surveying of the bay at ~100 m line spacing and then running the data through all of the steps detailed above. Contouring this data, maps of *Mysis* density in the bay were able to be generated (**Fig. 4.23** for 2018 and **Fig. 4.24** for 2019).

What these results generally show is that in March 2018 (**Fig. 4.23a**), the highest density of individuals were located around the littoral edges of the bay and likely corresponds to the presence of juvenile *Mysis*. The *Mysis* covered a greater extent of the central bay later in the season (**Fig. 4.23b**) but then, as the surface water warmed during the summer, they likely found cold water refugia (e.g. Degraeve & Reynolds 1975; Beeton & Bowers 1982; Rudstam et al. 1999) in the center of Emerald Bay and at greater depths (**Fig. 4.23c**) before dying off at the end of the summer season (**Fig. 4.23d**) in line with their 1-2 year life span. This pattern repeated itself the following year (**Fig. 4.24a** through **Fig. 4.24d**).

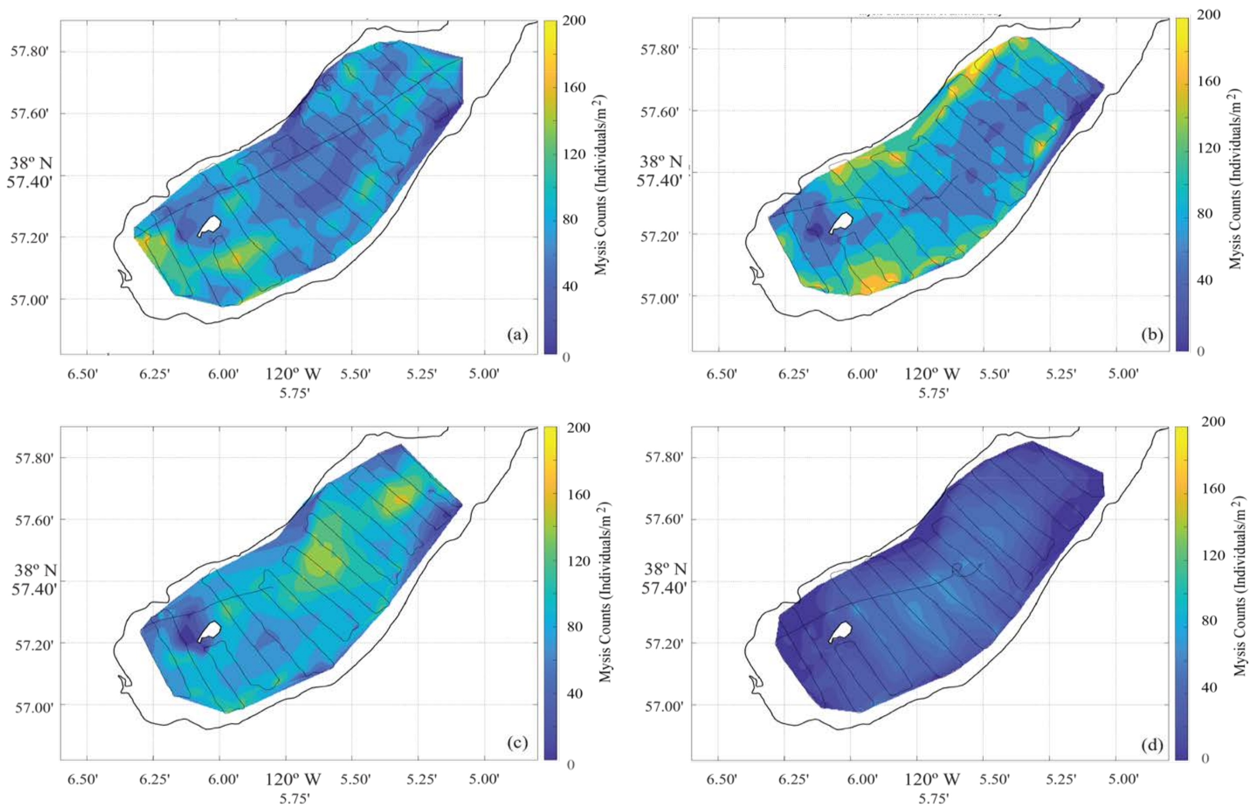

Fig. 4.23: Full synoptic mapping of Emerald Bay on (a) March 19, 2018; (b) June 6, 2018; (c) August 13, 2018; and, (d) October 23, 2018. The range on the color bar is from 0-200 individuals/m^2 in each of the subpanels in order to be consistent throughout.

There was clearly a decline in *Mysis* numbers between the two years, with average *Mysis* density appearing to be reduced from maximum values >160 individuals/m^2 in places to <80 individuals/m^2 in the following year. The extent to which this was due to trawl operations or

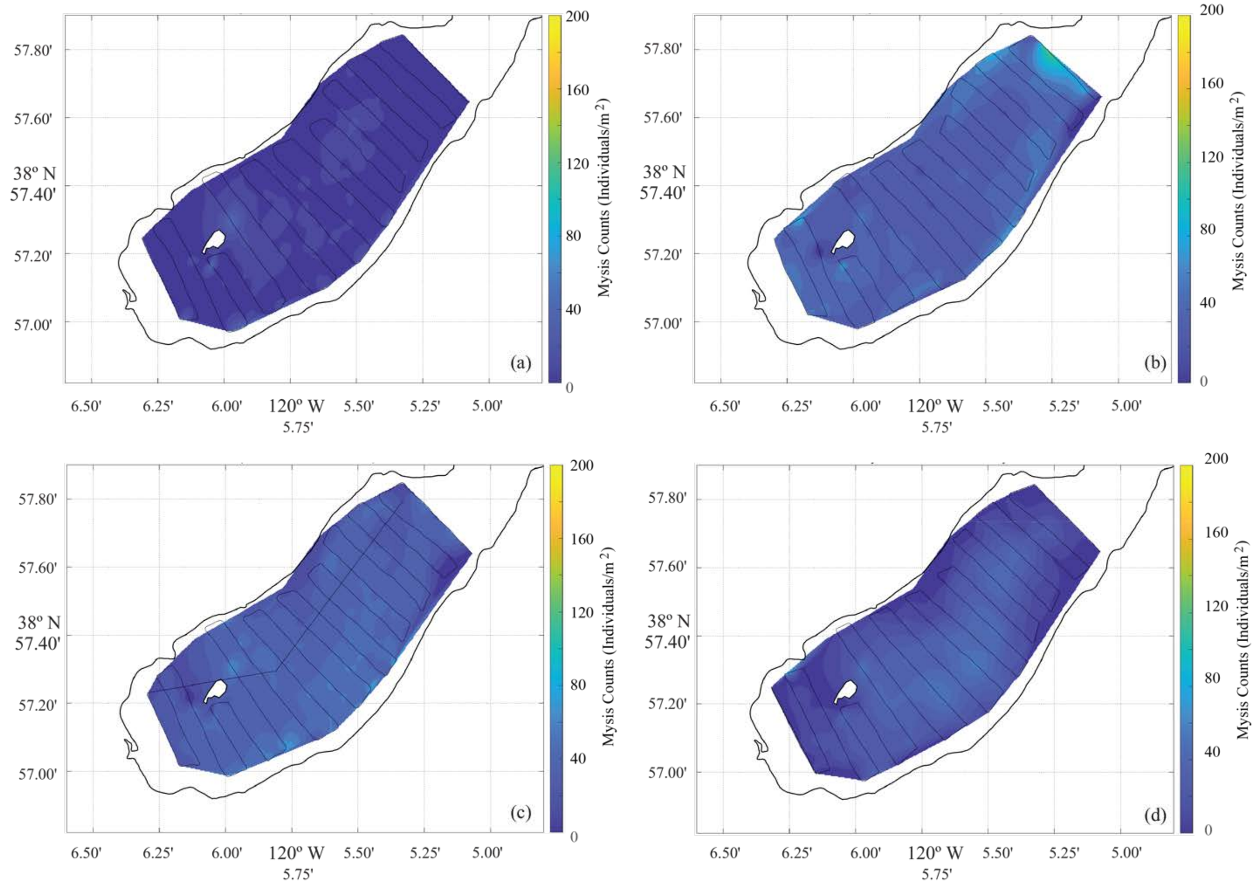

Fig. 4.24: Full synoptic mapping of Emerald Bay on (a) March 18, 2019; (b) June 10, 2019; (c) August 12, 2019; and (d) December 9, 2019. The range on the color bar is from 0-200 individuals/m^2 in each of the subpanels in order to be consistent throughout.

natural inter-annual demographic shifts cannot be ascertained. It does, however, indicate that more comprehensive and frequent synoptic surveys should be considered going forward to help understand these inter-annual trends.

4.2.3 Lake Tahoe Results

Using the same approach with the fish exclusion filter that was developed for Emerald Bay, the Biosonics system was deployed 15 times in Lake Tahoe. Four of those were adjacent to Camp Richardson, two were overnight deployments on fixed buoys, and nine were cross lake transects. An important operational difference for Lake Tahoe compared to Emerald Bay is that the system's vertical range (before the signal to noise ratio deteriorated) was smaller than the depth of the lake.

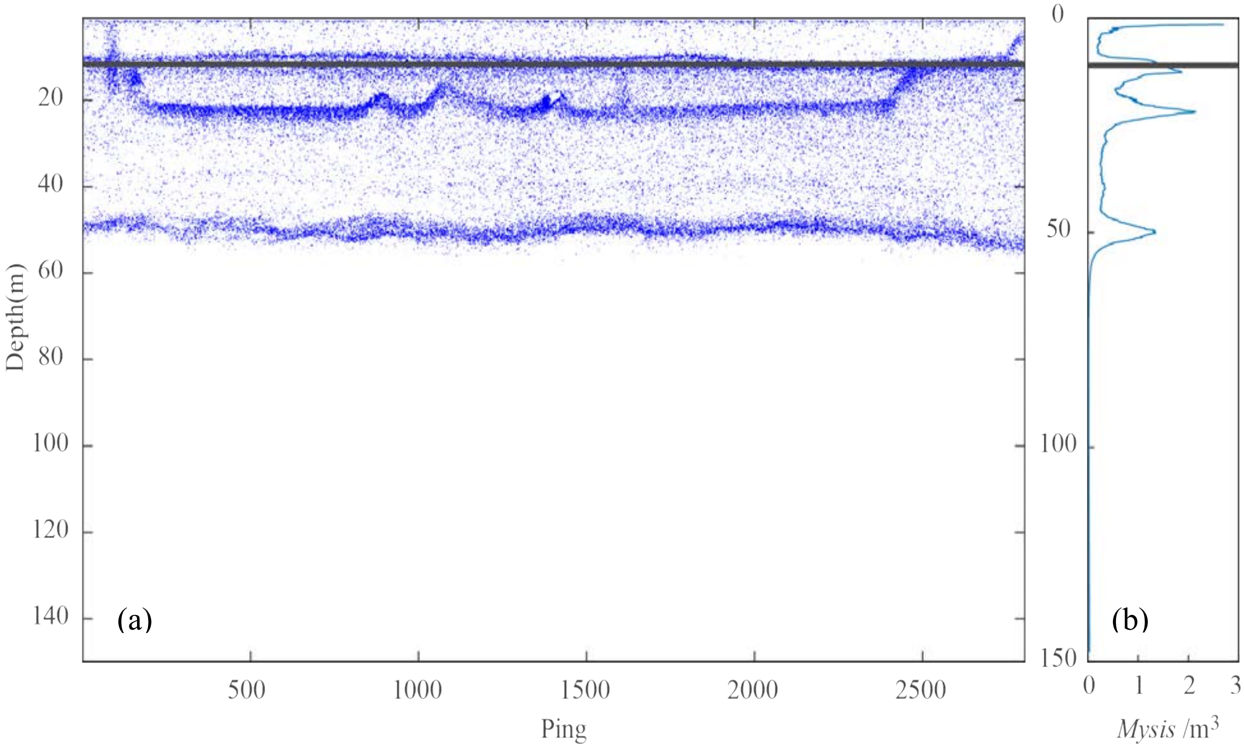

Fig. 4.25: (a) Backscatter return from a single transect on August 27, 2019 in the main body of Lake Tahoe (shown in blue) after removing the surface backscatter return and clipping the data at 150 m (greater than this tends to generate false positives at depth with depth of the thermocline (black horizontal line). (b) Vertical profile of average volumetric density (individuals/m^3) with depth with depth of the thermocline (black horizontal line) indicated. The length of the transect shown is approximately 4 km.

As a result, we only integrated the signal from a depth of 100 m to the depth of the thermocline. *Mysis* were observed to migrate upwards from an unknown depth (although previously estimated to be greater than 100 m; McCoy 2015). In a similar fashion to Emerald Bay, the individual *Mysis* in Lake Tahoe also congregated at the depth of the thermocline (**Fig. 4.25**), although there is evidently greater complexity than thermocline depth alone. Using the thermocline as an upper band, there are a number of continuous bands in the water column below.

Similar behavior was also observed near-shore as well (**Fig. 4.26**). This banding highlights some very important considerations for any future operations. First, it shows that real-time echosounding data need to be a part of trawl operations in order to locate the depth of the trawl

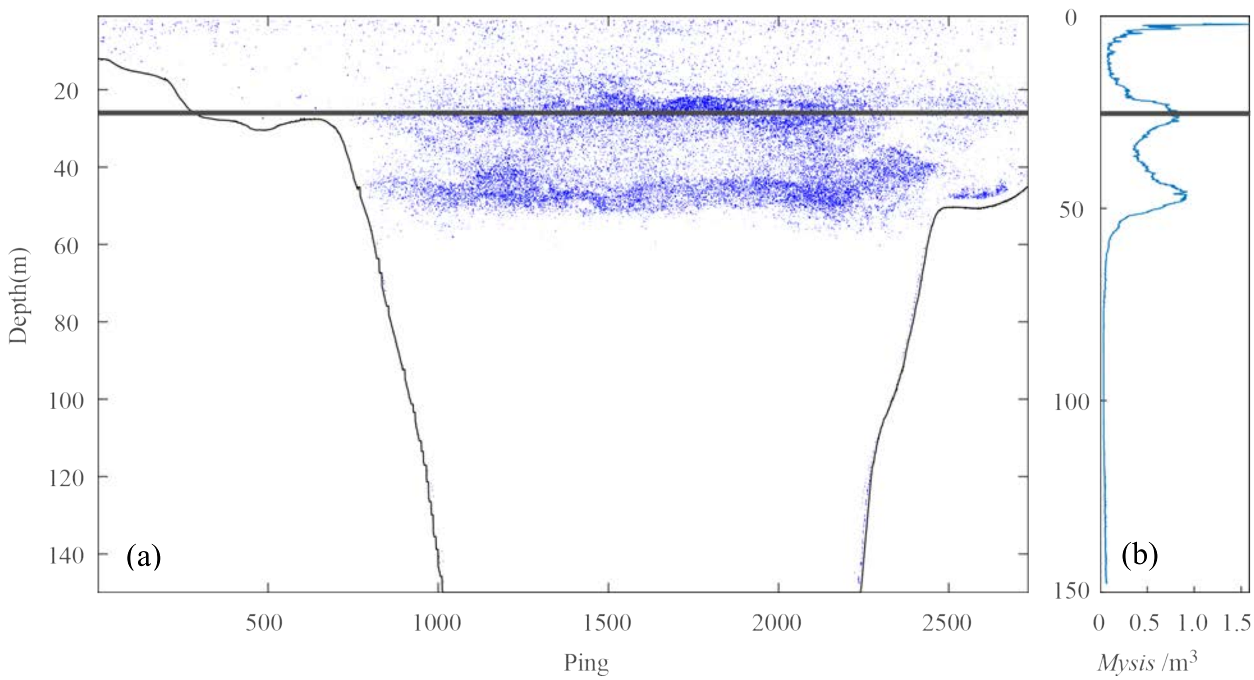

Fig. 4.26: (a) Backscatter return from a single transect on September 17, 2018 in the main body of Lake Tahoe (shown in blue) starting on shore at Camp Richardson and then turning around and returning to shore around Ping 1500 after removing the surface backscatter return and clipping the data at 150 m. Depth of the thermocline shown as a black horizontal line. (b) Vertical profile of average volumetric density (individuals/m^3) with depth with depth of the thermocline (black horizontal line) indicated.

net. Second, it indicates that there may be multiple depths at which high efficiency harvesting (catch per unit effort) could be conducted on a particular night. A further complicating factor arises due to Lake Tahoe's great areal extent. In Emerald Bay it was possible to operate with a great number of transect lines producing a more robust areal estimate of spatial density. In Lake Tahoe it was possible to only conduct paired cross-lake surveys in a single night's operation (**Fig. 4.27**). A full synoptic survey of the lake was not possible in the time available in a given evening. Boat speed is less than 4 knots and night-time conditions only persist for a maximum of 14 hours. Thus, theoretically we could only cover less than 50 linear survey miles.

It should be noted in **Fig. 4.26** that the individuals seem to be congregating in the pelagic zone as compared to the littoral. As will be shown in the detailed discussion of the Camp Richardson profiles, this lateral variability demonstrated a notable seasonal variability that was reproduced in both these profiles as well as the trawling efforts (next section). This points to the need to have more frequent surveys.

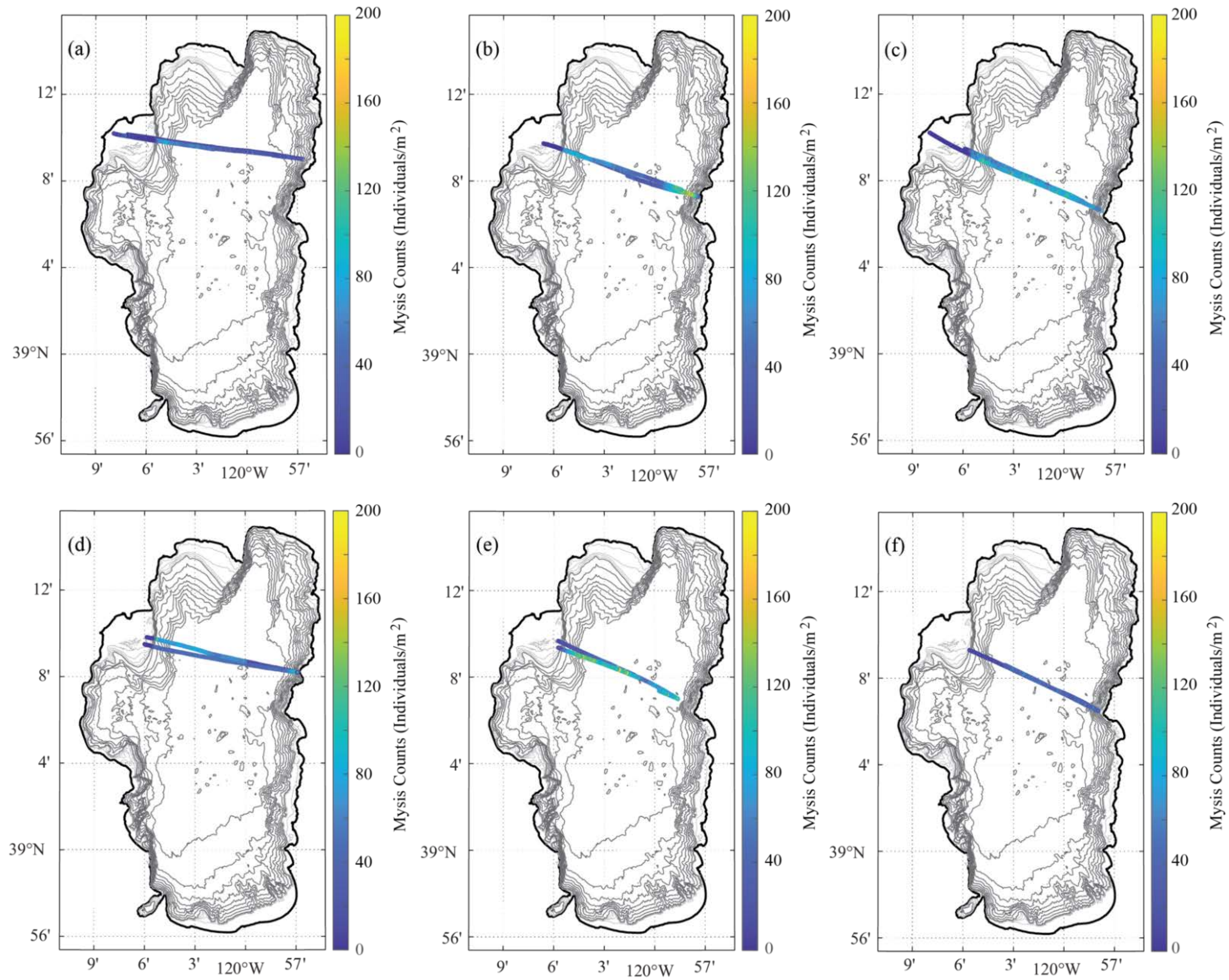

Fig. 4.27: Paired cross-lake transects across the northern end of Lake Tahoe on (a) July 10, 2018; (b) September 19, 2018; (c) November 13, 2018; (d) March 19, 2019; (e) August 27, 2019; and, (f) December 17, 2019. The limits on the color bar range from 0-200 individuals/m^2 in each of the subpanels.

In addition to these cross-lake transects, four sampling events were conducted in the southern end of the lake near Camp Richardson: September 17, 2018; March 18, 2019; June 6, 2019; and August 12, 2019. This site was selected in order to explore the spatial variation in *Mysis* in the transition from the nearshore to the pelagic. The importance of this linkage was hinted at in closer inspection in the regions where soundings were collected close to shore (e.g. the eastern edge of **Fig. 27**). **Fig. 4.28** shows a series of on-shore/off-shore transects from roughly 10 m of water (Ping 0) to roughly 150 m of water (Ping 1500). It should be noted that each of these subplots represent one half of the existing dataset that is mirrored on the return to shore (not shown for presentation purposes). Similar to Emerald Bay (**Fig. 4.23** and **Fig. 4.24**), there appears to be a seasonal dependence to this behavior. Starting in September 2018, *Mysis* were observed to be congregating in the pelagic region (**Fig. 4.28a**). Then, by March 2019, there are significantly fewer individuals but the ones that were there are clustered nearer to shore in the littoral region (**Fig. 4.28b**). This may be a function of their size class at this time of the year, although with Lake Tahoe's 3-4 year *Mysis* life span, size class is not as clear cut as it is in Emerald Bay. These numbers appear to increase through the rest of the season (**Fig. 4.28c-d**) and it is hypothesized that these larger individuals would then move into the pelagic region by the end of the stratification cycle (e.g. late fall).

One hypothesis of why this behavior is taking place with the older individuals is that, in regions where the depth of the shelf is deeper than the thermocline, the depths below the thermocline provide sufficient cold water refugia without the necessary energy expenditure to swim up from deeper depth. Further exploration would be required to understand how shelf depth limit influences the size and abundance of the *Mysis* populations but provides some insight on where to potentially trawl for individuals in the lake and different times of the year.

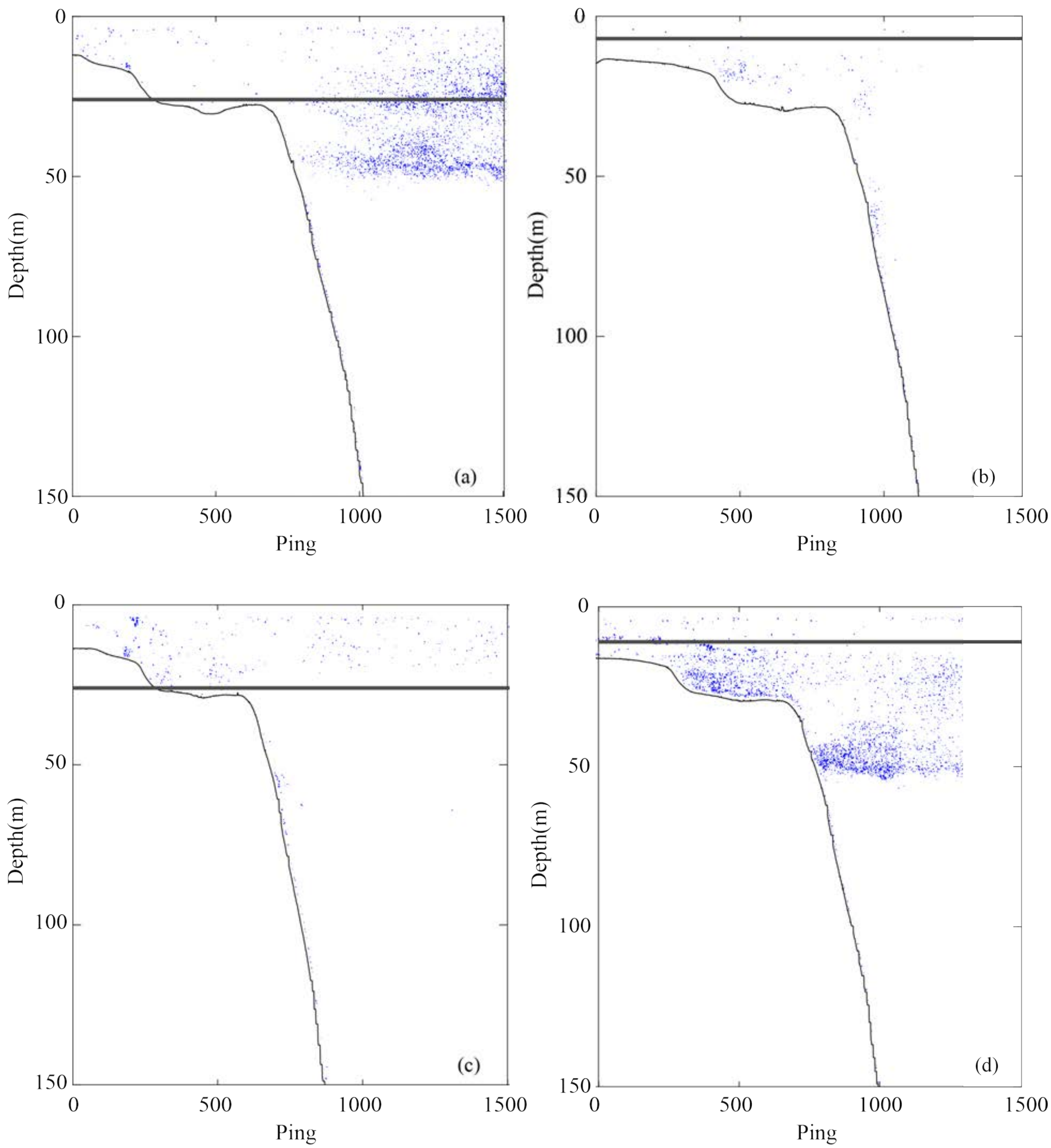

Fig. 4.28: On-shore/offshore transects collect at Camp Richardson on (a) Sept. 17, 2018; (b) March 18, 2019; (c) June 6, 2019; and (d) August 12, 2019 showing the relative density of individual *Mysis* at this site.

The results shown in **Fig. 4.28** highlight both the potential rewards of better understanding *Mysis* behavior and the complexity that still needs to be understood. The potential is that in **Fig. 4.28a** and **4.28d**, it is clear that in August and September that there is distinct layering (i.e. concentration) of *Mysis*. This behavior is precisely what is needed in order to make trawling efficient. On the other hand, the multiple possibilities for abundance is lower and more diffuse in March and June can only be surmised. It could be due to the physical mixing processes, demographic shifts, heterogeneity of the population in both space and time, as well as multiple other factors.

4.2.4 Biosonics Conclusions

Some technical conclusions regarding the use of echosounding for the measurement of *Mysis* may be made following our measurements through this project. Specific questions that are able to be addressed include are as follow: (1) What is an appropriate average Target Strength (TS) value for *Mysis?* (2) What is the size class and population density of *Mysis* that were able to be determined using this acoustic technique? (3) What error results from bottom reflections and larger fish detections?

(1) What is an appropriate average Target Strength (TS) value for *Mysis?*

It was concluded that typical target strength was on the order of -82 to -90 dB although this range reflected both a seasonal effect as well as a location within the lake. This is certainly in agreement with literature where the echo-location of specific species is based on a careful calibration of the return based on the localized conditions (e.g. Lavery et al. 2010). *The recommendation from this report is that the TS value should be calibrated quarterly by comparing vertical net trawls with acoustic measurements.*

(2) What is the size class and population density of *Mysis* that were able to be determined using this acoustic technique?

While there is evidence that this acoustic technique could resolve a broad range of size classes, it appears less reliable for the smaller sizes (e.g. <8 mm juveniles). In addition, low concentrations of individuals (<10 ind./m^2), were generally poorly resolved. *The recommendation from this report is to continue refining the acoustic technique, particularly during periods where smaller size classes or low population densities dominate.*

(3) What error results from bottom reflections and larger fish detections?

Overall, detection of both the larger fish as well as the bottom reflections was relatively straightforward. The bigger, unexpected challenge is how to get high quality results in deep regions where the bottom wasn't detected. This challenge arises because, unbounded by a bottom substrate, the acoustic energy will continue to spread spherically and result in a high signal to noise ratio in the return that is measured in the receiver. We've assumed in this report to cut this off at 100 m but that is somewhat arbitrary. *The recommendation from this report is to continue refining the acoustic technique in deep (i.e. bottom not observed) waters.*

4.3 Water Quality

Water sampling was conducted at monthly intervals in order to compare conditions between Emerald Bay and Lake Tahoe (which is being sampled under separate funding). This is an important part of this project, as sampling of Emerald Bay in the past has been very sparse and largely non-existent for the last 40 years. At that earlier time, the two systems were concluded to be very similar in many respects. In order to fully assess how comparable Emerald Bay and Lake Tahoe truly are in present times, the nutrient, biological and physical conditions needed to be compared.

4.3.1 Emerald Bay

Nitrate (NO_3, µg L^{-1}) concentrations were low (< 2.0 µg L^{-1}) throughout the water column during

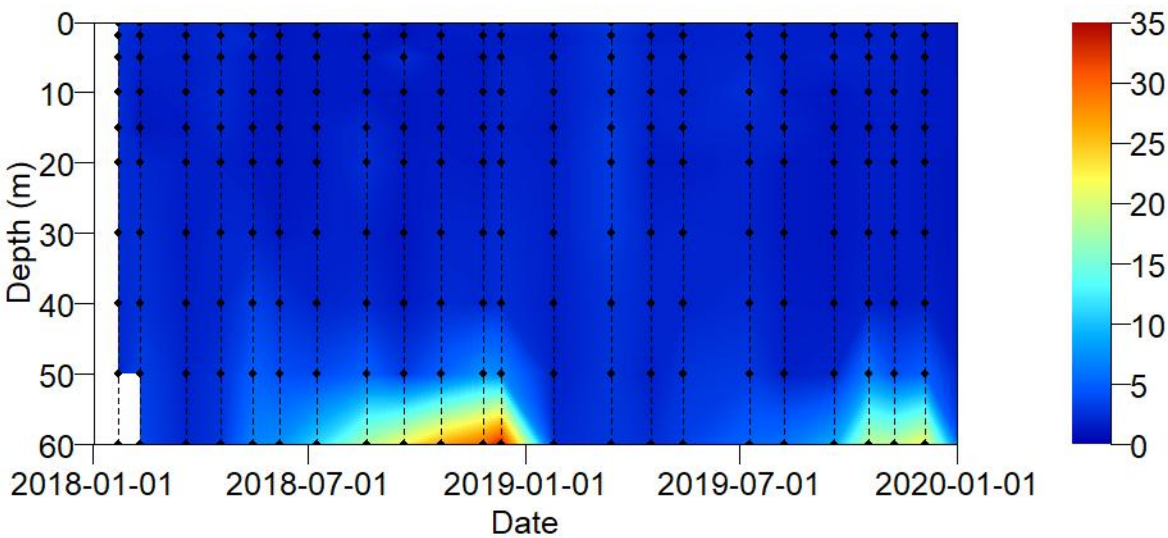

Fig. 4.29: Nitrate concentrations (NO_3, µg L^{-1}) in Emerald Bay in from January 2018 to December 2019. Dashed lines indicate the date samples were taken. Dots indicate the depth at which the water sample was taken.

our study (**Fig. 4.29**), with maximum concentrations approaching 35 µg L^{-1} at the bottom during summer to fall. Seasonally higher values near the bottom are likely associated with decomposition nitrification of organic sediments while the water column is stratified and the transfer of nutrients due to *Mysis* excretion at the bottom of the water column (Marjanovic 1989; Jassby et al. 1992).

Total Hydrolysable Phosphorus (THP, µg L^{-1}) ranges from 0.2 to 7.3 µg L^{-1}. Values are relatively higher at the bottom during stratified periods, likely as a result of sediment fluxes of SRP and settling of organic matter through the water column (**Fig. 4.30**). There are also high values in the middle of the water column seasonally.

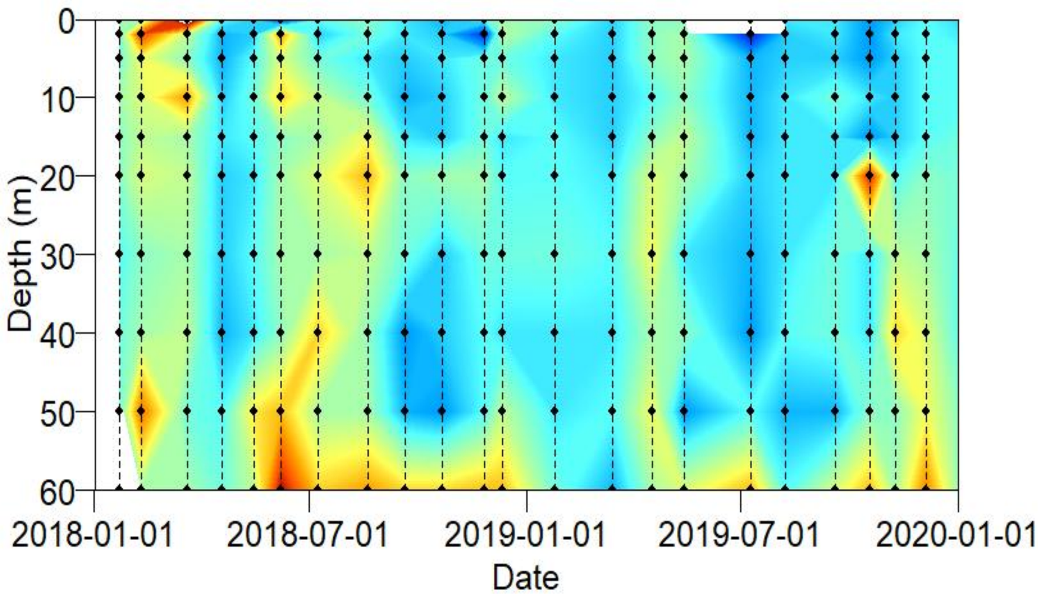

Fig. 4.30: Total Hydrolysable Phosphorus concentrations (THP, µg L^{-1}) in Emerald Bay in from January 2018 to December 2019. Dashed lines indicate the date samples were taken. Dots indicate the depth at which the water sample was taken.

Fine sediment particles (FSP) include all types of particles in the water column, i.e., organic particles, such as live/dead algal cells and terrigenous detritus, and inorganic particles washed in from the surrounding watershed. Particles in the size range of 1.0 to 4.76 µm are shown (**Fig. 4.31**) as these have the greatest impact on clarity. The concentration of particles was typically below 3,000 particles µg L^{-1} but with periods of higher concentration seasonally.

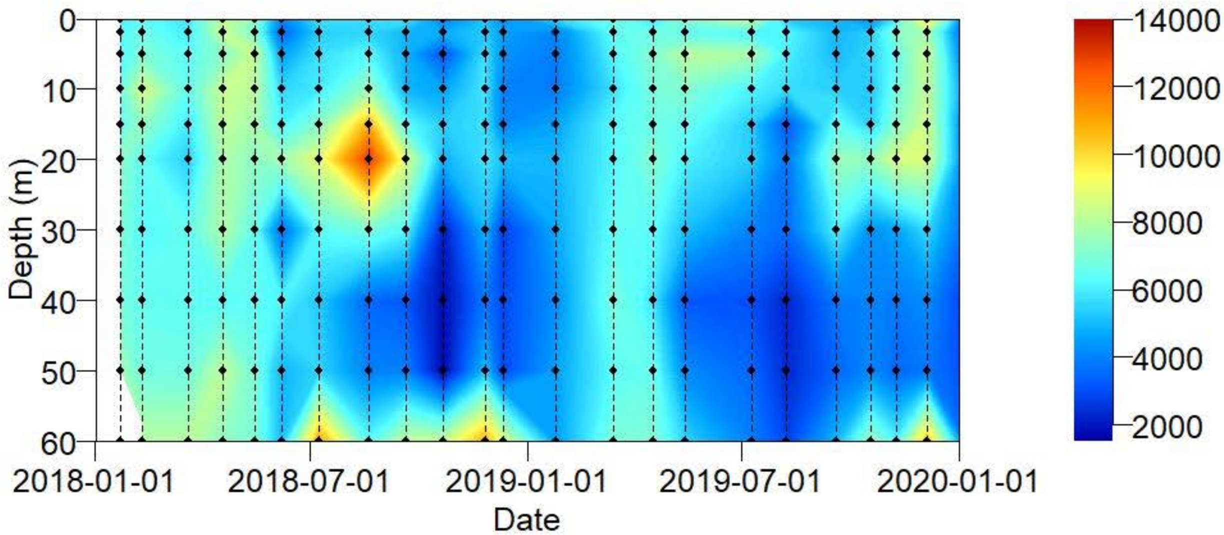

Fig. 4.31: Concentration of fine sediment particles in the size range of 1.0 to 4.76 μm, given in the number of particles per mL in Emerald Bay from January 2018 to December 2019. Dashed lines indicate the date samples were taken. Dots indicate the depth at which the sample was taken.

Chlorophyll-*a* concentrations showed a clear seasonal pattern (**Fig 4.32**). The values were vertically uniform when the bay was mixed from winter to spring. Once the water column was stratified a zone of relatively higher concentration appeared immediately below the thermocline and persisted throughout the summer at a depth of 20-30 m. This high chlorophyll-*a* zone (the deep chlorophyll maximum) would not affect the water clarity measured as Secchi depth because it occurs below the measured range of Secchi depth. The formation of a deep chlorophyll maximum is a feature that also occurs in Lake Tahoe every year, although the DCM at Tahoe is at depth of 50-70 m.

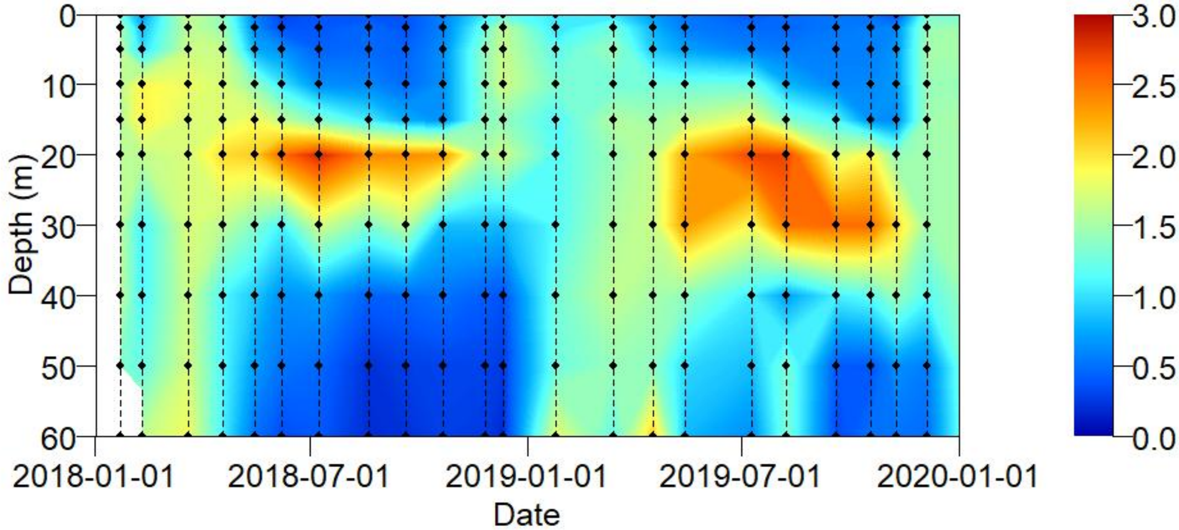

Fig. 4.32: Concentration of Chlorophyll-*a* (µg L^{-1}) in Emerald Bay from January 2018 to December 2019. Dashed lines indicate the date samples were taken. Dots indicate the depths at which samples were taken.

4.3.2 Lake Tahoe

Nitrate concentration for 2018 and 2019 in Lake Tahoe ranged from < 10 µg L^{-1} in surface waters to seasonal peak values approaching 40 µg L^{-1} at depth (**Fig. 4.33**). The values are in the same range as those occurring in Emerald Bay, with similar temporal distributions. The deeper hypolimnion of Lake Tahoe allows for the formation of a larger, deep water repository of nitrate.

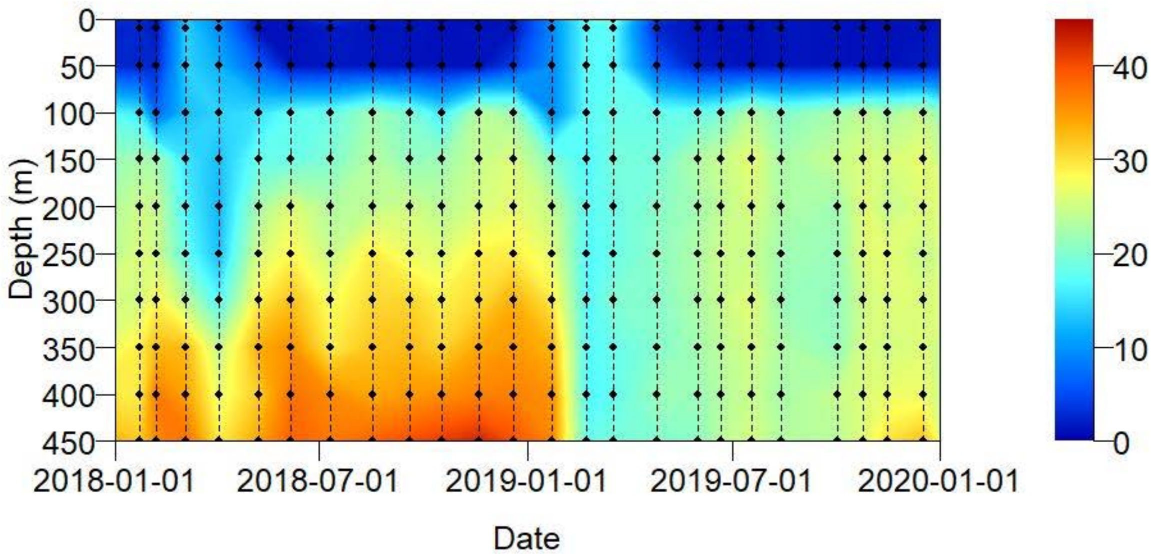

Fig. 4.33: Nitrate concentrations (NO3, µg L^{-1}) in Lake Tahoe from January 2018 to December 2019. Dashed lines indicate the date samples were taken. Dots indicate the depth at which the water sample was taken.

Total Hydrolysable Phosphorus concentrations in Lake Tahoe in 2018 and 2019 ranged from 1-5 µg L^{-1} (**Fig 4.34**). Concentrations tended to increase with depth and peak near the bottom, with a consistent seasonal pattern of increase during the winter months and decrease through the growing season.

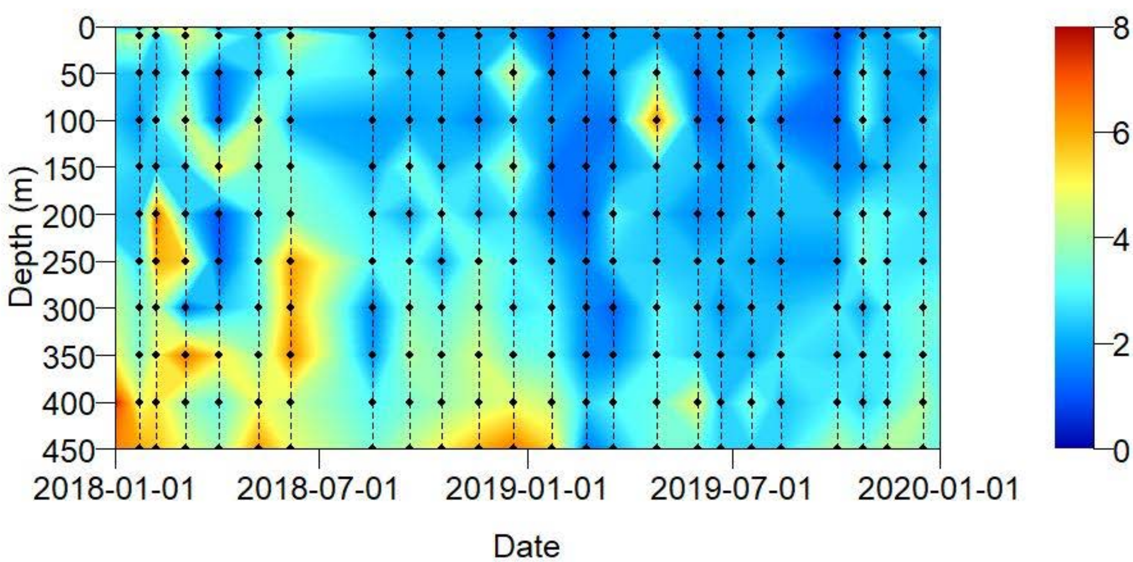

Fig. 4.34: Total Hydrolyzable Phosphorus (THP) concentrations (µg L^{-1}) in Lake Tahoe from January 2018 to December 2019. Dashed lines indicate the date samples were taken. Dots indicate the depth at which the water sample was taken.

Fine sediment particle concentrations in Lake Tahoe in the size range of 1.0 to 4.76 µm had peak concentrations of the same order of magnitude as found in Emerald Bay (**Fig 4.35**). However, there were substantial differences in the depth range over which fine particle concentrations were highest, with concentrations in Emerald Bay generally higher and distributed through a larger proportion of the entire water column. This difference in particle concentration could in part explain the generally higher water clarity in Lake Tahoe compared to Emerald Bay.

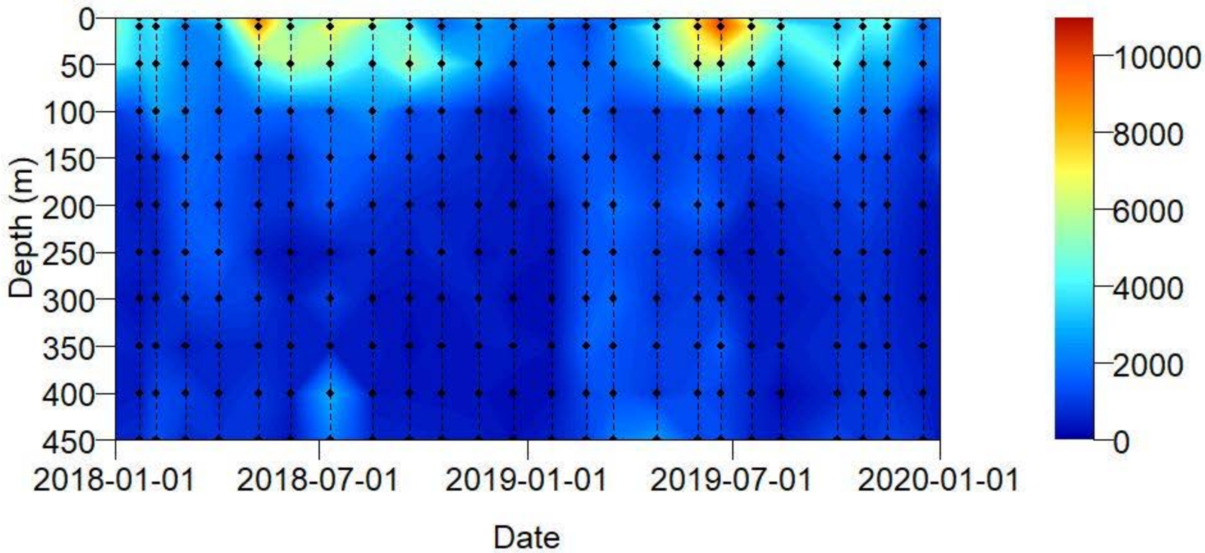

Fig. 4.35: Particle concentrations (numbers L^{-1}) in the size range 1.0 to 4.76 µm in Lake Tahoe from January 2018 to December 2019. Dashed lines indicate the date samples were taken. Dots indicate the depth at which the water sample was taken.

Chlorophyll-*a* concentrations for Lake Tahoe are shown in **Fig. 4.36**. Similar chlorophyll levels and the presence of a deep chlorophyll maximum is evident at a depth of 50 m. The depth of the deep chlorophyll maximum in Tahoe is greater than the deep chlorophyll maximum in Emerald Bay (20-30 m). This is likely the result of greater clarity in Lake Tahoe (allowing light needed for photosynthesis to penetrate deeper) and the greater thermocline depth on account of the greater wind exposure and fetch. Note that the depth interval of chlorophyll measurements at the deep site in Lake Tahoe were not sufficiently frequent to show the vertical distribution.

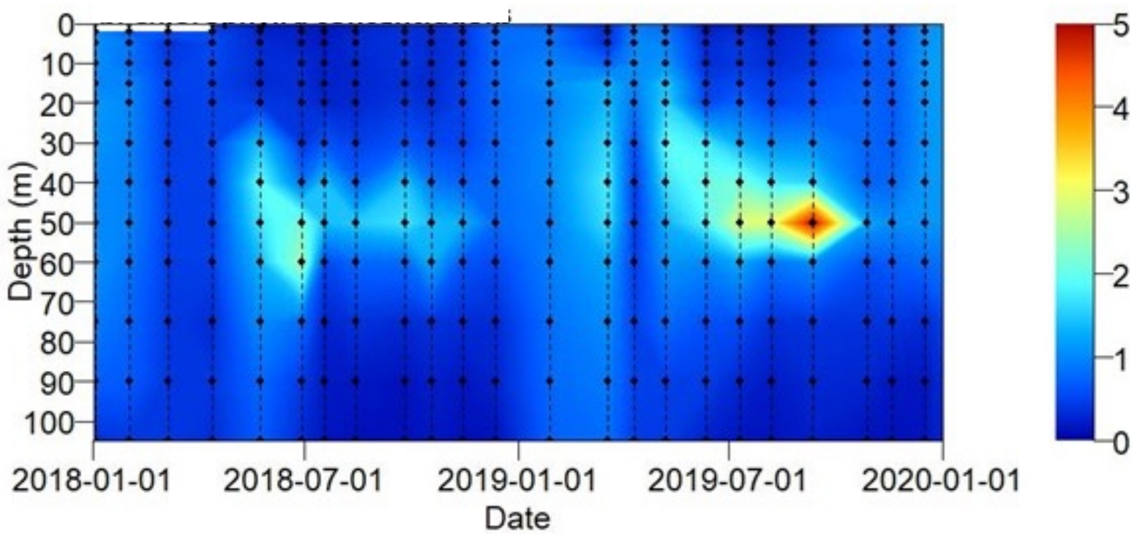

Fig. 4.36 Chlorophyll-*a* concentrations (µg L^{-1}) in the upper 100 m of Lake Tahoe from January 2018 to December 2019. Dashed lines indicate the date samples were taken. Dots indicate the depth at which the water sample was taken.

4.4 Physical Profiling

4.4.1 Emerald Bay

Physical and biological properties were characterized near the deepest point of Emerald Bay monthly during the project (see **Fig. 4.1**). Water clarity was measured as Secchi depth (m), by lowering a 25 cm all-white secchi disk from the shaded side of the boat. The depth is determined as the average of the depth where disk disappear from view when lowering and re-appears when recovering. Vertical profiles of water temperature (°C) were taken by either a Seabird 25 or Seabird 25plus multi-parameter profiler. Chlorophyll-*a* concentrations (Chl-*a*, $\mu g\ L^{-1}$) and fine sediment particle concentrations (number of particles per mL) were measured on discrete water samples taken from depths of 0, 5, 10, 15, 20, 30, 40, 50 and 60 m using a van Dorn sampler.

Secchi depth ranged from 10.5 to 16.0 m with a mean of 13.1 m in 2018 (n = 12) and ranged from 9.5 m to 16.6 m with a mean of 13.3 m in 2019 (n = 10). Each year showed a similar seasonal pattern. The annual minimum was attained in early summer, and gradually increased to the annual maximum in late summer. The clarity declined again during fall.

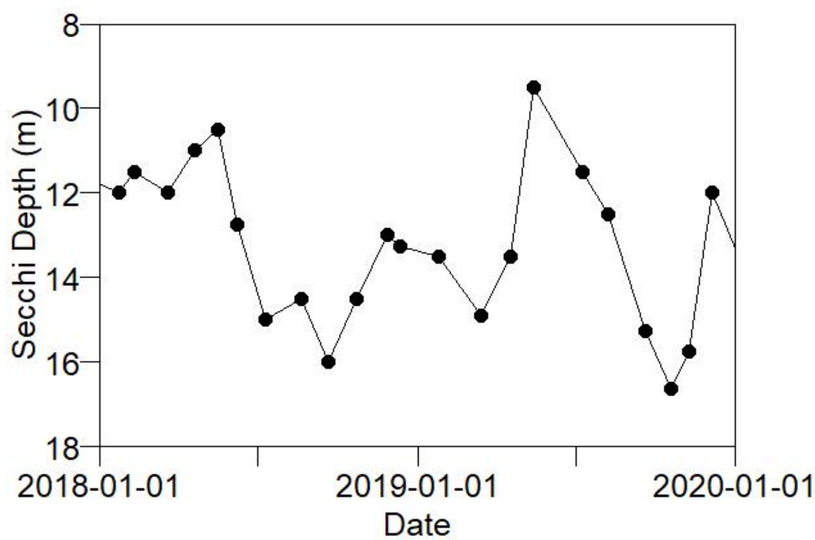

Fig. 4.37: Secchi depth in Emerald Bay from January 2018 to December 2019.

Temperature profiles allow the presentation of a time interpolated temperature distribution. It shows that Emerald Bay had a uniform temperature at around 5 °C throughout the water column during winter and stratified with surface temperature reaching almost 25 °C during the summer.

Stratification started between April and May and ended between December and January. 2019 was both warmer in summer and had a more sharply defined thermocline that 2018, although critical profiles in August may have missed the true thermal peak. The depth of the thermocline, determined as the depth where the rate of temperature change per unit depth (dT/dz) was a maximum, was less than 10 m in both summers and gradually deepened through the fall.

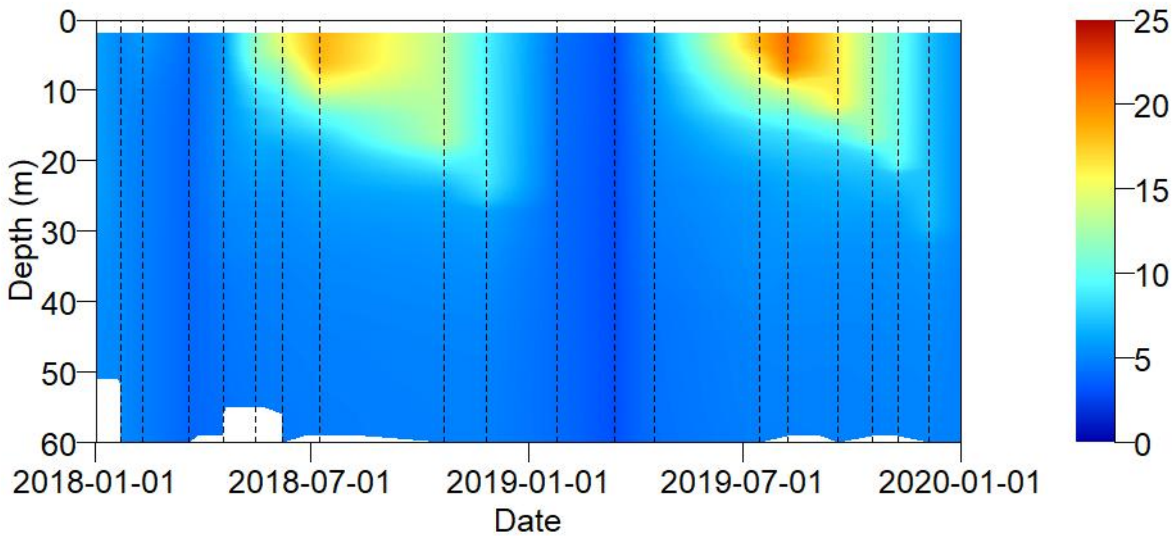

Fig. 4.38: Temperature distribution in °C in Emerald Bay from January 2018 to December 2019. Dashed lines indicate the date profiles were taken. Vertical resolution is 10 cm.

4.4.2 Lake Tahoe

Characterization of physical properties has been conducted at the Index station in Lake Tahoe. Secchi depth ranged from 15.2 to 30.5 m with annual average of 21.6 m in 2018, and from 11 to 34 m with average of 19.1 m in 2019 (**Fig. 4.39**). The clarity of Lake Tahoe was considerably greater than Emerald Bay, a pattern that has long been considered the norm. The Secchi depth distribution showed a bimodal pattern exhibiting clarity minima early in summer and later in fall as described in Jassby et al. (1999). This temporal variation is the same as what is observed in Emerald Bay.

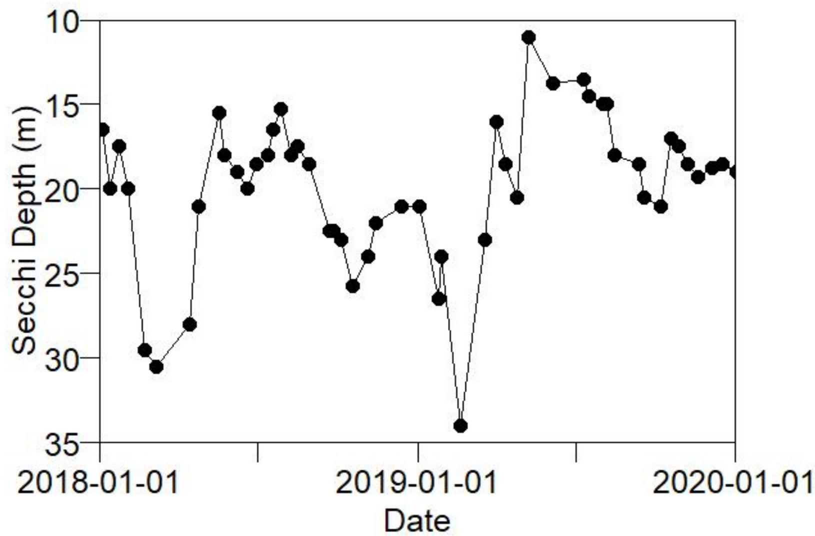

Fig. 4.39: Secchi depth at Index station in Lake Tahoe from January 2018 to December 2019.

Temperature profiles at the Index station show the stratification pattern of the lake (**Fig. 4.40**). Lake Tahoe starts to stratify between April and May and ends sometime between December and January. The depth of thermocline was at 15-20 m in the summer and deepened in late fall. This is considerably deeper than for Emerald Bay, as discussed above. The actual surface temperatures of Emerald Bay and Lake Tahoe were quite similar.

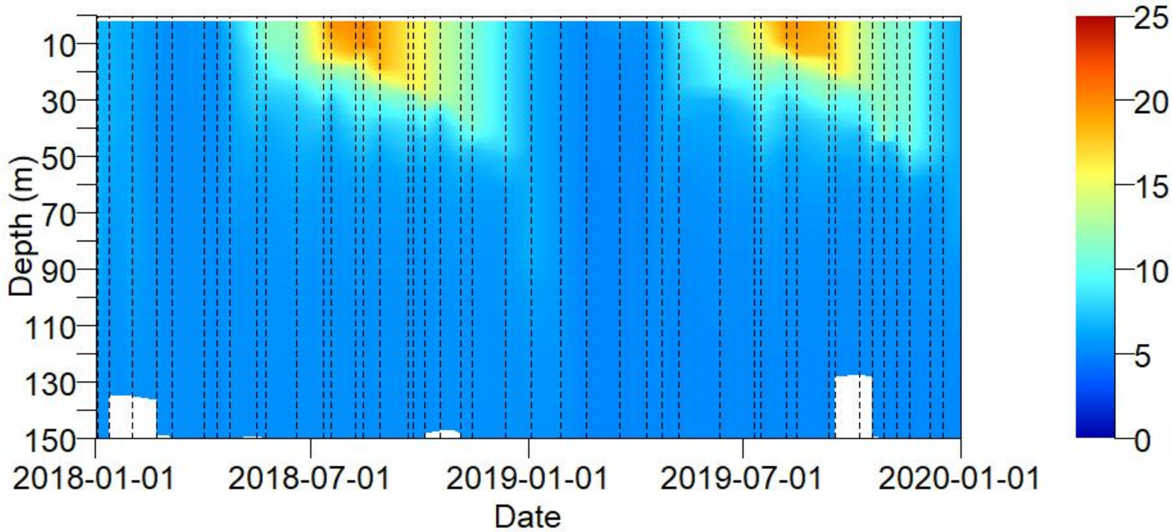

Fig. 4.40: Temperature profile in °C at the Index station in Lake Tahoe from January 2018 to December 2019. Dashed lines indicate the date profiles were taken. Vertical resolution is 10 cm.

4.4.3 Summary of Physical and Water Quality Variables in Emerald Bay and Lake Tahoe

The results of the two years of monitoring indicated the physical and chemical properties of Emerald Bay and Lake Tahoe are similar in important ways. Nutrients, chlorophyll-*a* and fine particle distributions are similar and appear to be controlled by the same range of driving forces. Being smaller and more sheltered, the thermocline depth in Emerald Bay is shallower. It also stratifies earlier and attains complete mixing sooner that Lake Tahoe. Summer surface temperatures in both water bodies are similar.

Clarity of Emerald Bay is lower than Lake Tahoe. The reasons for that are likely due to comparatively small but meaningful differences in particle concentrations (including small algae) and their specific location in the water column, and higher levels of colored dissolved organic matter (CDOM). CDOM was not measured as part of this project.

4.5 Phytoplankton

4.5.1 Phytoplankton in Emerald Bay

A total of 251 phytoplankton taxa belonging to 117 genera representing seven major taxonomic groups (Cryptophyta, Dinophyta, Chrysophyta, Haptophyta, Chlorophyta, Cyanophyta and Bacillariophyta) were identified. Over the study period, phytoplankton abundance ranged from 46 to 792 cell/ml, with an average of 287 cells/ml. Numerically small-sized (nanoplankton) and mixotrophic organisms comprised a considerable fraction of the total phytoplankton assemblage. In terms of biovolume, diatoms (Bacillariophytes), green algae (Chlorophytes) and cryptomonads (Cryptophytes) were the predominant the taxonomic groups.

The seasonal variation of total phytoplankton cell numbers throughout 2017-2019 is shown in **Fig. 4.40**. Maximum and minimum cell counts ranges were 179-219 cells/ml, 190-485 cells/ml and 129-620 cells/ml for 2017, 2018 and 2019, respectively. Cell numbers are in the range of oligotrophic conditions and within the range of low food availability for herbivorous consumers. Highest variation of cell numbers throughout a year was six-fold which was recorded in 2019.

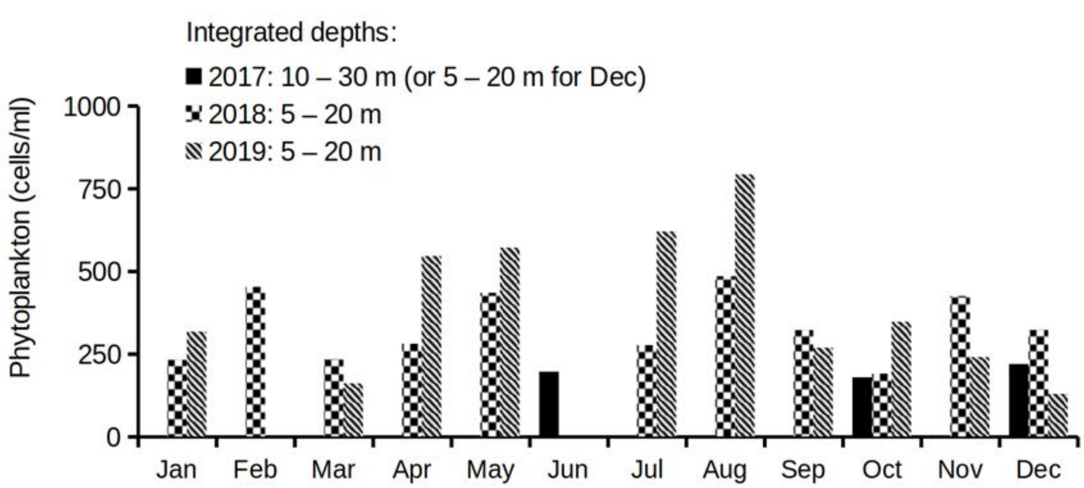

Fig. 4.41: Temporal changes in depth-integrated total phytoplankton abundance for samples collected from Emerald Bay during 2017-2019.

Seasonal variation in the taxonomic composition of phytoplankton in Emerald Bay is shown in **Fig. 4.42**. The proportion of Cryptophyta, Dinophyta, Chrysophyta, Haptophyta, Chlorophyta, Cyanophyta and Bacillariophyta represents the pelagic community at 10-30 m or 5-20 m depth range during 2017-2019.

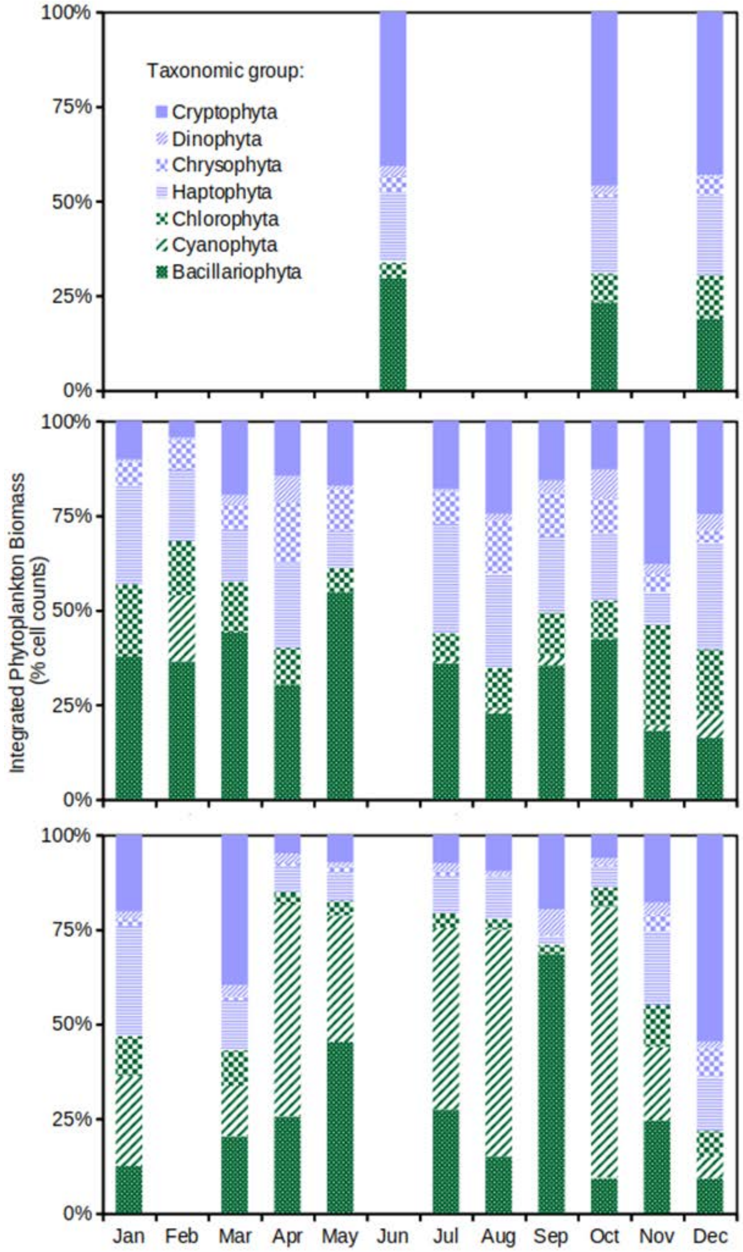

Fig. 4.42: Temporal changes in relative abundance (% of total cell counts) of the major taxonomic groups of phytoplankton in Emerald Bay during 2017 (top panel), 2018 (middle panel) and 2019 (bottom panel). Cell counts represent total phytoplankton of depth-integrated samples collected from 5, 10 and 20 m, except for June and October 2017, when water from 10, 20 and 30 m was combined. Color grouping represent trophic modes: photoautotrophic (in green), mixotrophic (in blue).

All phytoplankton photosynthesize, but some have the ability to supplement photosynthesis with ingestion of bacteria (phagocytosis). This mixture of nutrition mode is referred as mixotrophy. Mixotrophic organisms are diverse and comprise several taxonomic groups (**Fig. 4.42**). Moreover, most phytoplankton species are passive drifters or move relatively slowly, mixotrophs, however, are characteristically flagellated and thus capable of active locomotion. Switching from energy sources gives mixotrophic organisms a competitive advantage over autotrophs when light and/or nutrient availability is low (Palsson and Graneli 2004) and over heterotrophic flagellates when bacterial densities are low (Palsson and Daniels 2004). In Emerald Bay, mixotrophs comprised a considerable fraction of the total phytoplankton assemblage, and even surpassed autotrophs at many occasions throughout the study period. Temporal changes in the relative proportion of photosynthetic autotrophs (photoautotrophs) and mixotrophs in relation to total phytoplankton cell numbers during 2017-2019 is shown in **Fig. 4.43**.

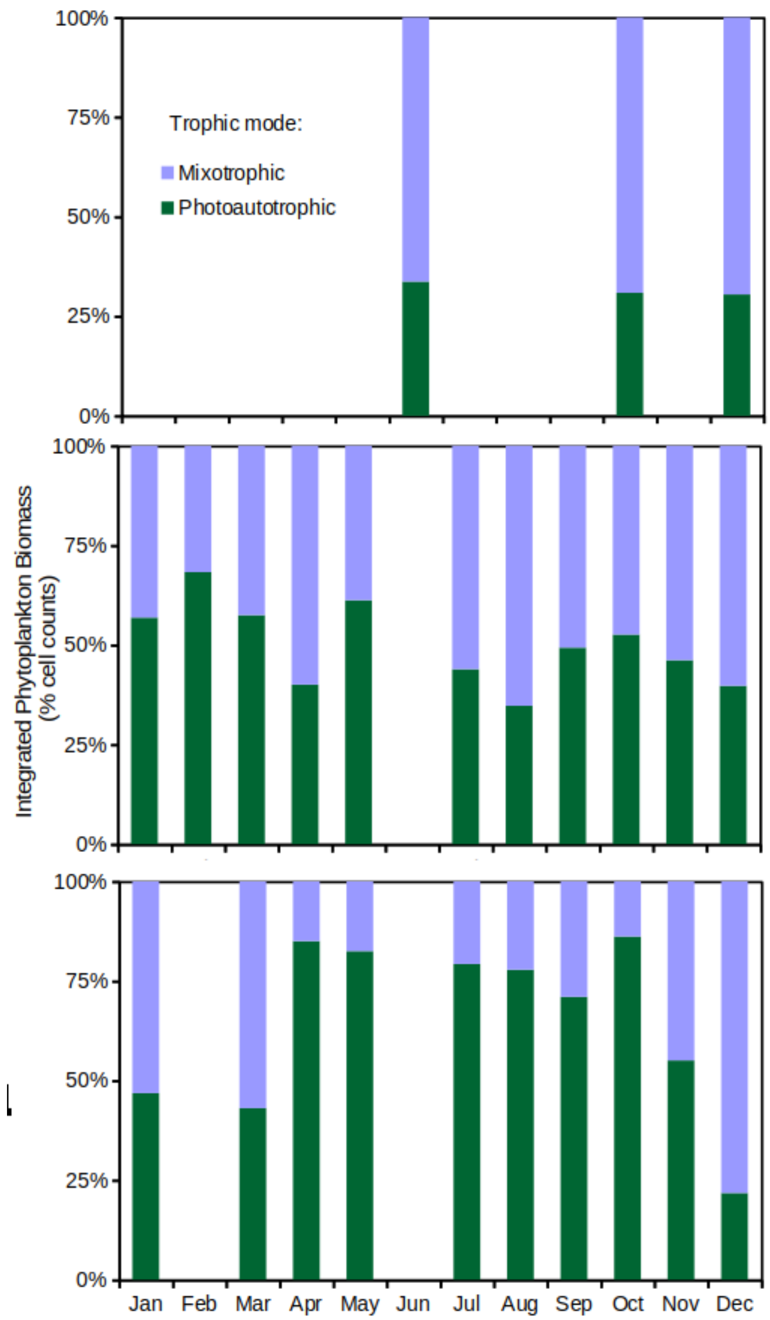

Fig. 4.43: Temporal changes in composition of phytoplankton in terms of trophic mode for depth-integrated samples collected at Station 4 in Emerald Bay during 2017 (top panel), 2018 (middle panel) and 2019 (bottom panel).

Research has shown that although cryptophyceans rarely comprise more than 15-20% of total phytoplankton biovolume in lakes they contribute considerably to plankton community dynamics

(Dokulil 1988). In Emerald Bay, cryptophyceans accounted for an average of 20% of total phytoplankton biovolume in the integrated 5-20 m (or 10-30 m) depth range.

Phytoplankton provide a source of energy (food) to herbivorous and/or omnivorous consumers (zooplankton), while zooplankton provide nutrients to phytoplankton through excretion. These interactions occur simultaneously at rapid time scales that are difficult to quantify in natural conditions. The importance of diatoms as good quality food resource for zooplankton is still largely controversial, but the high nutritional value of cryptophytes and the recognition of them as the preferred food resource of countless species of zooplankton and microcrustaceans seems to be almost unanimous. *Cryptomonas* in particular are considered an excellent quality food because their size range, their digestibility, and generally high food value due to high content of fatty acids. Among the freshwater phytoplankton, probably few exceed cryptophytes, particularly *Cryptomonas*, for its food value. Temporal changes in cell numbers of two cryptophytes taxa that are potentially important food sources in Emerald Bay, as links to higher trophic levels are shown in **Fig. 4.43**.

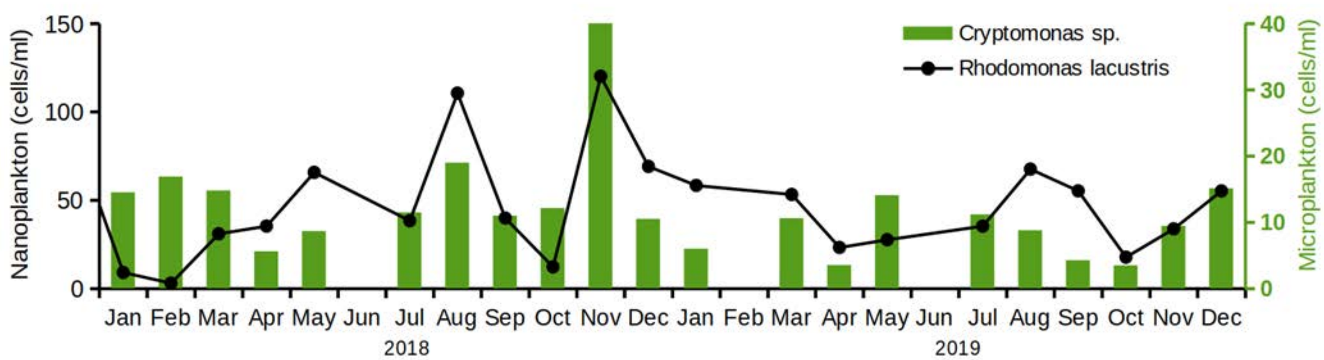

Fig. 4.44: Seasonal variation in abundance of *Cryptomonas* sp. and *Rhodomonas lacustris* present in depth-integrated samples at Station 4 in Emerald Bay, during 2018-2019.

On 26 November 2018, *Cryptomonas* sp. and *Rhodomonas lacustris* combined accounted for ~ 35% of total phytoplankton cell numbers (or ~22% of total phytoplankton biovolume) (**Fig. 4.44**). These peaks were observed prior to a large abundance peak of reproductive *Mysis* females, suggesting that phytoplankton community with cryptophyceans can constitute an important source of high quality food for subsequent consumers, which in this case, could be *Mysis* or zooplankton prey for *Mysis*. It is well known that *Cryptomonas* are ingested by a variety of rotifers and/or microcrustaceans. In **Fig. 4.44** it can be seen that *Cryptomonas* abundance in particular, dropped drastically following reproductive *Mysis* female abundance. Similar pattern with peaks of *Cryptomonas* and *Rhodomonas* followed by decline coincident with increase abundance in reproductive *Mysis* females was observed in December 2019. These observations suggest the consumption of Cryptophyceans contributes both to the biomass and reproductive growth potential of zooplankton species in Emerald Bay, including Mysis.

4.5.2 Phytoplankton in Lake Tahoe

The long-term data collected from Lake Tahoe's main basin revealed that the size structure of the pelagic phytoplankton community changed drastically over time. Phytoplankton community for four selected years (1969, 1985, 2002 and 2018) were sorted into two size classes. Temporal changes in size distribution at 5 m depth, representing conditions above the typical Secchi depth are shown in **Fig. 4.45**. The first category includes organisms smaller than 20 µm in length or diameter (excluded picoplankton). All phytoplankton species larger than 20 µm were assigned to the large phytoplankton cells category. Some consistent patterns have emerged. Large-sized species dominated by *Fragilaria crotonensis* made up over 86 % of the phytoplankton cell counts throughout 1969, but this ratio have changed drastically by 1985, when the small sized species dominated numerically. The number of cells decreased one order of magnitude from 1985 to 2002 and remained in the same range through 2018.

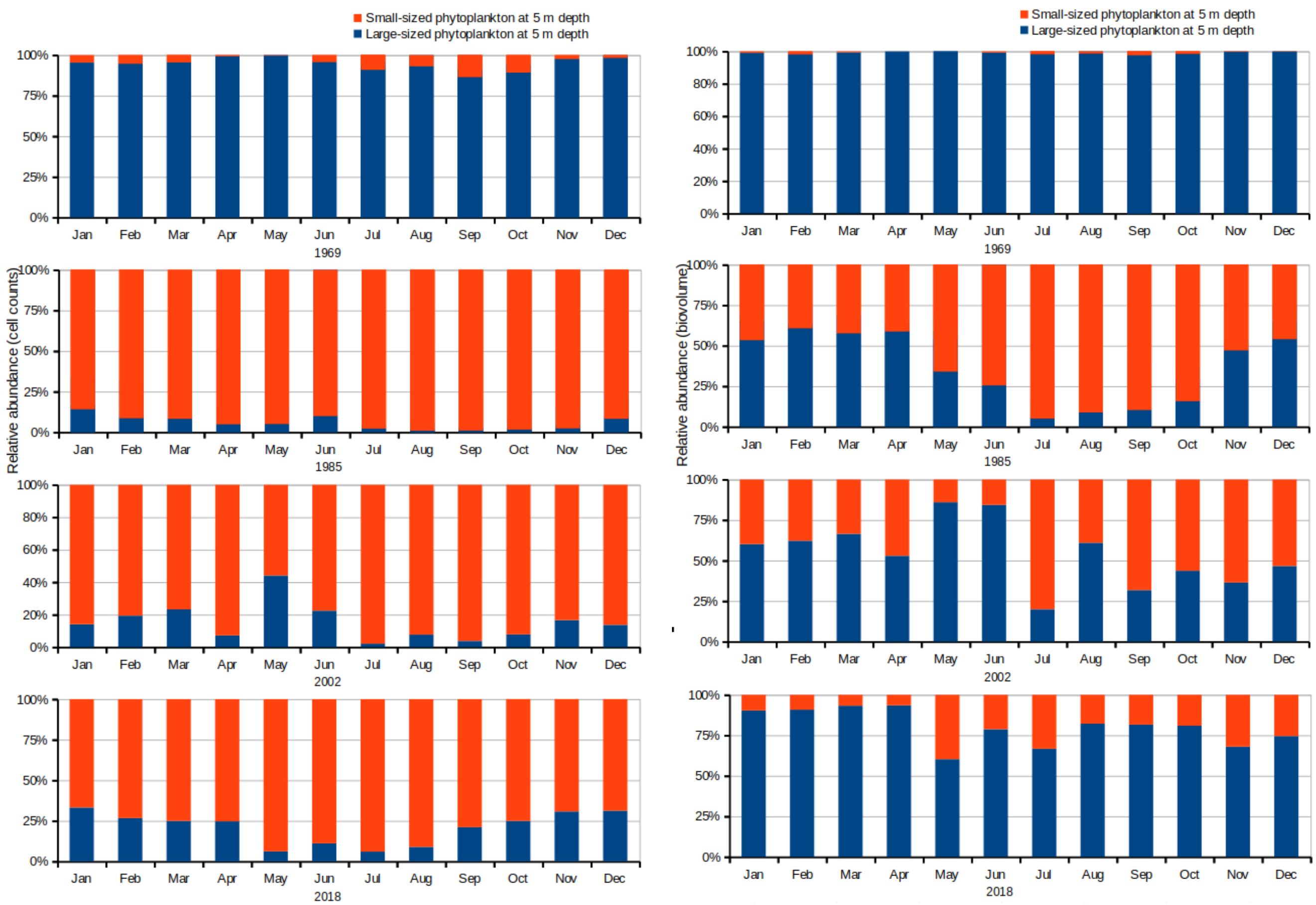

Fig. 4.45: Temporal changes in the relative abundance of two size classes of phytoplankton: large-sized (microplankton) and small-sized (nanoplankton) at Index Station in Lake Tahoe in 1969, 1985, 2002 and 2018. The graphs on the left are in terms of cell counts, while those on the right are in terms of biovolume.

Fragilaria crotonensis was the dominant phytoplankton between 1967 and mid-1970s, comprising over 90% of total number of cells (**Fig. 4.46**).

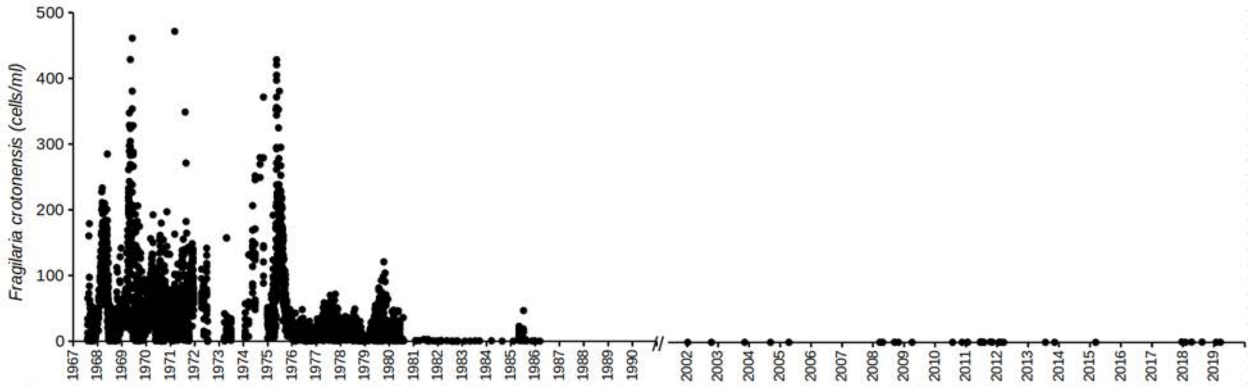

Fig. 4.46: Fluctuations in abundance (cells/ml) of the large sized diatom *Fragilaria crotonensis* in the main basin of Lake Tahoe. Data of all sampled depths (0-90m) plotted as individual data.

Long-term temporal variation of *Cryptomonas sp*. is shown in **Fig. 4.47**.

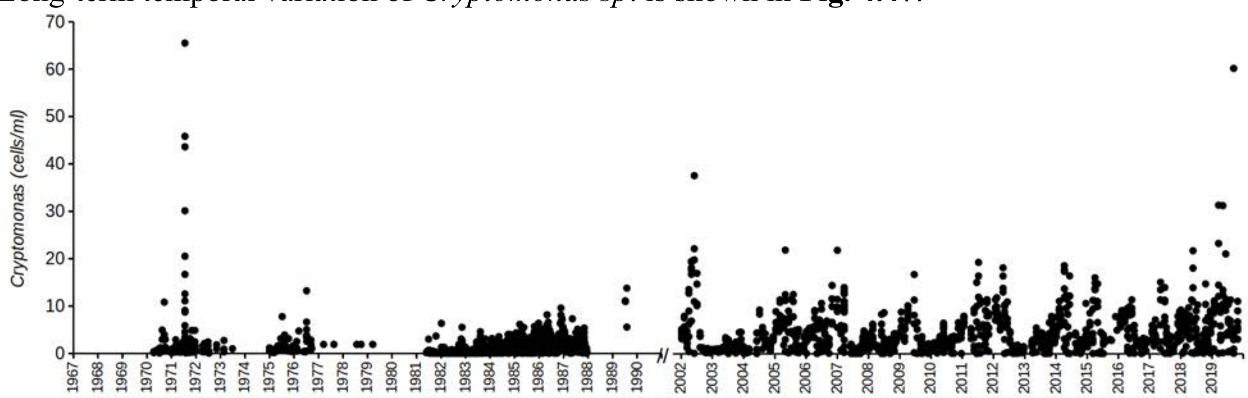

Fig. 4.47: Fluctuations in abundance (cells/ml) of the large sized phytoplankton *Cryptomonas* sp. in the main basin of Lake Tahoe. Data of all sampled depths (0-90m) plotted as individual data.

The historical long-term data revealed that *Cryptomonas* abundance peaked in 1971 and decreased sharply through 1975. In the same time period, according to Morgan (1979), the *Mysis* population in 1975 was overwhelmingly dominated by a single cohort which was produced in the spring of 1975 by the 1971 cohort. The overall population declined from 1975 to 1979 resulted primarily from mortality among individuals in the 1975 cohort. This suggests that food quality might have, in part, helped shape *Mysis* population fluctuation. Conditions in Lake Tahoe at one time must have been more favorable to *Mysis* growth and reproduction or they would never become established (Morgan 1979). *Cryptomonas* population dynamics may be linked to the abundance and reproductive success of *Mysis*.

4.5.3 Comparison between phytoplankton in Lake Tahoe's main basin and Emerald Bay

The relative phytoplankton community composition at the Index station located in Lake Tahoe's main basin and at station 4 in Emerald Bay is summarized in **Table 4.4**. Here we focus on observations of algae community over only two-years (2018-2019) because of the availability of data from the same depth range during this period. Our data show that the state of the two systems, and their constituent algal communities, present many similarities. For instance, the large contribution of diatoms (Bacillariophyta) and dominance of similar taxa in many instances. However, it should be noted such similarities represent a comparatively short snapshot in time, and a longer timeframe may show a different community structure.

Table 4.4: Relative phytoplankton community composition in Lake Tahoe's main basin and at Emerald Bay. Values of percentage algal cell density and biovolume represent averages generated from the 2018-2019 dataset of composite samples (5, 10, 20 m) for Emerald Bay Station 4 and discrete depth (5 m and 20 m) for the Index station LTP at the main lake basin.

Phylum	% of total abundance		% of total biovolume	
	Emerald Bay	Lake Tahoe's main basin	Emerald Bay	Lake Tahoe's main basin
Bacillariophyta	29.4	55.3	84.4	71.5
Chlorophyta	9.6	10.9	2.4	13.2
Chrysophyta	5.2	8.4	<1	1.2
Cryptophyta	14.9	7.2	7.0	<1
Cyanophyta	23.0	1.8	<1	<1
Dinophyta	2.2	1.3	4.9	4.9
Haptophyta	15.7	15.2	<1	<1
Dominant genera	(based on total algal cell density)		(based on total biovolume estimation)	
	Cyclotella *Pseudoanabaena* *Chrysocromulina* *Rhodomonas*	*Cyclotella* *Chrysocromulina* *Rhodomonas* *Synedra*	*Nitzschia* *Synedra* *Cryptomonas* *Gymnodinium*	*Synedra* *Nitzschia* *Botryococcus* *Cyclotella*

4.6 Thermistor Chain data

A thermistor chain was deployed 25 meters off the shore of Fannette Island in Emerald Bay from February 2018 to July 2020 (**fig. 4.48**). The intention of this data was to back up the monthly vertical profiling. Data collected show the extent and length of stratification in Emerald Bay, which impacts the diurnal migration of the *Mysis* population and the thermal habitability of

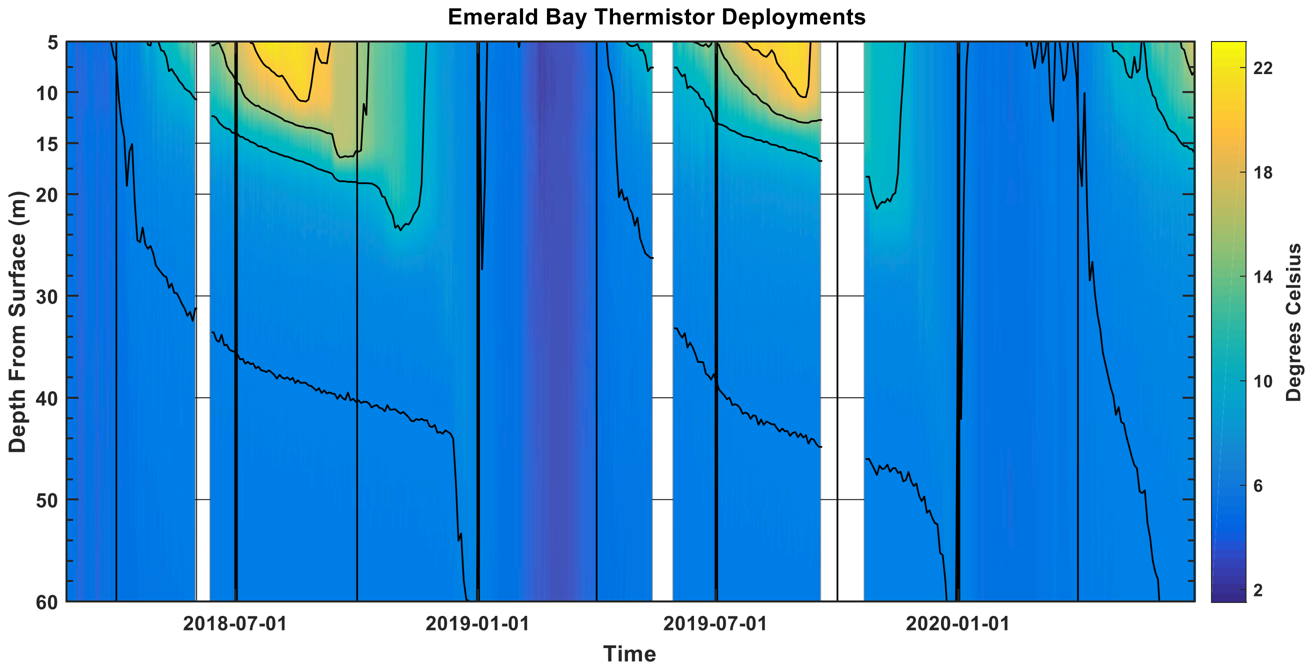

Fig. 4.48: Water temperature throughout the water column in Emerald Bay. Measurements were taken every ten minutes at 5, 10, 15, 20, 30, 40, 50 and 60 meters below the water surface from February 2018 to July 2020. Black vertical lines across the figure separate the data record into three-month periods, with thicker lines every 6 months. Isotherms of 5, 10, 15 and 20 °C have been smoothed into two-day averages and plotted across the full data record.

surface waters. The gaps indicate periods when the instruments were out of the water from downloading, calibration and subsequent redeployment.

The thermistor chain data record confirm that Emerald Bay was stratified from May to November and unstratified from December to April. The extent of stratification can be visualized with the 10, 15, and 20 °C isotherms. During each year, Emerald Bay was observed to fully mix between January and March as shown by the erosion of the 5 °C isotherm. A particularly cold year at Lake Tahoe, was 2019 when surface waters cooled to 1.5 °C setting up a short period of inverse stratification in February of that year (Schladow 2020).

The exact upper thermal tolerance of *Mysis* in Lake Tahoe is uncertain but from the literature it can be expected to peak at around 15 °C. Under this assumption, *Mysis* would not be expected within the upper 10 to 15 meters of the surface of Emerald Bay from June to October.

5.0 Emerald Bay Trawling results

5.1 Description of Trawl and Net Modifications

The trawling experiments for *Mysis* harvesting from Emerald Bay employed traditional trawling methods, adapted for a research vessel. A large conical net of decreasing mesh size was towed behind the R/V John LeConte, with the amount of cable out from a hydraulic winch determining the depth of the trawl. Basically, the weight and drag properties of the net, combined with the length of cable deployed and the speed of the vessel determines the depth of the net. Commercially available trawls are generally designed for either scientific sampling (1 m^2 opening) or, large scale, marine harvesting (>150 m^2 opening). The former design was deemed too small to effectively cover the volume of water needed to assess trawling impact on the *Mysis* population while the latter were too large to be towed by our research vessel. For this reason, we reached out to other institutions known to employ larger scale trawling methods for the scientific collection of similar size species.

An Aluette Pelagic trawl was loaned from Florida International University (North Miami, FL) and shipped from Woods Hole Oceanographic Institution (Woods Hole, MA). An Aluette trawl is light weight, quick to deploy, and designed for use where tight turns are needed. This seemed like the best option for use in Emerald Bay which is both narrow and complex on account of Fannette Island and the bay's steep sidewalls. The trawl net itself was 20 m in length with a rectangular opening of 75 m^2 (10 m width x 7.5 m height), effectively fishing an area of 56 m^2 while underway (see **Fig. 5.1**). It was held open while underway by Hendricksson trawl doors (1 m x 0.5 m) weighing 20.5 kg each. As with all trawl nets, the mesh started relatively large (38 mm) and tapered toward the cod end (2 mm), reducing drag forces while still capturing small sized organisms. The TERC research vessel was able to tow the trawl net at 1.5 knots.

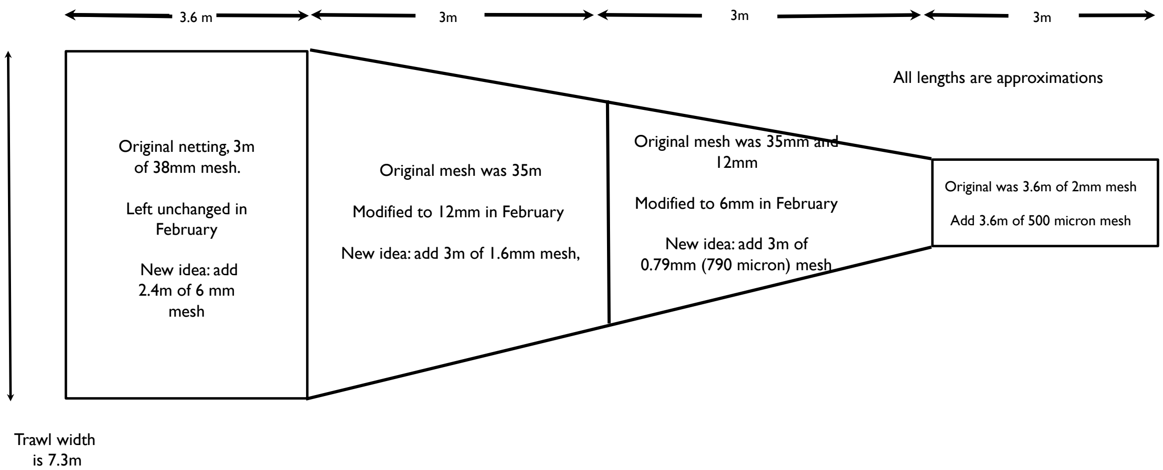

Fig. 5.1: Initial trawl net configuration with each of the sections and modifications as shown.

Our initial efforts with the trawl proved ineffective. While the trawl was specified as being able to capture organisms down to 2 mm in size, the mysids were too small to be captured in any quantity (despite their 15 mm length). We therefore had a smaller mesh sewn and inserted inside the original Aluette trawl. Our catch rate of mysids rose significantly but the two trawls together proved too cumbersome, reducing boat speed and increasing the turning radius.

We ultimately settled on modifying our newly designed, fine-mesh Aluette trawl to function on its own while still using the original Hendricksson doors. This trawl was 11.5 m in length with a functional opening of 11 m^2 while fishing (**Fig. 5.2**). Images of these modifications can be seen in **Fig. 5.3**. Mesh size ranged from 6 mm at the front to 0.5 mm at the cod end. The smaller mesh size forced the reduction in overall trawl dimensions in order to maintain boat speed and maneuverability with the TERC research vessel.

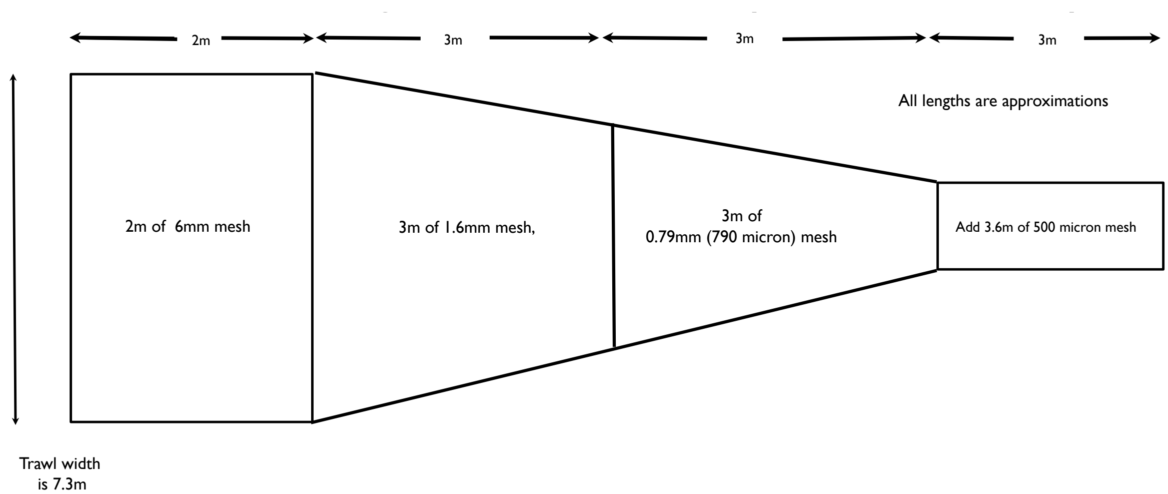

Fig. 5.2: Final modified trawl net design, fabricated by Tahoe Canvas Co., and implemented for the remainder of the study.

The development and testing of a functional trawl for harvesting Emerald Bay *Mysis* shrimp occurred from January through October 2018. During this time, 10 nights (66 hours) were spent testing designs, developing methods, and evaluating the catch. The trawl was deployed to the depth of maximum mysid density as indicated by the Biosonics Echosounder. The length of hydraulic cable deployed was calibrated to trawl depth and boat speed using a continuously recording pressure sensor (HOBO U20L) attached to the leading edge of the trawl. The height of the trawl opening was calculated by attaching a pressure sensor to the float line and lead line (top and bottom of trawl) during successive deployments. During harvest trawling, the pressure sensor was deployed on the float line and downloaded using an Onset coupler upon recovery to determine actual trawl depth. By the end of October, the catch per unit effort was deemed

Fig. 5.3: Fabricating the finer mesh insert (left). The combined coarse and fine mesh nets (right).

satisfactory to implement harvest within Emerald Bay with the goal of reducing the standing crop of mysid shrimp.

For the harvesting of juvenile *Mysis* in shallower water in Emerald Bay, we utilized a different net configuration. It was comprised of two commercially available trawls coupled within a custom-built frame. Each trawl opening measured 0.5 m x 1.5 m for a combined trawl opening of 1.5 m^2. Mesh size tapered from 6 mm to 0.5 mm along the 4 m length (**Fig. 5.4**). This was pulled by the *R/V Bob Richards* at a speed of 1.4 knots in shallow water during the spring and summer months to target juvenile mysids. The system required a crew of two to safely operate.

The larger trawl described previously, was used to harvest mysids in open water. The trawl had an effective opening of 13 m^2. The mesh size tapered from 6 mm at the front end to 0.5 mm at the cod end. Overall trawl length was 11.6 m. The trawl was towed behind the *R/V John LeConte* at a speed of 1.1 knots. This system utilized steel trawl doors (0.3 x 1 m) to hold the net open while underway and required a crew of three to operate.

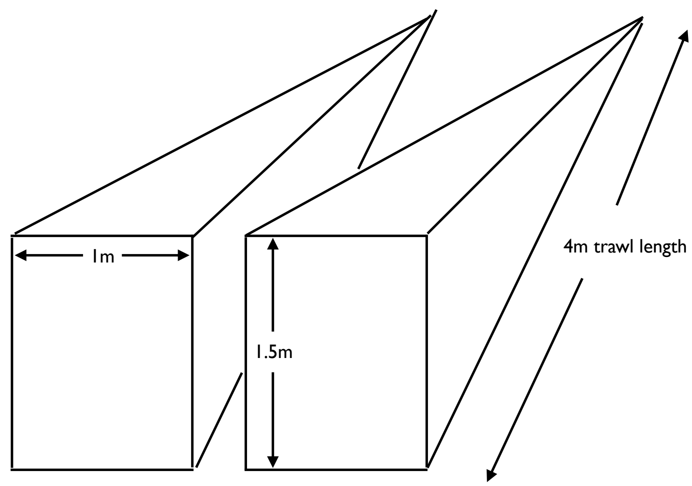

Fig. 5.4: Two commercially available scientific trawls coupled within a custom frame were used to harvest juvenile shrimp in shallow water in Emerald Bay during spring.

It was determined during the trawl evaluation phase of the project that trawling within the confines of Emerald Bay was not feasible during peak summer months (July and August) due to excessive boat traffic both day and night. High density of recreational boats after dark was observed on several occasions while conducting mysid density assessments (stationary vertical net tows).

5.2 Trawling Schedule and Operations

The yield of *Mysis* shrimp harvested from an open water body depends on several factors, including the abundance of shrimp and the efficiency of the trawling device. The abundance of shrimp can be greatly affected by their life history characteristics and seasonal environmental change. *Mysis* display shifts in vertical distribution based on seasonal stratification of the water body, selecting temperatures below 15 ºC (Rudstam et al. 2008), although the precise upper limit is not known for Lake Tahoe and Emerald Bay the extent of the water column where. During winter and spring, the *Mysis* formed lose aggregations spanning 20 m or more of water depth starting 20 m below the surface. This was likely due to the absence of a thermocline and a widely distributed prey base. Under these conditions, trawl harvest was greatly reduced. In summer and fall, during stratified lake conditions, mysids are more likely to accumulate in narrow depth strata (2-5 m) just below the thermocline depth (12 m in Emerald Bay) thereby facilitating harvest efficiency.

Additionally, *Mysis* populations in Emerald Bay display horizontal separation between adults and juveniles. With young-of-year (YOY) *Mysis* having greater light and heat tolerance, they seek nearshore shallow water habitat to avoid cannibalistic predation (Morgan and Threlkeld 1982). *Mysis* in Emerald Bay exhibited these behaviors during this study, dramatically altering the available harvest and catch per unit effort through spring and early summer. By mid-summer, juvenile mysids have grown enough to avoid cannibalistic predation (15 mm) and move to the center of the bay in preparation for fall-winter breeding.

Trawling strategies may differ depending on the goals of the harvest. For instance, harvesting to maximize biomass removal (large adults) may employ a seasonally different strategy than targeting specific segments of the population to decrease year class abundance. As seen in all commercial fisheries around the world, harvesting mature adults before they can reproduce and repopulate juvenile life stages has brought about population crashes. While this is not a sustainable model for commercial fishing, it may be the best strategy for population depletion of an invasive species.

Our trawling strategy shifted seasonally to target the greatest abundance of individuals rather than the largest biomass of shrimp available. During late summer, fall and early winter, trawling efforts focused on the adult portion of the population in the center of the bay over deep water. These were the large (15-18 mm) breeding adults and females that were already carrying eggs. With each female holding 8-18 eggs, removing these individuals would dramatically decrease breeding success and therefore the following year's adult population. During late winter, spring, and early summer, the population of adults is naturally reduced as a result of mature males and females dying after breeding or release of young held in the brood pouch (**Figs. 4.7-4.12**). During this time, the maximum population abundance (number of individual *Mysis*) shifts to shallow (<30 m), nearshore areas where the density of juveniles can be four times that of adults in the center of the bay. Focusing trawling efforts in this region decreases the number of *Mysis* reaching adulthood later in the year even though total harvested biomass may be lower based on the small size of the juvenile *Mysis* (juvenile = 26,704 ind./lb vs adult = 18,477 ind./lb wet weight).

Our understanding of the seasonally dynamic distribution of the *Mysis* population allowed us to target the greatest number of individual *Mysis* with the goal of reducing natural recruitment. The trawling strategy would need to be different if the effort were to have focused on reducing the Lake Tahoe population. Lake Tahoe *Mysis* have a 3-4 year life cycle and do not appear to display the onshore-offshore movement observed in Emerald Bay (Morgan and Threlkeld, 1982). However, the limited amount of Biosonics Echosounder measurements that were available for Tahoe make these conclusions still very tentative, and an important focus area for future research and monitoring.

Harvest trawling commenced in November 2018 as soon as the modified trawl design proved large numbers of shrimp could be removed from the bay. Trawling continued through the end of the year when shrimp dispersed throughout the water column with the erosion of the thermocline. During this time, the trawl described above was towed for 49 hours at an average depth of 27 m (range 23.5-31m). Catch per unit effort averaged 6.9 lbs/hr. (range 3.9-11.7 lbs/hr. wet weight) with lower catches occurring when the trawl depth had a mismatch to the depth of *Mysis* aggregation as reported by the Biosonics echo sounder. The average harvest equated to 62,500 individuals every hour. Based on the high density of adult *Mysis* over a narrow depth band this was expected to yield the highest biomass of shrimp throughout the year.

Following the fall-winter harvest, the catch per unit effort (CPUE) was used to assess the probability that our trawling with the size of vessel and net we had could reach the target mysid abundance of 27 ind./m^2. At the harvest rate described it would take months of effort to achieve the target abundance, an unrealistic duration. For this reason, the focus of the trawling effort shifted to determine the most effective means of removing mysid numbers during each season of the year, or more accurately, during each stage in the shrimp life cycle.

The spring and summer harvest focused on removal of juveniles prior to them reaching maturity, thereby diminishing future reproductive success. During this time of year, juveniles occupy shallow water (<30 m) around the perimeter of Emerald Bay. Densities exceeding 400 individuals per m^2 have been recorded during June in 15-20 m of water (Morgan and Threlkeld, 1982). This density is up to ten times that of adult abundance occupying the offshore habitat at the same time.

In order to effectively trawl in shallow water, a small trawl (1.5 m x 1 m opening) was used behind a nimbler vessel. This technique proved effective at capturing juvenile *Mysis* but the small trawl opening and the small size of the *Mysis* greatly diminished the overall biomass harvested.

The shift to shallow water harvest does not appear to be an issue for the main body of Lake Tahoe. Only a small percentage of the lake is characterized by depths that could serve as a refuge for juvenile mysids (<30m). Additionally, with the 3-4 year life cycle of *Mysis* in Tahoe versus the single year life cycle of the Emerald Bay population, targeting a single cohort would not have the impact it could in Emerald Bay. Therefore, a strategy of targeting the highest biomass, regardless of year class, would be expected to have the greatest impact on the overall mysid population over time. Generally, seeking mysid populations covering a narrow vertical distribution with high abundance would lead to the greatest harvest.

5.3 Catch per Unit Effort

Trawling in Emerald Bay with our larger trawl proved most effective during the fall and early winter when the highest density of adult *Mysis* were located offshore over deep water (2.9 and 3.2 kg/hr respectively). Spring harvest using the same techniques showed a greatly reduced catch (0.6 kg/hr.). This is likely due to the loss of post reproductive adults from the population and the nearshore habitat selectivity of juveniles recently released into the water body.

Use of the small trawl, nearshore in shallow water proved successful at capturing the new cohort of *Mysis*. While the small opening diminished overall harvest (0.4 kg/hr), relative catch was similar to that of the large trawl when yield was normalized to a trawl opening of 1 m^2. These results are summarized in **Table 4.2**.

Table 5.1: Catch Per Unit Effort in Emerald Bay Using Two Trawl Designs

Season	Catch per Hour (Kg)	Trawl Size (m^2)	Normalized Catch Kg/hr (1 m^2)
Winter	3.2	13.2	0.24
Spring	0.6	13.2	0.04
Summer*	0.4	1.5	0.27
Fall	2.9	13.2	0.22

* Note that summer trawling utilized a 1.5 m^2 trawl in shallow water.

5.4 Bycatch

Any trawling effort is likely to have some level of bycatch associated with the harvest of target species. In the case of trawling for Mysis in Emerald Bay, both fish and native zooplankton were of concern. Due to the relatively large mesh size of the trawl, cladocerans were able to pass through unrestrained while some pelagic fish species were collected.

During trawling operations, the bycatch of fish species was noted. Each time the trawl was recovered, the catch was sifted by hand to remove, identify, and release any fish. Fish inadvertently captured were immediately released back to the lake. Observations of released fish indicated they swam off shortly after release.

In the interest of returning fish back to the lake as quickly as possible, identification was done visually in the field. Key anatomical features were used (adipose fin, par marks, depth of fork in caudal fin, orientation of mouth, etc.) to separate Tahoe's known species. Only two individuals were listed as an unidentified juvenile trout.

By catch was calculated to be 2.8 fish per hour during the fall-winter harvest trawling with no fish being caught during the spring effort. The majority of the bycatch was made up of juvenile kokanee salmon (76%) and adult Lahontan redside shiners (17%). Adult game fish only made up 4% of the total bycatch.

Echosounder surveys showed vertical separation of fish and Mysis around the thermocline. It is suspected that the majority of fish were captured during the deployment and retrieval of the trawl ass it passed through shallower depths. It is reasonable to assume that a larger trawl moving at faster speeds will increase bycatch. However, if the bycatch is captured during deployment and recovery, the increased catch would not be proportional to the total increase in volume of water fished during trawling. It may be possible to adjust trawl deployment and recovery strategies to further reduce bycatch.

5.5 Scaling up to Commercial Harvest

The *Mysis* harvesting operations that were conducted as part of this project were undertaken with a small, under-sized research vessel. Both the speed of the vessel and the size of the net greatly constrained the catch. Additionally, the crew could only work for 4-5 hours at a time, with a large fraction of the trawling time taken up by bringing in or letting out the net. High speed winches and real-time net depth data, equipment that is standard on a commercial trawler were not available. These factors constrained the CPUE.

At the same time, a tremendous amount of new knowledge was acquired in understanding (1) the seasonal spatial distributions of *Mysis* in both Emerald Bay and Lake Tahoe; (2) the seasonal size distribution and life stages of *Mysis*; (3) the zooplankton and phytoplankton communities of Emerald Bay, along with the nutrient and physical descriptions of the bay (of importance when assessing the impacts of a future, commercial scale trawling effort).

There were several limitations to the *Mysis* harvest that could likely be overcome by a commercial operation. Trawling by researchers was limited to 4-6 hours per night due to daytime research obligations and travel time to and from Emerald Bay. The power of the research vessel limited the overall trawl size. A more powerful vessel designed specifically for trawling could tow a larger net at an appropriate speed (>1.5 knots) while still maneuvering within the confines of Emerald Bay. Additionally, by switching to a flow-through trawl design or a high-speed net retrieval system, greater efficiency can be realized by avoiding repeated recovery and deployment of the trawl.

As a preliminary estimate, based on discussions with commercial trawl operators and designers, a commercial trawler would be able to operate for eight hours per night with minimal net retrieval time losses), at a similar trawl speed, and using a trawl net with 15x the opening. That represents a 30x increase in trawl effort. We have estimated that with our limitations it would take 290 nights to achieve *Mysis* reduction below 27 ind./m^2. With this 30x increase, it would take less than 20 nights (one month) to achieve this target. Factors such as reduced efficiency as the number of *Mysis* reduce have not been taken into account, but the possibility of reducing Emerald Bay to less than 27 ind./m^2 within 3 months seems within reason.

By way of comparison and cross-check, a commercial operation at Okanagan Lake, BC, using a continually harvesting trawl system (mother ship and trailing barge) was able to remove an average of 273-455 kg of *Mysis* nightly (hours unknown) with peak harvests of 910-1,364 kg per night (Kay 2002). During this period, *Mysis* densities at Okanagan were roughly double that of Emerald Bay (Rae and Andrusak 2006). Assuming a reduction in harvest of 50% based on available *Mysis* density, one could expect an average nightly harvest of 150 kg. This corresponds to one month (22 days).

The precise time needed would depend on daily harvest efficiency, which would diminish as the target abundance approached, as well as weather conditions that permit safe boat operations. Still, it remains promising that verified harvest rates of *Mysis* from a large water body could reduce Emerald Bay mysids to target values within a single season.

Further up-scaling to Lake Tahoe poses further unknowns. The limited number of Biosonics surveys have not yet provided a reliable estimate of horizontal distribution of *Mysis*. Given the vast size of Tahoe, this information could best be obtained by a combination of autonomous Biosonics profiles (allowing for unmanned operations) and computer modeling to understand the complex interactions between the basin-scale horizontal gyres that are known to exist in Lake Tahoe and the diurnal vertical migrations undertaken by the *Mysis*.

6.0 Experiment to compare and contrast the influence of invasive mysid shrimp and native zooplankton on the properties of the pelagic environment of Lake Tahoe and Emerald Bay

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To examine how zooplankton affect pelagic nutrient cycling and particle sizes in this oligotrophic system, we conducted semi-natural mesocosm experiments with cladocerans (*Daphnia spp*.), calanoid copepods (*Epischura nevadensis*), and mysids using the oligotrophic waters of Lake Tahoe and its more productive embayment, Emerald Bay. Previous studies have demonstrated differences in both feeding behaviors and diets between these taxa. *Daphnia* are filter-feeders preying on phytoplankton, bacterioplankton, and microzooplankton (Lampert 1987; Adrian and Schneider-Olt 1999). *Epischura* in Lake Tahoe is omnivorous (Richards et al. 1991), and calanoid copepods generally use both filter-feeding as well as raptorial feeding (Mauchline 1998). Similarly, mysids are omnivorous and use filter-feeding and raptorial feeding (Rybock 1978; Cooper and Goldman 1980; Grossnickle 1982; Ramcharan et al. 1985; Sawyer 1985; Johannsson et al. 2001). To understand the effects of zooplankton on concentrations of algae, particles (an indicator of water clarity), and nutrients, we compared water incubated with each taxon to water that lacked macrozooplankton. Additionally, we compared the aggregated effects of these taxa in Lake Tahoe water to their effects in the more productive water of Emerald Bay.

We hypothesized that *Daphnia* would have the greatest effect on phytoplankton concentrations, algal productivity and particle concentrations because of its indiscriminate grazing behavior, while copepods and mysids feed more selectively upon their prey items. We predicted that adult mysids should have the greatest effect on dissolved carbon species because of the release of dissolved organic carbon through “sloppy feeding” (Sierszen and Brooks 1982). Finally, because of the stoichiometric differences between the excretions of these taxa, copepods and mysids should lead to lower nitrogen:phosphorus ratios (measured as dissolved inorganic nitrogen/soluble reactive phosphorus), while cladocerans should increase these ratios (Madeira et al. 1982; Andersen and Hessen 1991; Brett et al. 1994; Walve and Larson 1999; McCarthy et al. 2005). Additionally, we predicted that the difference between the effects of the taxa should be greater in Emerald Bay than in Lake Tahoe because of the bay's greater productivity.

6.1 Materials & Methods

We conducted two semi-natural, mesocosm experiments: an experiment using zooplankton and water collected from Lake Tahoe in October 2019 and an experiment using zooplankton and water collected from Emerald Bay in July 2019. We modeled our experiment design after that used in Brett et al. 1994. We collected the study organisms from each system using a 500-micron zooplankton net. Due to the low density of *Daphnia spp*. in Lake Tahoe and Emerald Bay, we used *Daphnia spp.* from mesotrophic Castle Lake, CA. Because individual size can influence the grazing rate and size selection of particles (Burns 1968), we compared the size of the *Daphnia* from Castle Lake to those from Emerald Bay. We randomly selected ten *Daphnia* from each system and measured their lengths from the eye to the base of the tail spine. The average lengths of Castle Lake *Daphnia* were not significantly different from Emerald Bay *Daphnia* (Student's t-test p-value > 0.05; Castle Lake mean size: $1.2 \pm$ SD 0.154 mm, Emerald Bay mean size: $1.1 \pm$ SD 0.161 mm).

We incubated the study organisms in 10-liter plastic containers filled with water collected from Lake Tahoe and Emerald Bay. For the Lake Tahoe experiment, we used water collected from the Glenbrook water pumping facility that retrieves water from 18 m below the lake's surface. For the Emerald Bay experiment, we used water collected with a modified bilge pump from an 11-meter depth in the bay. We screened the water through an 80-micron filter prior to adding it to the containers to remove zooplankton. We employed five treatments in each experiment: Control (no macrozooplankton added), *Daphnia*, *Epischura*, Juvenile Mysid (< 12 mm length), and Adult Mysid (≥ 12 mm length). We included treatments for both adults and juvenile mysids because the size of food items influences whether mysids ingest their prey through suspension or raptorial feeding (Grossnickle 1982; Metillo 1995) and because adult mysids rely more heavily than juveniles on zooplankton prey (Rybock 1978; Lesutiene et al. 2007). Five replicates were used for each of these treatments in each experiment.

We used the same dry-weight biomass for each of the zooplankton treatments in our experiments (as in Cottingham et al. 1997). We used biomass equivalents, rather than counts of each zooplankton, because a similar study (Brett et al. 1994) noted that their species-dependent results were obscured by the use of treatments that contained unequal biomasses. We measured the average dry weight of each taxa and inoculated each mesocosm (except the Control) with the number of individuals that is equivalent to the dry-weight biomass of three adult mysids (**Table 6.3**) using hand-pipettes. Therefore, the results of our experiments are all biomass-specific.

The experiments were maintained in a temperature-controlled chamber programmed with a mean temperature of 9.7 °C and a 16-hr fluorescent light: 8-hr dark diel cycle. We re-arranged the containers in the chamber daily to randomize the amount of light that each container received, and we gently rotated the containers to discourage settling of particulate matter. We incubated the Lake Tahoe experiment for eight days and the Emerald Bay experiment for seven days.

6.1.1 Phytoplankton concentrations, grazing indices, and PPR

To determine chlorophyll-*a* concentrations in each treatment, we filtered water from each container through a Whatman 1825-047 GF/F filter, and the filters were submerged in methanol in individual film canisters for 24 hours in a dark environment (Arar and Collins 1997). We determined chlorophyll-*a* concentrations in the methanol solution with a Turner 10-AU fluorometer, and pheophytin concentrations were determined from the same samples following acidification. Because pheophytin is a degradation product of chlorophyll-*a*, the ratio of these compounds can be used as an indicator of grazing intensity (Carpenter and Bergquist 1984; Brett et al. 1994). In the Lake Tahoe experiment, biomass-specific primary productivity (PPR) was determined for four of the five replicates from each treatment. PPR was determined with radioactive C^{14} using light and dark bottles incubated for 6 hours (Steeman-Nielsen 1951; Goldman 1960), and these values were divided by the chlorophyll-*a* concentrations to calculate biomass-specific PPR.

The following formula was used to determine the relative primary productivity (PPR):

Relative PPR = ((^{14}C Assimilated$_{Light\ Bottle}$ – 14C Assimilated$_{Dark\ Bottle}$) * 1.06 * DIC * (1000 liters/m^3) * (Volume$_{Bottle}$)) / ((^{14}C Added) * 2.22×10^6 * (Geiger Efficiency) * Time * Volume$_{Filtered}$ * ChlA)

where

Relative PPR = primary productivity relative to chlorophyll a concentration; units: mg carbon/ mg chlorophyll-*a*/hr

14C Assimilated$_{Light\ Bottle}$ = radioactivity of light bottle filter; units: counts per minute (cpm)

14C Assimilated$_{Dark\ Bottle}$ = radioactivity of dark bottle filter; units: counts per minute (cpm)

1.06 = isotopic discrimination factor of radioactively-labeled carbon

DIC = DIC concentration; units: mg carbon/liter

1000 liters/m^3 = conversion factor to convert cubic meters to liters

Volume$_{Bottle}$ = volume of borosilicate glass bottles (145 ml)

14C Added = amount of 14C added to bottle (5 microcuries)

2.22 x 106 = conversion factor to convert microcuries into disintegrations per minute (DPM)

Geiger Efficiency = efficiency of Planchet counter (0.33)

Time = length of incubation (6 hours)

VolumeFiltered = volume of water filtered (50 ml)

ChlA = concentration of chlorophyll-*a*; units: mg chlorophyll-*a*/m^3

6.1.2 Particle concentrations

We measured the concentration and sizes of organic and inorganic particles from each container with a Liquilaz LS-200. The instrument measured concentrations of all particles with diameters of 0.5 microns and larger. Each sample was serially diluted with ultrapure deionized water to achieve a concentration less than 10,000 particles/milliliter so as to minimize interference in the analyzer (Heyvaert et al. 2011). A magnetic stir bar was used to stir the sample and suspend particles during measurement. Between sample measurements, we flushed the analyzer with ultrapure deionized water until the readings fell below 20 particles/milliliter. We classified all particles with diameters smaller than 5 microns as small particles and particles with diameters of 5 microns and greater as large particles.

6.1.3 Nutrient concentrations

To analyze nutrient concentrations, we first filtered water from each container through Whatman 1825-025 GF/F filters (pre-combusted at 450 °C for 4 hours). Total dissolved carbon (TDC) concentrations were measured with a Shimadzu TOC-V, and dissolved organic carbon (DOC) concentrations were measured from the same samples following acidification (Shimadzu 2003). Concentrations of ammonium, nitrate and soluble reactive phosphorus (SRP) were determined spectrophotometrically. We measured nitrate following reduction with a hydrazine-copper solution (Lamphake et al. 1967). This method determines the combined concentration of nitrate and nitrite, and we hereafter refer to these measurements as nitrate concentrations. We measured ammonium concentrations following reaction with phenol, sodium hypochlorite, and potassium nitroferrocyanide (U.S. Environmental Protection Agency 1993). We measured soluble reactive phosphorus (SRP) concentrations following acidification with ammonium molybdate and reduction with ascorbic acid (Murphy & Riley 1962). The (dissolved inorganic nitrogen) DIN/SRP ratio was calculated by dividing the sum of the ammonium and nitrate concentrations by the SRP concentrations.

6.1.4 Statistical Analyses

We used pairwise comparisons to compare each post-hoc measurement from each of the zooplankton treatments to the Control. Prior to analyzing these comparisons, a Shapiro-Wilk test (Shapiro and Wilk 1965) was used to determine if the residuals of each response variable were normally distributed, and a Bartlett's test (Snedecor and Cochran 1989) was used to test for homoscedasticity. The data were log-transformed if they did not initially pass these tests. If the data passed these tests, they were analyzed with a Dunnett's test to compare each of the response variables from each of the zooplankton treatments (*Daphnia*, *Epischura*, Juvenile Mysid, Adult

Mysid) with those of the Control. A Dunnett's test is a multiple comparison test that compares the dependent values of any number of treatments with those of a control (Dunnett 1955). We used the DescTools package (Andri Signorell et al. 2019) in R version 3.6.3 the Dunnett's tests.

If the data did not pass either the Shapiro-Wilk and Bartlett's tests following log transformation, we instead used two-sample permutation tests to test for differences in each response variable between each treatment and the Control. A two-sample permutation test is a non-parametric test that calculates a test statistic by re-assigning the observations of the dependent variable to the two sample groups N! times, in which N is the pooled number of observations of the dependent variable in both of the treatment groups (Ross 2014). We used the coin package (Hothorn et al. 2006) in R version 3.6.3 for these two-sample permutation tests.

Analyses of similarities (ANOSIM) were used to determine the differences between the treatments. ANOSIM is a nonparametric test that calculates a test statistic from within-group and among-group dissimilarities (Clarke 1993). We used separate tests for the Lake Tahoe and Emerald Bay experiments to determine if among-species dissimilarities differed between the two systems. The ANOSIMs considered the concentrations of chlorophyll-*a*, pheophytin, small and large particles, nitrate, ammonium, and SRP, and we measured the dissimilarities with Euclidean distances. We excluded measurements that were not independent. For example, the DIN/SRP ratios were excluded from the analysis because the ammonium, nitrate, and SRP concentrations were included instead. The vegan package (Oksanen et al. 2019) in R version 3.6.3 was used for these ANOSIMs and for the non-metric multidimensional scaling (NMDS) graphic presented in **Fig. 6.5**.

6.2 Results

6.2.1 Lake Tahoe

None of the zooplankton taxa significantly affected phytoplankton biovolume, phytoplankton productivity, or particle concentrations in the Lake Tahoe experiment. Specifically, none of the zooplankton treatments yielded chlorophyll-*a* concentrations, pheophytin/chlorophyll-*a* ratios, or biomass-specific PPRs that were significantly different from those of the Control (**Fig. 6.1a, c, e; Table 6.3**). However, juvenile mysids did have a marginal effect on large particle concentration.

The average concentration of large particles in the Juvenile Mysid treatment was 52% lower ($p = 0.06$) than that in the Control (**Fig. 6.2c; Table 6.3**).

Overall, the effects of these taxa on the nutrient concentrations in the Lake Tahoe experiment were minimal. However, *Daphnia* and adult mysids significantly influenced DOC concentrations. Specifically, relative to the control, DOC was 25% lower in the *Daphnia* treatment and 26% lower in the Adult Mysid treatment ($p = 0.04$, $p = 0.04$, respectively). Additionally, DOC concentrations were marginally lower in the Juvenile Mysid treatment compared to the Control (by 22%; $p = 0.06$) (**Fig 3c**; **Table 3**). Additionally, *Epischura* significantly reduced SRP concentrations, which were 35% lower ($p < 0.01$) than in the Control (**Fig. 6.4e; Table 6.3**).

6.2.2 Emerald Bay

In the Emerald Bay experiment, *Daphnia* significantly affected phytoplankton biovolume and particle concentrations, and juvenile mysids also affected small particle concentrations. The *Daphnia* treatment contained an average chlorophyll-*a* concentration that was 64% lower than the Control ($p < 0.01$) (**Fig. 6.1b**, **Table 6.4**). Additionally, the average pheophytin/chlorophyll-*a* ratio, which is an indicator for grazing rates, for the *Daphnia* treatment was marginally higher (by 33%) than the Control ($p = 0.08$). (**Fig 6.1c**, **Table 6.4**). Average small particle concentrations were 53% lower ($p = 0.03$) in the *Daphnia* treatment and 23% lower ($p = 0.02$) in the Juvenile Mysid treatment relative to the Control (**Fig 6.2a**; **Table 6.4**). Large particle concentrations were also marginally lower by 44% in the *Daphnia* treatment than in the Control ($p = 0.05$) (**Fig. 6.2b**; **Table 6.4**).

In the Emerald Bay experiment, ammonium was the only nutrient influenced by the taxa. The average ammonium concentration in each of the zooplankton treatments was significantly higher than that of the Control (**Fig. 6.4d**; **Table 6.4**). Ammonium concentrations were 428% ($p < 0.01$), 216% ($p < 0.01$), 572% ($p < 0.01$), and 391% ($p = 0.01$) higher in the *Daphnia*, *Epischura*, Juvenile Mysid, and Adult Mysid treatments, respectively, relative to the Control. Consequently, the average DIN/SRP ratio for each of the zooplankton treatments were also significantly higher than those of the Control (**Fig. 6.4h**; **Table 6.4**). Average DIN/SRP ratios were 222% ($p < 0.01$), 240% ($p < 0.01$), 230% ($p < 0.01$), and 174% ($p < 0.01$) higher in the *Daphnia*, *Epischura*, Juvenile Mysid, and Adult Mysid treatments, respectively, relative to the Control.

6.2.3 Comparisons between Lake Tahoe and Emerald Bay

The overall effects of these taxa were significantly different in the Emerald Bay experiment, but not in the Lake Tahoe experiment. The ANOSIM results for the Lake Tahoe experiment indicate that the differences between the treatments were statistically indistinguishable from the differences between the replicates ($R = 0.05$, p-value = 0.19). In contrast, the ANOSIM results for the Emerald Bay experiment indicate that these taxa generated different pelagic ecosystem structures ($R = 0.35$, p-value < 0.01). **Fig 6.5** illustrates with a non-metric multidimensional scaling (NMDS) that the treatments have a lesser degree of overlap in the Emerald Bay experiment than the Lake Tahoe experiment.

6.3 Discussion

Because *Daphnia* significantly depleted phytoplankton biovolume in the Emerald Bay experiment but not in the Lake Tahoe experiment, we suggest that *Daphnia*'s ability to reduce algal biovolume depends on the trophic state of the water. This finding is consistent with other studies showing that *Daphnia* is a less effective grazer in low-nutrient oligotrophic systems (DeMott 1982; DeMott and Kerfoot 1982; Cottingham et al. 1997; Cyr 1998; Cyr and Curtis 1999). DeMott 1982 found that *Daphnia*'s inability to specifically target suitable prey items while filtering made the taxa ill-adapted to systems with low concentrations of phytoplankton. For this reason, *Daphnia* has recolonized Lake Tahoe only during times of relatively high algal primary productivity that supported *Daphnia*'s filtering limitations (Byron et al. 1986). Given that the final chlorophyll-*a* concentrations in the Lake Tahoe and Emerald Bay *Daphnia* treatments were similar (Lake Tahoe *Daphnia* mean: 0.58 ± 0.37 µg chlorophyll-*a* / milliliter; Emerald Bay *Daphnia* mean: 0.44 ± 0.11 µg chlorophyll-*a* / milliliter), *Daphnia* may not have been able to graze phytoplankton to a lower concentration than was present in the ambient Lake Tahoe water.

Our results contrast with those of Elser et al. 1990, which found that algal biomass in Lake Tahoe decreased with increasing *Daphnia* densities. Because the *Daphnia* densities in our experiments were higher than those used in the experiments of Elser et al. 1990, it is possible that nutrient recycling by *Daphnia* may have stimulated algal growth in the Lake Tahoe experiment enough to obscure any grazing effects. Because the phytoplankton concentrations and grazing indices in the other zooplankton treatments (*Epischura*, Juvenile Mysid, Adult

Mysid) were not significantly different from the Controls in either experiment, this suggests that these organisms likely relied upon microzooplankton prey (rotifers and protozoa) to a greater extent than *Daphnia* did.

Through grazing, *Daphnia* concomitantly increased water clarity in the Emerald Bay experiment by removing small particles (diameter < 5 microns) that account for 75% of light scattering in the lake (Swift et al. 2006). Using a chlorophyll-*a*-to-particle conversion factor developed for the lake (Swift et al. 2006) along with the chlorophyll-*a* and particle concentrations of the Controls, we determined that 17% of particles were algal in the Emerald Bay experiment. Only 10% of particles were algal in the Lake Tahoe experiment. Previous studies have found that *Daphnia* remove food items from their carapace gap when unsuitable items are ingested, and these removed items can even include edible algae (Lampert 1987; Kirk 1991). Therefore, the abundance of non-algal particles may have further contributed to the ineffectiveness of *Daphnia* in controlling particle size in Lake Tahoe.

The results for the *Daphnia* and Juvenile Mysid treatments in each experiment suggest that these organisms selected different particle types. While both *Daphnia* and juvenile mysids were effective in removing small particles in the Emerald Bay experiment, juvenile mysids did not affect chlorophyll-*a* concentrations, suggesting that juvenile mysids may have selected for non-algal particles when grazing. Likewise, in the Lake Tahoe experiment, juvenile mysids were marginally effective at removing large particles. They also led to the lowest concentration of small particles despite (although this difference was not significantly different from the Control). Lasenby and Langford 1973 suggested that mysids may select non-algal particles with high surface areas, passing the particles through the digestive system and gleaning microbiota from the particle surface. Similarly, other studies have shown that mysids select detrital and inorganic particles from the benthos during daytime feeding (Van Duyn-Henderson and Lasenby 1986; Bigelow and Lasenby 1991), and this same behavior may explain mysid particle selection in the pelagic zone. Further studies into particle selection by mysids in low-nutrient water can help to further illuminate the role of mysids in removing light-attenuating and light-scattering particles.

Contrary to our predictions, none of the zooplankton treatments contributed to an elevation of TDC or DOC concentrations. Instead, *Daphnia* and juvenile mysids led to a decrease in DOC concentrations in the Lake Tahoe experiment. Furthermore, the average DOC concentrations for each of the treatments containing macrozooplankton were lower than the Controls in both experiments, though not all of these differences were statistically significant. The lack of large zooplankton in the Controls may have allowed for an accumulation of DOC that did not occur in the treatments containing the macrozooplankton, and the results suggest that these organisms may have utilized particulate organic carbon as a food source before this matter disaggregated into dissolved forms. This contrasts with previous studies showing that elevated DOC concentrations can result from *Daphnia* and mysid feeding (Lampert 1978; Sierszen and Brooks 1982). The lack of difference in TDC concentrations when compared to the Controls suggests that the release of carbon dioxide from the macrozooplankton through cellular respiration was insignificant in the experiments.

The results for nitrogen and phosphorus compounds did not conform with the differences in tissue stoichiometry and excretion stoichiometry of these taxa found by previous studies (Madeira et al. 1982; Andersen and Hessen 1991; Brett et al. 1994; Walve and Larson 1999; McCarthy et al. 2005). For instance, while we predicted that *Epischura* would lead to an increase in SRP because of the relatively phosphorus-rich excretions from calanoid copepods noted in other studies, we instead found that *Epischura* led to low SRP concentrations. The relatively low concentrations of nitrogen and phosphorus compounds in the low-nutrient waters of Lake Tahoe and Emerald Bay may have negated the stoichiometric differences between these taxa found in other studies. Considering that Lake Tahoe is primarily limited by phosphorus and secondarily limited by nitrogen (Goldman et al. 1993), our results suggest that there is no general difference between these taxa in their abilities to stimulate algal growth through nutrient excretions.

As eutrophication increases for freshwater systems, lake ecosystem processes will change as a result (Smith 2003; Vadeboncoeur et al. 2003). As Lake Tahoe continues to undergo progressive cultural eutrophication (Van Landingham 1987; Goldman 1988; Schladow 2019), its pelagic ecosystem may eventually resemble that of Emerald Bay (**Fig. 6.6**). As the lake's trophic state increases, the differences between the effects of its zooplankton taxa may magnify, as suggested by their general effects on Emerald Bay. For instance, despite that the lake currently lacks a *Daphnia* population, the results of this study suggest that this taxa could play an important role in managing the lake's algal concentrations and its clarity in the future when there is sufficient productivity to support their growth and reproduction. When this could occur is not currently understood. Additionally, because these taxa and mysid life stages dominate during different seasons (Richerson 1969; Morgan and Threlkeld 1982), these zooplankton may generate greater temporal heterogeneity in Lake Tahoe's planktonic ecosystem with increasing eutrophication. These differences may also be reflected in other eutrophying oligotrophic lakes.

Table 6.1: Secchi depth, chlorophyll-*a* concentrations, and SRP concentrations for Lake Tahoe and Emerald Bay. Lake Tahoe chlorophyll-*a* and SRP concentrations were determined from an 18-meter depth; Emerald Bay chlorophyll-*a* and SRP concentrations were determined from an 11-meter depth. Lake Tahoe chlorophyll-*a* and Emerald Bay Secchi measurements were measured in October 2019; all other measurements were performed in July 2019 (Schladow 2019).

	Secchi Depth (meters)	**Chlorophyll-*a* (micrograms/liter)**	**SRP (micrograms/liter)**
Lake Tahoe	21.6[1]	0.4	3.0[1]
Emerald Bay	14	0.92	3.0

Table 6.2: Description of the five treatments in each experiment. Each treatment consisted of five replicates.

Treatment	**Description**
Control	No zooplankton added
Daphnia	360 *Daphnia spp.* individuals
Epischura	1,680 *E. nevadensis* individuals
Juvenile Mysid	21 juvenile mysid individuals (length < 12 mm)
Adult Mysid	3 adult mysid individuals (length ≥ 12 mm)

Table 6.3: Results for the Lake Tahoe experiment. The values outside of the parentheses are the percent increase (positive value) or decrease (negative value) in the parameter relative to the Control of the experiment. The values in parentheses are the p-values of the statistical tests. Values marked with a P indicate that a permutation test was used, all other values were determined with a Dunnett's test. Values that are statistically significant ($p < 0.05$) are bolded.

	Daphnia	***Epischura***	**Juvenile Mysid**	**Adult Mysid**
Chlorophyll-*a*	-20 (0.98)	87 (0.17)	98 (0.10)	5 (1)
Pheophytin / Chlorophyll-*a* Ratio	28 (0.77^{P})	95 (0.49^{P})	-68 (0.24^{P})	96 (0.20^{P})
Biomass-specific PPR	93 (0.22^{P})	-180 (0.12^{P})	-61 (0.20^{P})	25 (0.32^{P})
Small Particles (≥ 0.5 µm & < 5 µm)	-19 (0.96)	15 (1)	-48 (0.11)	3 (1)
Large Particles (≥ 5 µm)	-45 (0.38)	-31 (0.38)	-52 (0.06)	-2 (1)
TDC	-4 (0.48^{P})	1 (0.79^{P})	-7 (0.17^{P})	-3 (0.48^{P})
DOC	**-25 (0.04^{P})**	-17 (0.12^{P})	-22 (0.06^{P})	**-26 (0.04^{P})**
NO_3	9 (0.55^{P})	0 (1^{P})	17 (0.25^{P})	4 (0.74^{P})
NH_4	-47 (0.27)	-43 (0.49)	-4 (1)	-24 (0.70)
SRP	-7 (0.91)	**-35 (< 0.01)**	12 (0.65)	-14 (0.51)
DIN/SRP Ratio	-25 (0.56)	9 (0.98)	-12 (0.94)	-5 (1)

Table 6.4: Results for Emerald Bay experiment. Details are the same as for Table 6.3.

	Daphnia	*Epischura*	Juvenile Mysid	Adult Mysid
Chlorophyll-*a*	**-64 (< 0.01)**	25 (0.81)	5 (1)	16 (0.88)
Pheophytin / Chlorophyll-*a* Ratio	33 (0.08)	2 (1)	2 (1)	4 (0.99)
Small Particles (≥ 0.5 µm & < 5 µm)	**-53 (0.03^{P})**	-17 (0.18^{P})	**-23 (0.02^{P})**	-9 (0.12^{P})
Large Particles (≥ 5 µm)	-44 (0.05)	11 (0.99)	-37 (0.25)	-13 (0.98)
TDC	-8 (0.16)	1 (1)	-5 (0.51)	-1 (1)
DOC	-10 (0.22^{P})	-12 (0.13^{P})	-12 (0.12^{P})	-13 (0.11^{P})
NO_3	3 (0.93^{P})	-29 (0.35^{P})	14 (0.64^{P})	0 (1^{P})
NH_4	**428 ($< 0.01^{P}$)**	**216 ($< 0.01^{P}$)**	**572 ($< 0.01^{P}$)**	**391 (0.01^{P})**
SRP	-26 (0.43)	-35 (0.19)	-10 (0.96)	-13 (0.88)
DIN/SRP Ratio	**222 (< 0.01)**	**240 (< 0.01)**	**230 (< 0.01)**	**174 (< 0.01)**

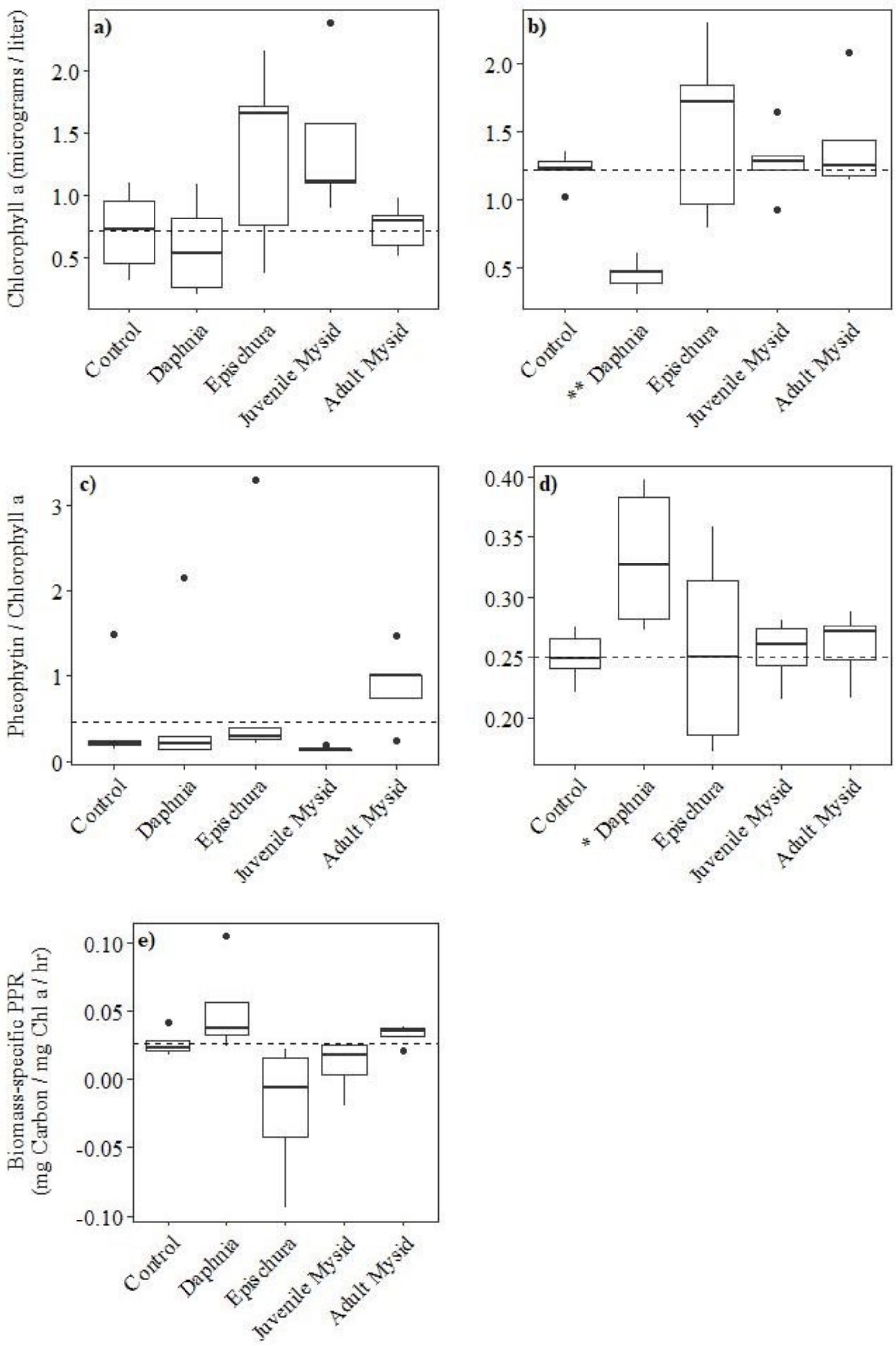


Fig. 6.1: Chlorophyll-*a* concentrations, pheophytin / chlorophyll-*a* ratios, and biomass-specific PPR. The Lake Tahoe experiment values are shown in the graphs in the left-hand column, and the Emerald Bay experiment values are shown in the graphs in the right-hand column. Treatments that are significantly different ($p < 0.05$) from the Control are marked with ** below the x-axis label; treatments that are marginally different ($p < 0.1$) are marked with *. The average value of the Control is marked with a dashed line. For each boxplot, the solid horizontal line in the box signifies the median value for that treatment, while the edges of the box signify the first and third quartiles. Note that the y-axis scales are dissimilar.

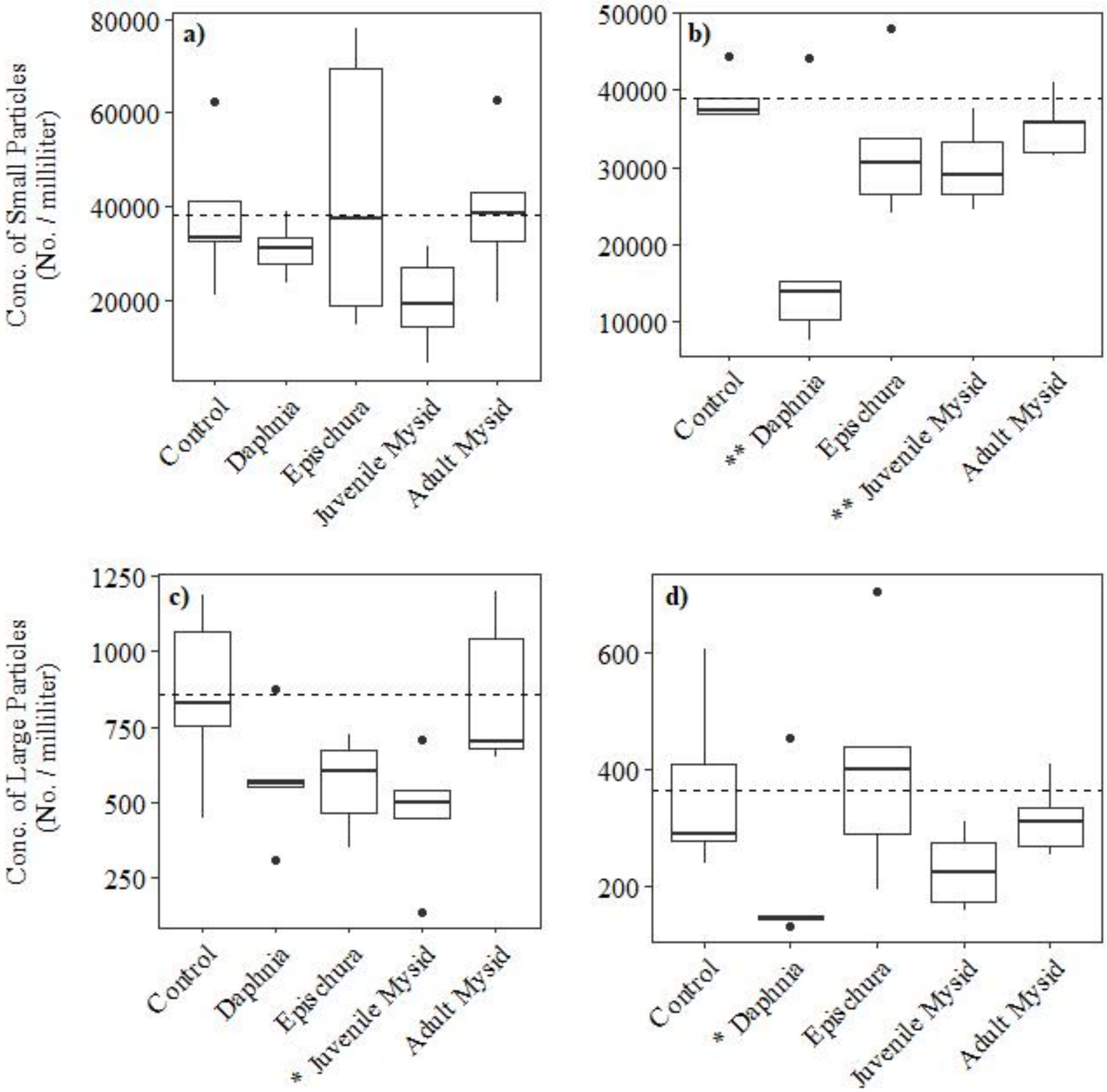


Fig. 6.2: Concentrations of small (≥ 0.5 µm & < 5 µm), large (≥ 5 µm) and cumulative (≥ 0.5 µm) particles in the Lake Tahoe and Emerald Bay experiments. Details are the same as for Fig 6.1. Note that the y-axis scales differ between subplots.

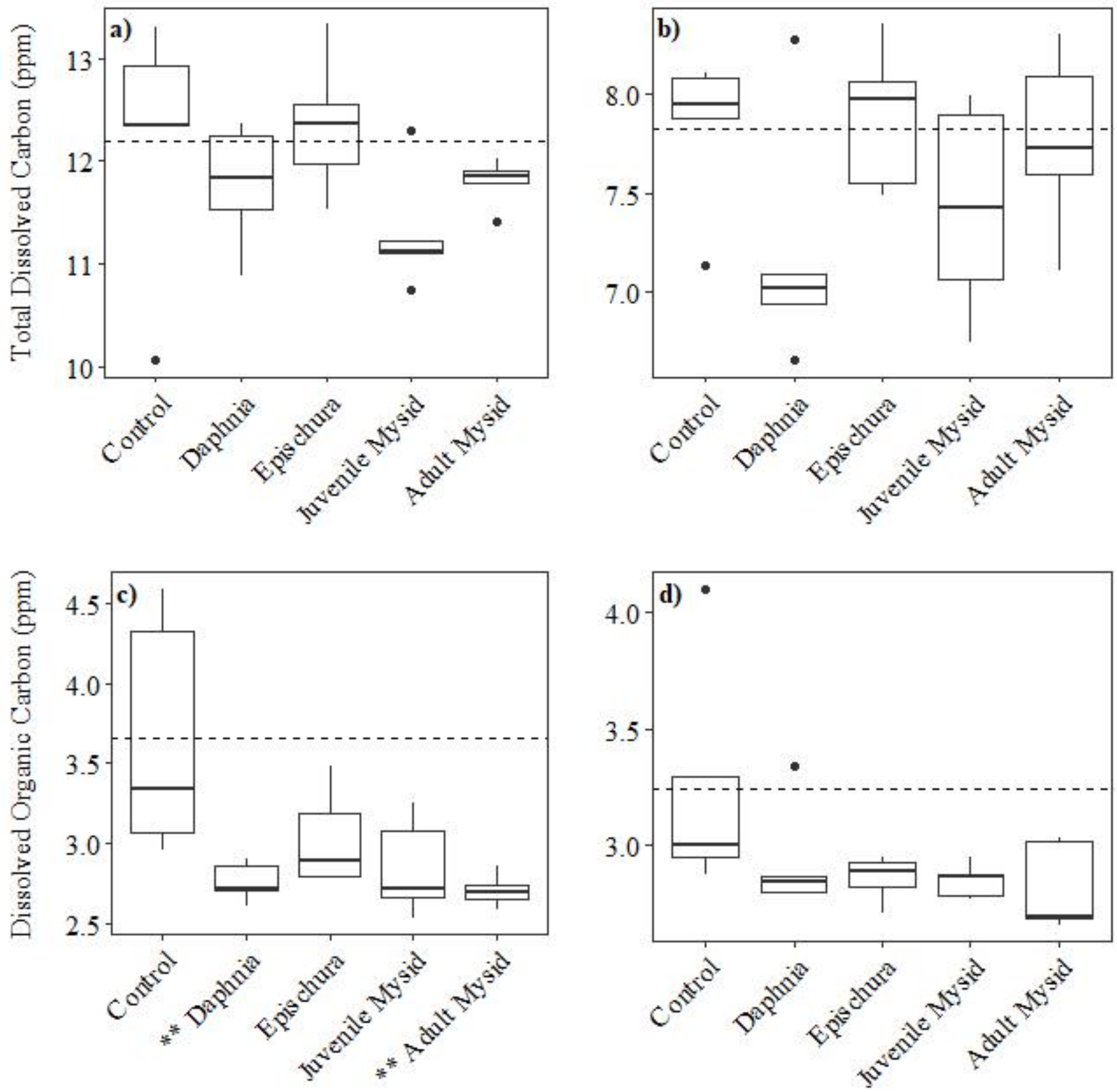


Fig. 6.3: Concentrations of TDC and DOC in the Lake Tahoe and Emerald Bay experiments. Details are the same as for Fig 6.1. Note that the y-axis scales are dissimilar.

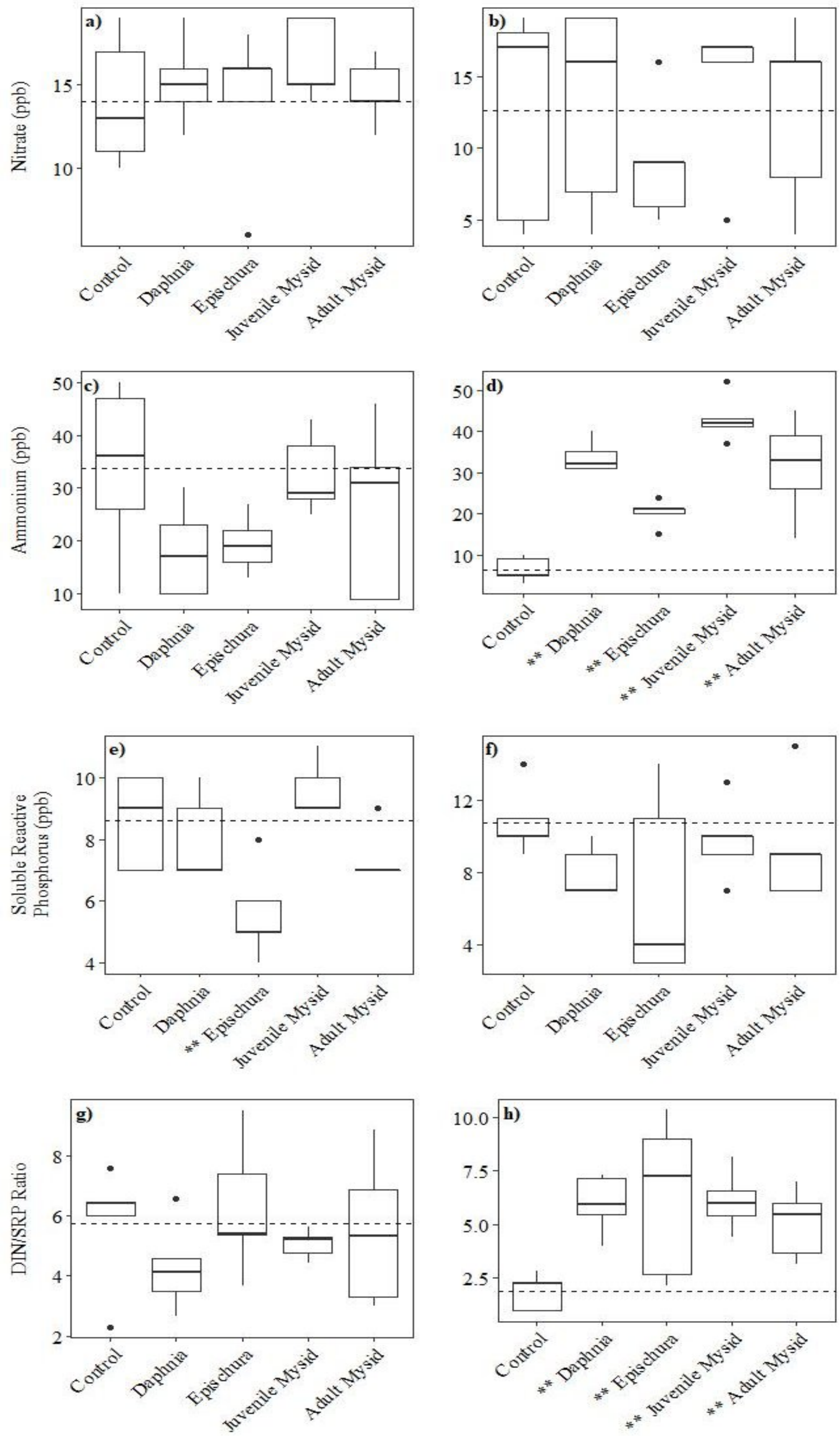


Fig. 6.4: Concentrations of nitrate, ammonium, SRP, and the DIN/SRP ratios in the Lake Tahoe and Emerald Bay experiments. Details are the same as for Fig 6.1. Note that the y-axis scales are dissimilar.

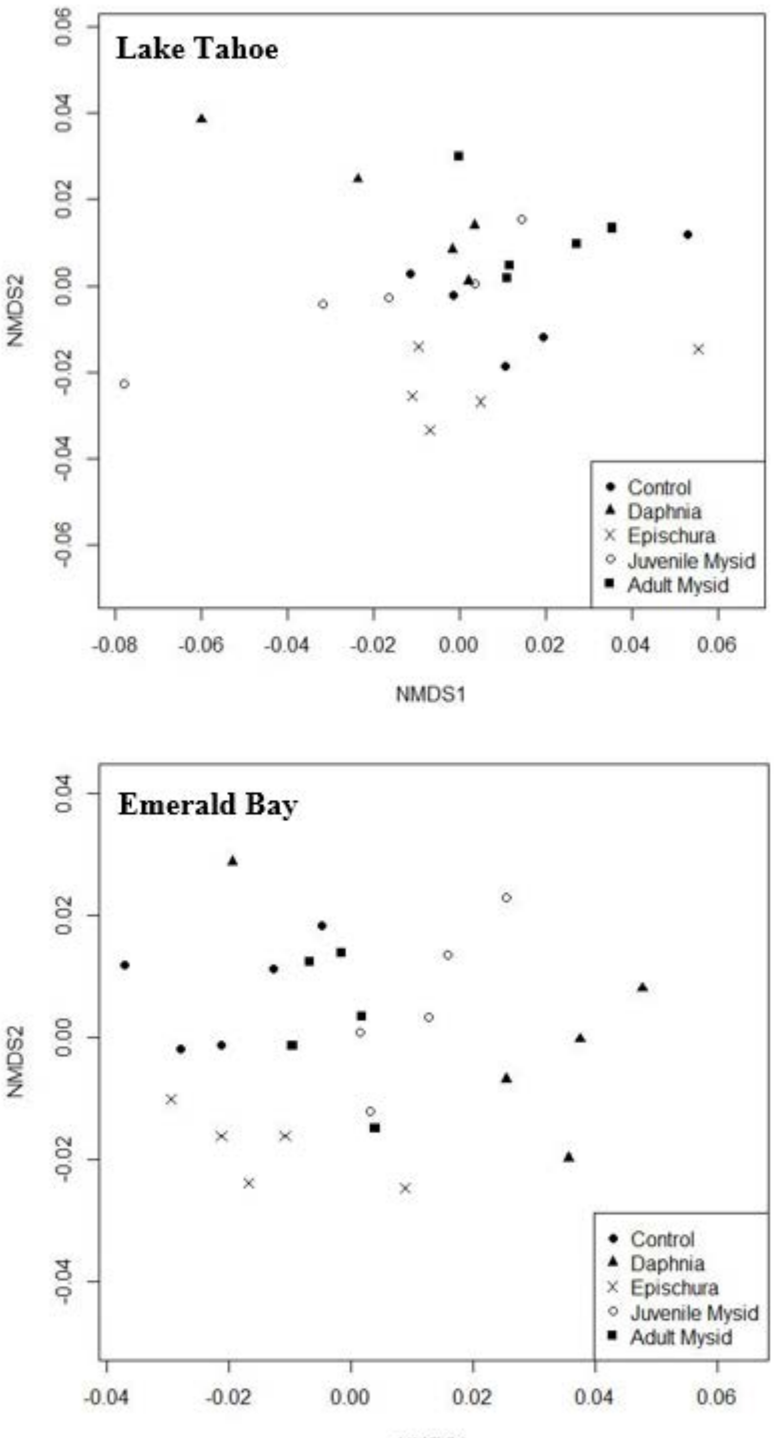


Fig. 6.5: Non-metric multidimensional scaling (NMDS) analysis of the Lake Tahoe and Emerald Bay experiments. Dissimilarities were measured with Euclidean distances.

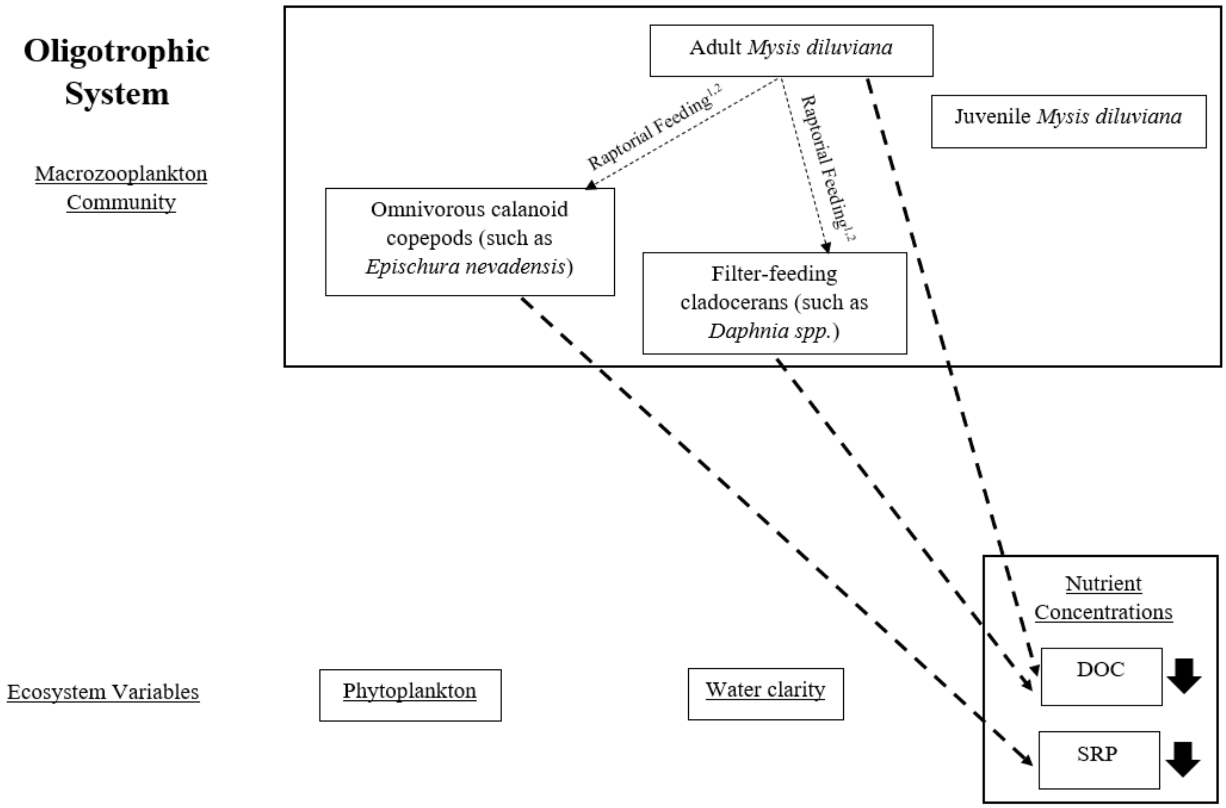


Fig. 6.6: Conceptual model showing the relationships among macrozooplankton and pelagic processes as demonstrated in the Lake Tahoe (oligotrophic) experiment. 1) Rybock 1978; 2) Sawyer 1985.

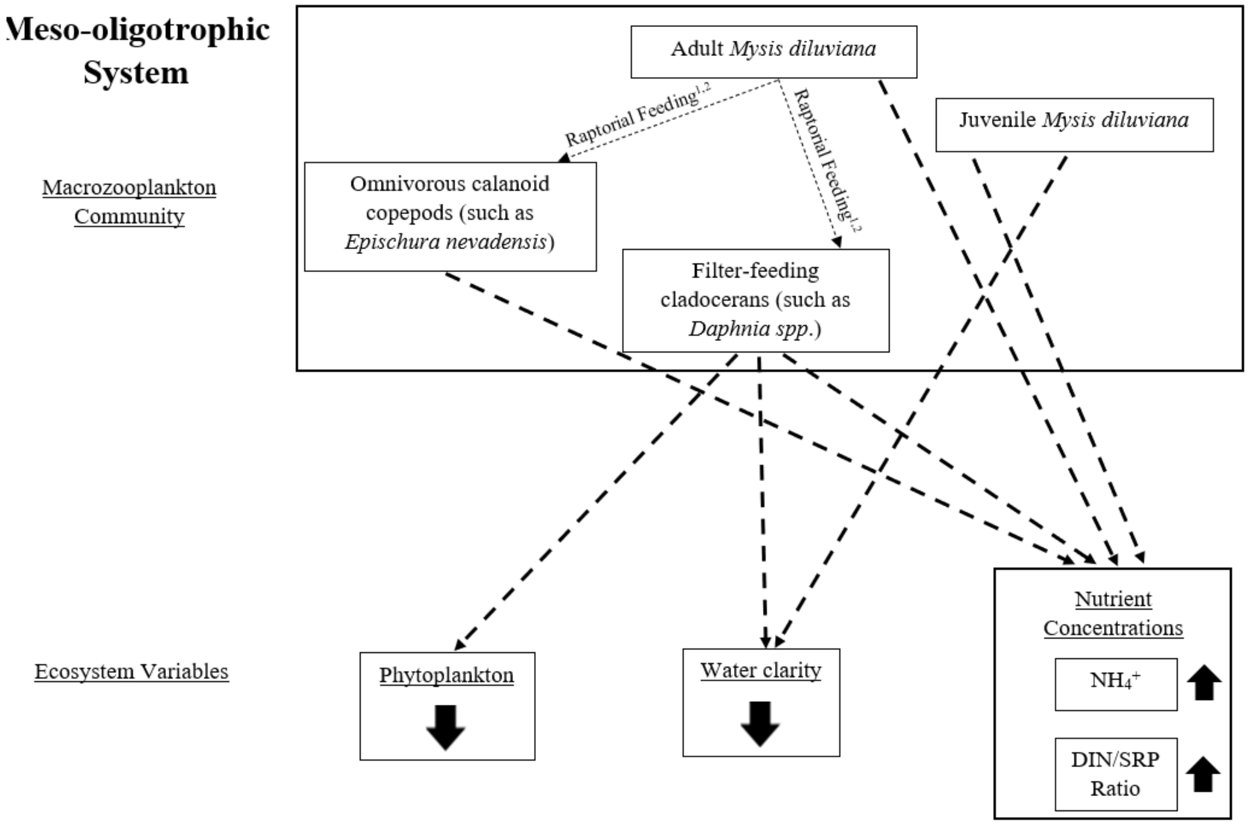


Fig. 6.7: Conceptual model showing the relationships among macrozooplankton and pelagic processes as demonstrated in the Emerald Bay (meso-oligotrophic) experiment. Sources are the same as for Fig 6.6.

7.0 Quantify the feeding behavior of mysids in two locations, Emerald Bay and Lake Tahoe from early and late summer

7.0.1 Diet Analysis

The mysid shrimp collected by UC Davis TERC from spring, summer, fall, and winter of 2018 and 2019 from Emerald Bay and Lake Tahoe were analyzed for their diet. Samples were preserved initially with 10% sucrose formalin and then transferred to 90% ethanol for long-term storage. The foreguts were removed from the mysids and smeared in heated glycerin gel mounted to a microscope slide. Preliminary dissections of dozens of macrozooplankton from the Lake Tahoe zooplankton community were used to corroborate the presence of mandibles in the mysid guts. Relatively small organisms that were ingested whole (rotifers, phytoplankton, pollen) were identified and enumerated. Relatively larger individuals that were not ingested whole (copepods, cladocerans) were instead enumerated by counting the occurrences of mandibles identified to species (Rybock 1978). For these individuals, the number of prey individuals ingested was determined by dividing the number of mandibles of the species by two, using the assumption that two mandibles indicated that one individual was consumed (Caldwell et al. 2016). Any other body parts of larger organisms were not counted or considered but were instead considered unidentified organic matter. The organisms were identified to the following general groups: *Daphnia spp.*, *Bosmina longirostris*, unidentified cyclopoids, *Epischura nevadensis*, *Diaptomus tyrelli*, *Kellicottia longispina*, *Keratella cochlearis*, non-diatom algal cells, diatom cells, pollen grains, and unidentified organic matter using a compound microscope. The relative percent contributions of the prey items to the stomach contents of the individual mysid was also estimated. Pictures of typical prey items are shown in **Fig. 7.3**.

7.0.2 Stable Isotope Analyses

Amino acid stable isotope analysis was used to determine the long-term energetic feeding behavior of mysid shrimp. Amino acid stable isotope analysis is capable of minimizing the variability in isotopic signatures by utilizing δ13C and δ15N signatures that are conserved across taxonomic groups (Bowes & Thorp 2015; Bowes and Thorp 2017). Samples were dried at 50 °C for 1 hour and then ground to a powder. Because of the relatively low dry weight biomass of mysid shrimp in Lake Tahoe, the dried tissues of three separate adult mysids were analyzed as one merged sample. Three individuals from each of the following groups were analyzed: a)

Emerald Bay individuals collected in May 2018, b) Emerald Bay individuals collected in August 2018, c) Lake Tahoe individuals collected in May 2018, d) Lake Tahoe individuals collected in August 2018, e) Emerald Bay individuals collected in May 2019, f) Emerald Bay individuals collected in August 2019, and g) Lake Tahoe individuals collected in May 2019. Samples were analyzed by the UC Davis Stable Isotope Facility using mass spectrometry (Thorp and Bowes 2017; stableisotopefacility.ucdavis.edu/compoundspecific.html).

The δ13C results (mean values and standard deviations) were analyzed with FRUITS, a Bayesian mixing model used to determine the relative contributions of carbon sources to the diets of organisms (Fernandes 2014). δ13C values for alanine, asparagine, aspartic acid, glutamine, glutamic acid, glycine, isoleucine, leucine, phenylalanine, proline, and valine and the associated standard deviations were included in the model. Published δ13C values for these amino acids in cyanobacterial, green algal, fungal, C3 plants (an average of terrestrial, aquatic, crop, and grasses), and terrestrial C4 plants were used as sources in the model (Thorp and Bowes 2017). Trophic position of the organisms was determined using the following equation (Bowes and Thorp 2015; Thorp and Bowes 2017):

$$\text{Trophic Position} = (((\delta^{15}N_{\text{Glutamic Acid + Glutamine}} - \delta^{15}N_{\text{Phenylalanine}}) - 3.4) / 7.6) + 1$$

7.1 Results

7.1.1 Diet analysis

In Lake Tahoe, for all seasons analyzed, most of the content in mysid foreguts is unidentifiable organic matter. Pollen, *Epischura* and *Kellicottia* are the most common identifiable components of the diet, and pollen is a particularly large component of mysids collected in May 2018 (21.7%), while *Epischura* is a large component in mysids from May 2019 (32.5%). Other prey items (cladocerans, *Keratella*, algae, *Diaptomus*) are only a small contribution to the diets of the mysids (**Table 1**). In Emerald Bay, pollen and *Kellicottia* are the most common identifiable diet components, and most of the content in the foreguts is unidentifiable organic matter. Pollen is a particularly large component of diet in mysids collected from May 2018 and October 2019 (20% and 23.8%, respectively). The overall diets of mysids collected from 2018 and 2019 are largely similar (**Table 7.2**).

7.1.2 Stable isotope analysis

The carbon sources of mysids varied dramatically between seasons and between systems. For instance, in May 2018, C3 plants were the dominant basal carbon source in Lake Tahoe, contributing approximately 60% of basal carbon (**Fig. 7.1**). However, during this same period, green algae was the dominant basal carbon source for mysids in Emerald Bay, contributing approximately 80% of basal carbon in that system (**Fig. 7.2**). This contrasts with results from August 2018 which indicated that green algae contributed approximately 80% of basal carbon in Lake Tahoe mysids and C3 plants contributed approximately 70% of basal carbon in Emerald Bay mysids. For both spring measurements (May 2018 and May 2019), C3 plants were the dominant primary carbon source for Lake Tahoe mysids and green algae were the dominant basal carbon source in Emerald Bay mysids. A general pattern in primary carbon sources in the summer is not apparent from the results. Carbon sourcing from terrestrial C4 plants and cyanobacteria was consistently low in both Emerald Bay and Lake Tahoe for all seasons. Fungus consistently contributed approximately 10% of basal carbon across the measurement periods and for both systems. Analysis of the trophic positions of mysids consistently indicated that mysids occupied trophic level 3 (secondary consumers) in summer and fall and in both Emerald Bay and Lake Tahoe (**Fig. 7.3**).

Table 7.1: Average percent contributions of prey items to mysid diets in Lake Tahoe.

		Feb 2018 (n = 5)	May 2018 (n = 3)	August 2018 (n = 5)	December 2018 (n = 5)	March 2019 (n = 5)	May 2019 (n = 5)	October 2019 (n = 5)
Cladocerans	*Daphnia*	0	0	0	0	0	0	0
	Bosmina	0	0	0	5	0	1.25	26.5
	Unidentified	0	0	0	0	0	0	0
Copepods	*Epischura*	6	3.33	5	5.56	1	32.5	3
	Diaptomus	0	0	0	3.33	0	0	2
	Cyclopoid	0	0	0	0.56	0	0	0
	Unknown	0	0	0	0	0	0	0
Rotifers	*Kellicottia*	6	3.33	9	7.22	1.5	0.63	1
	Keratella	0	0	0	0	0	0	0
Algae	Diatom	0	0	0	0	0	0	0
	Non-diatom	4	3.33	2	1.67	0	0	0
	Pollen	5	21.67	10	6.11	4	4.38	0.5
	Unidentified Organic Matter	87	70	70	67.8	93.5	60.63	47.7

Table 7.2: Average percent contributions of prey items to mysid diets in Lake Tahoe.

		February 2018 (n = 4)	May 2018 (n = 5)	August 2018 (n = 5)	December 2018 (n = 5)	March 2019 (n = 5)	May 2019 (n = 6)	October 2019 (n = 4)
Cladocerans	*Daphnia*	0	0	1	0	0	0	0
	Bosmina	0	0	7	2	0	0	0
	Unknown	0	0	0	0	0	0	0
Copepods	*Epischura*	0	0	3	1	0	4.58	0
	Diaptomus	0	0	2	0	0	0	0
	Cyclopoid	1.25	1	1	2	0	1.25	0
	Unknown	0	0	0	0	0	0	0
Rotifers	*Kellicottia*	1.25	4	6	5	1	7.92	0.63
	Keratella	0	1	2	1	1	9.17	0
Algae	Diatom	0	0	0	2	0	0	0
	Non-diatom	1.25	3	2	2	0	0	0
	Pollen	5	20	14	6	12	5.42	23.75
	Unidentified Organic Matter	95	78	70	90	86	71.7	75.63

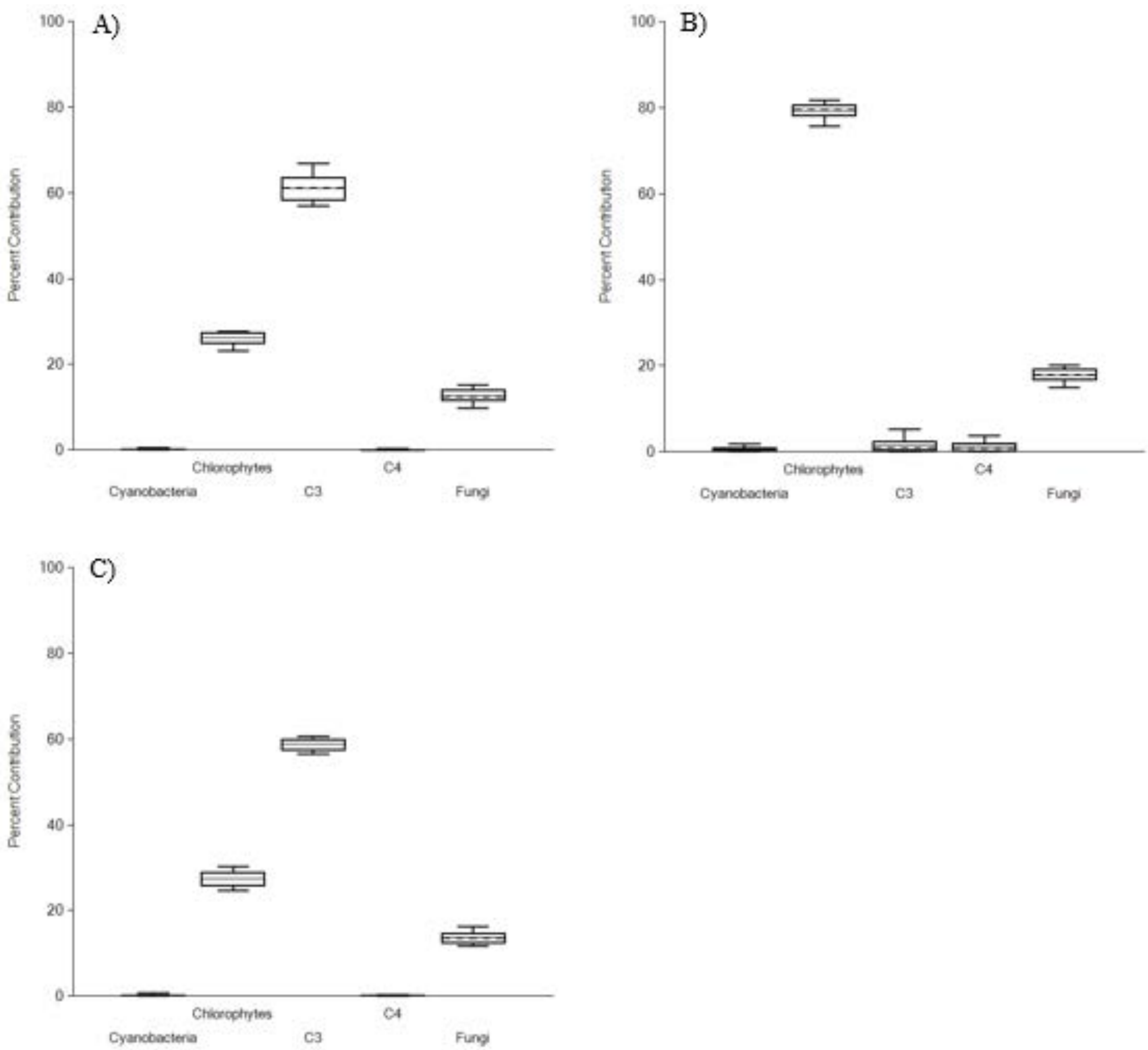


Fig. 7.1: FRUITS model output for basal carbon sources of Lake Tahoe mysids determined through amino-acid-specific stable isotope analysis. Collected in A) May 2018, B) August 2018, and C) May 2019. Each month's results are based upon the analysis of one sample containing the combined tissues of three adult (>12 mm) mysids. Each box contains the mean (solid horizontal line), median (dashed horizontal line), 68% confidence interval (box edges), and 95% confidence interval (whiskers) generated from the posterior distribution of the model.

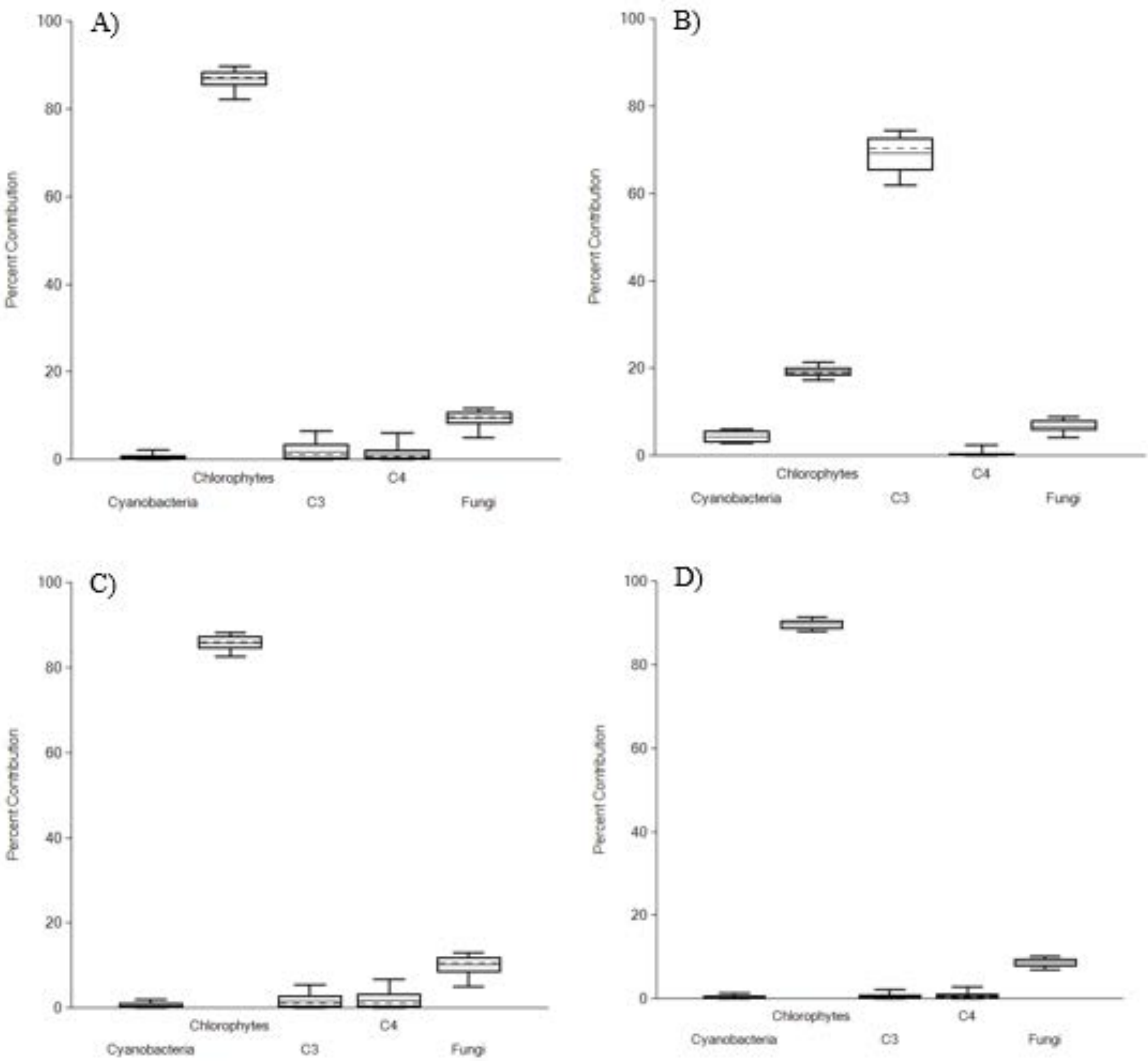


Fig. 7.2: FRUITS model output for basal carbon sources of Emerald Bay mysids determined through amino-acid-specific stable isotope analysis. Collected in A) May 2018, B) August 2018, C) May 2019, and D) August 2019. Each month's results are from analysis of one sample containing the combined tissues of three adult (>12 mm) mysids. Each box contains the mean (solid horizontal line), median (dashed horizontal line), 68% confidence interval (box edges) and 95% confidence interval (whiskers) generated from the posterior distribution of the model.

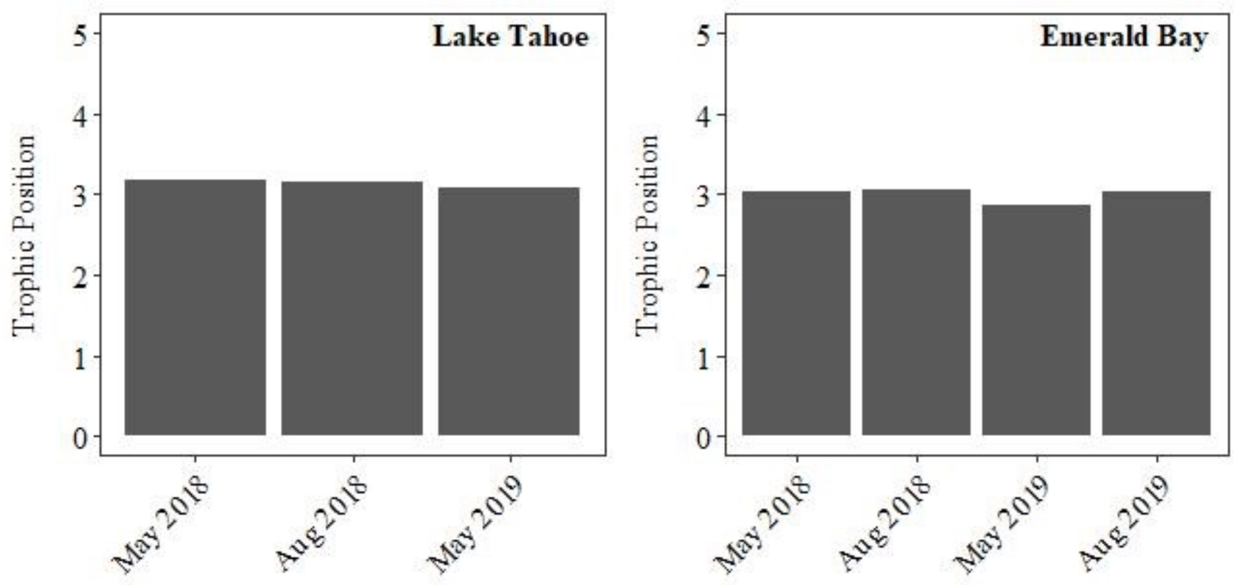


Fig. 7.3: Trophic positions of mysids determined with amino-acid-specific stable isotope analysis. One sample containing tissues from 3 adult (>12 mm) mysids were analyzed from each system and from each month. Trophic position 1 = primary producer; trophic position 2 = primary consumer; trophic position 3 = secondary consumer.

7.2 Discussion

$\delta^{15}N$ analysis indicated that mysids were consistent secondary consumers or eating zooplankton or invertebrates which eat algae (trophic position ~ 3) in Emerald Bay and Lake Tahoe (**Fig. 7.3A, B**). The lowest positions occurred in Lake Tahoe mysids in January (2.8 ± 0.2) and May 2012 (2.8 ± 0.3), while the highest, occurred in mysids in Lake Tahoe mysids in November 2011 (3.2 ± 0.2). values indicate an omnivorous diet consisting of primary producers but predominantly primary consumers like zooplankton or benthic invertebrates.

In Emerald Bay and Lake Tahoe, C3 plants (terrestrial or aquatic plants), or green algae consistently provided the majority of primary carbon for mysids (**Fig. 7.2**). C3 plants were the dominant primary carbon source for Lake Tahoe mysids in May 2018 and May 2019 and for Emerald Bay mysids in August 2018, providing 61% ± 2.4, 59% ± 1.1, and 69% ± 3.5 of primary carbon, respectively. In Lake Tahoe, green algae provided 79% ± 1.5 of primary carbon for mysids in August 2018. In Emerald Bay, green algae provided 87% ± 1.8, 86% ± 1.4, and 90% ± 0.9 of primary carbon for Emerald Bay mysids in May 2018, May 2019 and August 2019, respectively. Fungus contributed 9-18% of primary carbon. Cyanobacteria and C4 plants contributed minimally as a source of carbon, and the highest contribution from these carbon sources was a 4% ± 0.01 contribution from cyanobacteria to Emerald Bay mysids in August 2018. The relevant diets data support these findings suggesting that terrestrial pollen may play an important role in augmenting mysid energy budgets for Tahoe.

8.0 Summary of Lessons Learned from Emerald Bay and Lake Tahoe: 2018-2020

This project, when combined with the earlier six-year monitoring (2011-2017) program, constitutes the largest, continuous body of scientific data assembled for Emerald Bay. Previous data collections for Emerald Bay were conducted as part of Tahoe-wide monitoring projects in the late 1960s (Goldman 1974), and a doctoral dissertation (Morgan 1979) conducted in the 1970s. Ecosystem and physical mixing dynamics in Lake Tahoe have changed in the last half century, and it is almost certain that Emerald Bay has changed as well, although it has had far less scientific attention. Fortunately, the continuous Lake Tahoe data collected since 1968 provides a baseline reference to compare and quantify changes that may have transpired in Emerald Bay during the time periods where no direct measurements were made.

One thing that is evident, and will be addressed below, is that there are many scientifically interesting and ecologically important questions with direct management implications that remain to be studied in Emerald Bay and in Lake Tahoe. While some of the specific questions are addressed through the objectives of this work, several outstanding scientific questions are called out in the recommendations for future work below. The intent of this project was not to be the definitive study of Emerald Bay, but to address a very specific task, namely to: "*plan, test and optimize a strategy to improve water clarity in Lake Tahoe by reducing the abundance of Mysis shrimp.*"

As described in the body of the report, this project was motivated by the major perturbation that was fortuitously observed in Emerald Bay from 2011-2017. Many things were not monitored during that period (it was an unfunded project), but the long-established, well documented *Mysis*-cladoceran interaction appeared to be playing out. An outcome of this interaction was the dramatic increase in water clarity that followed the increase in population of Daphnia. The magnitude and duration of the clarity shift was an unambiguous ecosystem response to the zooplankton dynamics. Although such a clarity shift had not previously been reported before at Lake Tahoe in response to *Daphnia*, it has been reported in other systems (e.g. Lake Washington, where return of *Daphnia* resulted in a doubling of clarity). This new understanding and the extensive literature on the grazing behavior of cladocerans, when combined with the vast body of work at Tahoe that has demonstrated the link between clarity and fine particle concentration, has strengthened our conceptual model of ecosystem dynamics in Lake Tahoe.

This bi-State funded project was aimed at exploring whether the effects of the "natural" removal of *Mysis* that was witnessed in Emerald Bay could be repeated through a program of deliberate trawling for *Mysis*, and whether that approach could be extended to the far larger Lake Tahoe water body. With the realization that the physical speed and maneuverability limitations of our research vessel, combined with the net requirements for Emerald Bay, would not allow for the complete removal of *Mysis* as planned, we focused on conducting the work necessary for planning, testing and optimizing the strategy for Emerald Bay and Lake Tahoe. The major lessons learned, together with suggested actions, are summarized below. A plan for the controlled removal of *Mysis* from Emerald Bay and Lake Tahoe is presented in Chapter 9.0.

Lesson 1 – Limnological Attributes of Emerald Bay are Analogous to Lake Tahoe -– For the purposes of the *Mysis*-Cladoceran-Clarity relationship, Emerald Bay can be viewed as an analog of the larger lake dynamics and can be used to guide future trawling operations in Lake Tahoe. Despite their large depth and area differences, there are many similarities between the two water bodies including: the key forcing dynamics (meteorology and hydrology); the physics associated with mixing and especially seasonal deepening of the thermocline; the nutrient chemistry (NO_3 and THP); the phytoplankton community makeup; and the zooplankton community makeup. This was confirmed by the suite of monthly and other measurements taken in Emerald Bay during the project and the ongoing Lake Tahoe monitoring. The similarities of the biology were first noted by Goldman (1974) and so it is almost certain that both water bodies have been changing concomitantly over the decades. The mesocosm experiments described in Section 6.0 suggested there may be a difference in feeding behavior between Lake Tahoe and Emerald Bay need to be interpreted with extreme caution. The water samples were taken on only one occasion at one point in each water body, and no determination of the algal speciation (and size) was made.

The observed differences in the clarity of the two water bodies is likely the result of a slightly larger concentration of fine particles in Emerald Bay (observed as part of this project). This likely stems from the larger watershed to lake volume ratio of Emerald Bay compared to Lake Tahoe. Secchi depth in Emerald Bay is on the order of 10 m, far higher than most lakes.

Emerald Bay's primary production (PP) has been said to be higher than Lake Tahoe's. An extensive search of the existing literature showed that conclusion was derived from two very limited sets of measurements. PP measurements were first collected at several sites in Tahoe and Emerald Bay in the summers of 1967-1971, during the time period when *Mysis* introduction was disrupting the entire lake food web. In 1978 a second set of summer PP measurements were taken in Emerald Bay. Both sets of measurements showed higher summer PP in Emerald Bay, but it is unknown if this holds true presently, and whether it extends over the annual cycle and across the euphotic zone. In the 40 years since those measurements, during which time PP in Lake Tahoe has increased by a factor of four, no major shift in zooplankton populations have been observed. This suggests that the impacts of PP on the relationship between *Mysis* and cladocerans may not be significant in this system.

The final area where the two water bodies differ is in the *Mysis* life histories. It has long been documented that in Emerald Bay, *Mysis* have a 1-2 year life span, whereas in Lake Tahoe they have a 3-4 year lifespan. This is expected to have little impact on the *Mysis* removal plans, as it is a feature that can be incorporated into population models for determining trawling rates. However, it does indicate that there may well be differences in the feeding patterns of the two populations, and the flow of carbon and nutrients related to differences in population demographics.

Suggested Actions

- As Emerald Bay could be used as an analog for Tahoe for future management and scientific questions, a baseline monitoring program should be established in Emerald Bay in order to identify deviations and synchronies between the two. Emerald Bay will be quicker to respond to changes than the main lake and could serve as a sentinel for Lake Tahoe.

- The measurement of annual PP in Emerald Bay should be considered as part of future monitoring, including the relative contributions of benthic and pelagic sources of production to ecosystem totals.
- The monitoring program in both Lake Tahoe and Emerald Bay should be expanded to capture more of the food-web interactions.

Lesson 2 – Utilizing Seasonal Stratification Dynamics Increases Harvest Efficiency – The thermocline that forms in the spring and persists till the late fall acts to focus *Mysis* populations in concentrated layers at night, and therefore provides a high abundance layer that can be trawled with greater efficiency. *Mysis* were consistently observed to migrate to a level within the thermocline such that they did not exceed their thermal tolerance. Focusing on the fall season for trawling had additional advantages. First, the *Mysis* in Emerald Bay were adults by the fall, and often gravid. Therefore, harvesting at this time reduced both the current year's adult population and the following year's population. By fall, boat traffic was also greatly reduced, allowing for greater unfettered access to the lake and less potential disturbance to recreational users of the system.

The spring stratification period also presented a unique opportunity in Emerald Bay. Juvenile *Mysis* were present in far higher concentration nearer to shore at this time but were still constrained vertically by the thermocline. This presents the possibility of a separate trawling season for juveniles (possibly with a modified strategy) occurring at a second time of the year.

Insufficient data exists to know whether this two-season potential exists at Lake Tahoe and whether it can be utilized to further optimize the removal of *Mysis*.

Suggested Actions

- More thoroughly characterize the seasonal areal distribution and age distribution of *Mysis* within Lake Tahoe. This will enable the determination of whether *Mysis* can be efficiently harvested in the spring in Lake Tahoe and how long the summer/fall sampling can continue for.

- Develop a population model for *Mysis*, particularly for Lake Tahoe, where the 3-4 year life cycle introduces greater complexity. The inter-annual variability that the *Mysis* shows that the system is dynamic.

- Develop population models for *Daphnia* and *Bosmina* to better allow planning in a future condition where *Mysis*, *Daphnia* and *Bosmina* could coexist. These models would need to take into account future temperature changes (as water temperature provides different barriers to each species).

Lesson 3 – Bioacoustics Successfully Determined *Mysis* Location – Adapting methods pioneered by others, boat-mounted bioacoustics were an extremely valuable method for determining both the vertical and horizontal position of *Mysis* in the water column. For Emerald Bay fairly complete areal maps, as well as vertical profiles of *Mysis* distribution, could be produced, with far greater accuracy than using vertical trawls alone (the interpolation and extrapolation is greatly reduced). For Lake Tahoe, the size of the lake presented a challenge. Only 50-60 km of bioacoustics survey could be completed in a very long night, so horizontal distribution and variability could not be assessed very well.

Alternatives that could be explored to reduce costs and increase areal coverage is using combinations of Autonomous Surface Vehicles (ASVs) or Autonomous Underwater Vehicles (AUVs) as the platforms for the Echosounder. These vehicles would permit complete night surveys under all conditions with no need for a two-person crew to be present the entire time. A surface-vessel mounted Echosounder has an effective vertical range of 100-200 m, meaning the lower 300 m of Lake Tahoe (potentially half the resource) remains completely inaccessible for surface mounted technology. AUVs could help fill that void in the future.

As well as the potential for resource assessment using bioacoustics, there is also a need to utilize bioacoustics for guidance of future trawling operations (operational bioacoustics). Presuming that there is horizontal heterogeneity (a critical unknown for Tahoe, but well demonstrated for Emerald Bay), then a trawl vessel would not know where to operate in the absence of real-time bioacoustics data in order maximize the Catch Per Unit Effort (CUPE). This could readily be provided with ASVs. In Emerald Bay the heterogeneity is less of an issue as the size of the bay and its steep sides largely confines where a trawler can operate. In Lake Tahoe, however, a trawl operator has unlimited choices on where to go, and data-based, real-time guidance is considered essential to maximize operations.

Bioacoustic data, both in its operational mode and its assessment mode, are providing critical datasets on the distribution of *Mysis* in Lake Tahoe. While *Mysis* are known to control their vertical movements, horizontally they are more likely to be transported by horizontal gyres, jets and other features of the lake circulation dynamics. All of these operate on length scales of kilometers to tens of kilometers. Data on the position over time of *Mysis* are therefore invaluable in the calibration of three-dimensional modeling tools that could further help locate *Mysis* ahead of time. This is discussed further below.

Suggested Actions

- ASV and/or AUV surveys of *Mysis* distribution in Lake Tahoe would help address a critical unknown. The size of the resource needs to be better quantified, and in particular its areal distribution and how this changes seasonally. The detailed planning, optimizing and costing of *Mysis* removal depends directly on knowing this.

- Develop real-time, operational bioacoustics to optimize harvest efficiency.

- Archiving bioacoustic data for calibration and validation of three-dimensional particle tracking models, along with other physical data needed for modeling studies.

Lesson 4 – The Life Cycles of *Mysis* and Zooplankton – The life cycle studies performed indicated that the *Mysis* populations in both Emerald Bay and Lake Tahoe have changed little in the last 40 years. Similarly, the make-up of the zooplankton communities in each water body shared most characteristics and were reminiscent of the make-up from previous decades. These results serve to highlight the large magnitude of the perturbation observed from 2011-2017 in a historic context.

An important result from both the *Mysis* tow data and the bioacoustics data was the finding that juvenile *Mysis* heavily favored the littoral zone of Emerald Bay in the spring. This permits the consideration of harvesting juveniles during that time of year, possibly using different technologies, in addition to conducting summer-fall pelagic harvesting of adults. The extent to which this occurs in Lake Tahoe is unknown. The more varied morphology of Lake Tahoe's littoral zone precludes simple extrapolation. Given the large size of the lake, if similar seasonal behavior was found in parts of the littoral zone it could have a large impact on optimizing trawling strategy and economics (see Section 9.0). The 3-4 year *Mysis* life cycle in Lake Tahoe makes this a particularly interesting and important question.

Suggested Actions

- Conduct bioacoustic surveys of Lake Tahoe focusing on littoral – pelagic seasonality differences and differentiating between *Mysis* age classes.

- Determine the littoral *Mysis* populations around the lake.

- Undertake population modeling of Mysis and Zooplankton to better understand the system and to allow refinement of resource quantification for harvesting.

Lesson 5 – *Daphnia* in Emerald Bay preferentially consume fine particles – Mesocosm experiments performed on *Daphnia* indicated that they were very effective at grazing down to very small sized particles (both organic such as *Cyclotella* and inorganic silt) that are known to have significant impacts on Lake Tahoe's clarity. This result was consistent with observations in the literature over the last 50 years, and the disappearance of *Daphnia* from Lake Tahoe immediately preceded the long-term clarity loss. This result confirmed that monitoring of particle size distribution and algal speciation is an important metric for future monitoring.

Suggested Actions

- A high priority will be to monitor the abundance of *Cyclotella* and fine inorganic particles both before and after future *Mysis* removal efforts in both Emerald Bay and Lake Tahoe.

Lesson 6 – The Catch Per Unit Effort (CPUE) for *Mysis* – this was a very difficult lesson to learn, as it could only be learned at night due to the *Mysis* vertical migration. After using a loaned "*Mysis* net" it was learned that the mesh size was too large for *Mysis* in Emerald Bay. Several time-intensive iterations of net fabrication and testing were needed to arrive at an acceptable balance between drag and catch. The size of the research vessel being used for trawling was only adequate for a relatively small net, once the mesh size had been optimized at a much finer mesh opening. This net and vessel combination was too small to reduce the Emerald Bay *Mysis* population in a reasonable length of time, and thus could not meet the hoped for performance metrics. But the critical knowledge that was gained and will permit substantive trawling to be fully evaluated, is the optimum combination of net size and trawl vessel size that is required.

A preliminary design for the optimum vessel size (45' length, 15' beam, 350 HP diesel engine) and net size (28 m long x 13.3 m wide with refined mesh sizes) has now been determined with the assistance of Hickey Bros Research, LLC, and Baileys Harbor Fish Company, LLC, from Wisconsin. A reconnaissance trip in early 2021 is planned to permit refinement of these recommendations. That net size has a 14 times large opening than the net used in this project, and the vessel is 30% larger and more powerful. When combined with real time sensing of net depth and video of *Mysis* entering the net (both techniques used by commercial trawlers), together with the use of high speed winches, and operational bioacoustics, a 20-30 fold increase in the CPUE for Emerald Bay appears attainable. In Lake Tahoe, where the operating space is less confined, larger yields may be possible.

Flume testing and hydraulic analysis (not funded by this project) have shown that hydraulic drag is the greatest single impediment to the rate of shrimp removal. An alternative harvesting approach using a novel low drag hydrofoil, V-Net and pump-assistance has been conceptualized and is currently being patented. Depending on the CPUE achieved by "conventional trawling" and whether the actual cost on board of *Mysis* meets the desired financial target, it may be advantageous to look at alternative harvesting technologies. Any reduction in CPUE reduces the costs of *Mysis* removal.

Suggested Actions

- A "full-scale" commercial trawling experiment in Emerald Bay is the logical next step to evaluate the economics and benefits of *Mysis* removal, and necessary steps for up-scaling to Lake Tahoe. It had originally been anticipated that a "research-scale" approach could provide that information, but that proved to be insufficient effort. Based on the experience of this project, the actual trawling for Emerald Bay may take on the order of one month if the appropriately designed equipment is used, and allow time for a proper assessment of Lake Tahoe.

- A monitoring program to determine the changes to physical, chemical, biological and ecological components should be integrated with the above experiment, with monitoring both before and after trawling. The monitoring should include the entire food web (phytoplankton to fish), and the harvested *Mysis* should be used toward developing a marketable product that can either offset or fully cover *Mysis* control costs in the future.

Lesson 7 – Harvesting *Mysis* in the Absence of Real-time Data and Predictive Tools is Inefficient – The narrowness of Emerald Bay meant that the boat operator had few options to choose between when planning trawl runs. Essentially the boat conducted parallel runs along the longitudinal axis of the bay. The only choice was the depth at which the nets should be positioned, and that depth was provided by the on-board bioacoustics system.

Lake Tahoe is completely different. At a trawl speed of 2.7 km/hr (1.5 knots), a vessel could travel 22 km in an 8-hour night. With a surface area of 600 km^2, where should the vessel start and end? On-board bioacoustics are of limited value as the vessel will only know what is below it, but nothing about *Mysis* distributions all around it.

Based on the algorithms that were developed in this project, it is possible to have a data collection vessel moving in advance of the trawl vessel and producing *Mysis* depth and abundance maps that are transmitted to the trawl vessel in real time. Based on this information, the trawl vessel could constantly refine its optimum path to intercept the greatest abundance of *Mysis* during its night's work. It is currently feasible to have the data collection vessel be totally autonomous (no crew) and to be operating with a full obstacle avoidance system.

The project was able to confirm the consistency and the actual rise and fall rates of *Mysis* in Lake Tahoe and Emerald Bay. These were shown to occur with a characteristic velocity of 1-2 cm/s. The motions of *Mysis* are also acted upon by the basin-scale horizontal motions (currents, gyres, jets) in the lake, which have characteristic velocities an order of magnitude larger. This suggests that the areal distribution of *Mysis* is likely to be heavily controlled by the hydrodynamics. Combining these processes, the lake hydrodynamics and the *Mysis* behavioral dynamics, through a three-dimensional model driven by local meteorology and hydrology is very feasible. With calibration data provided by bioacoustics surveys, it would allow for the development of both a knowledge base of *Mysis* distribution patterns annually, seasonally and after episodic (e.g. storm) events, and a predictive capacity for *Mysis* distribution across the entire lake. Combined with the real-time, operation bioacoustics this would allow for the optimization of *Mysis* control on both a seasonal and a nightly basis.

Suggested Actions

- Develop a three-dimensional *Mysis* distribution model to be operated both in conjunction with the operational bioacoustics and to permit longer term planning.

9.0 A 15-Year Lake Tahoe *Mysis* Control Plan

Since the disappearance of Mysis from Emerald Bay and the large and sustained clarity improvement that followed over the next two years, the idea of pursuing the deliberate removal of *Mysis* shrimp to help achieve the federally mandated clarity goals, to remove an invasive species and to restore the native food web has attracted attention. When coupled with the potential commercial value of *Mysis* shrimp, the idea becomes more compelling. In the absence of future public funding at prior levels to achieve Lake Tahoe's restoration goals, it is extremely important to strive to have environmental restoration become increasingly financially self-sufficient. This project was a first step toward that, by gathering the data needed to "plan, test and optimize a strategy to improve water clarity in Lake Tahoe by reducing the abundance of *Mysis* shrimp".

What follows is a plan to control *Mysis* in both Emerald Bay and Lake Tahoe that in the long term may actually generate net revenues. That alone distinguishes it from all other restoration concepts currently available. The Plan also has many other unique advantages. Some of these include:

- The opportunity for a combination of both public and private investment, as there are specific investment opportunities for private investors.

- The level of total expenditures in the initial Phase is at a level that is on the order of 10% of what has traditionally been spent at Lake Tahoe for clarity control annually.

- The unique opportunity of creating a new industry at Lake Tahoe that both trains and employs a workforce that may grow to over 100 people.

- The potential to change the entire Tahoe ecosystem in a direction closer to what is was before the major disturbances started in the 1950s. The likely advantages of this are still a topic of active research.

The plan is specific, both in its duration and in its revenue projections. These are both based on the best available information, but we would expect that as more information becomes available (particularly estimates on the CPUE of a professional trawling operation, and better estimates of the size of the resource) that some of the specifics may change. The proposed start of operations in Emerald Bay, which allows for the results to become evident in less than a year, would allow those lessons learned to be immediately applied to a Lake Tahoe operation over the next 14 years. Those lessons would be both for the physical trawling operations, the necessary monitoring, and the overall financing of the operation.

It should be noted that the Plan could be both shortened or lengthened, although that would impact the financial profitability. An assessment of that would best be made at the end of Phase I.

9.1 The Plan

We recommend the following set of steps and actions based on the findings of this research project, the 50 years of research done on Lake Tahoe, consultation with experts and colleagues from a range of disciplines including invasive species control, food sciences, veterinary science, the commercial fishing industry, venture capital, the pet food industry, and primary consumer research with pet owners throughout California. We have also incorporated the suggestions from the independent peer review that was facilitated by the Tahoe Science Advisory Council. Our proposed timeline for the reduction of *Mysis* populations in Lake Tahoe, shown in **Fig. 9.1**, is sensitive to a few key variables, notably the commercial catch per unit effort (CPUE), *Mysis* population models (including natural variability), and the market demand for *Mysis* based

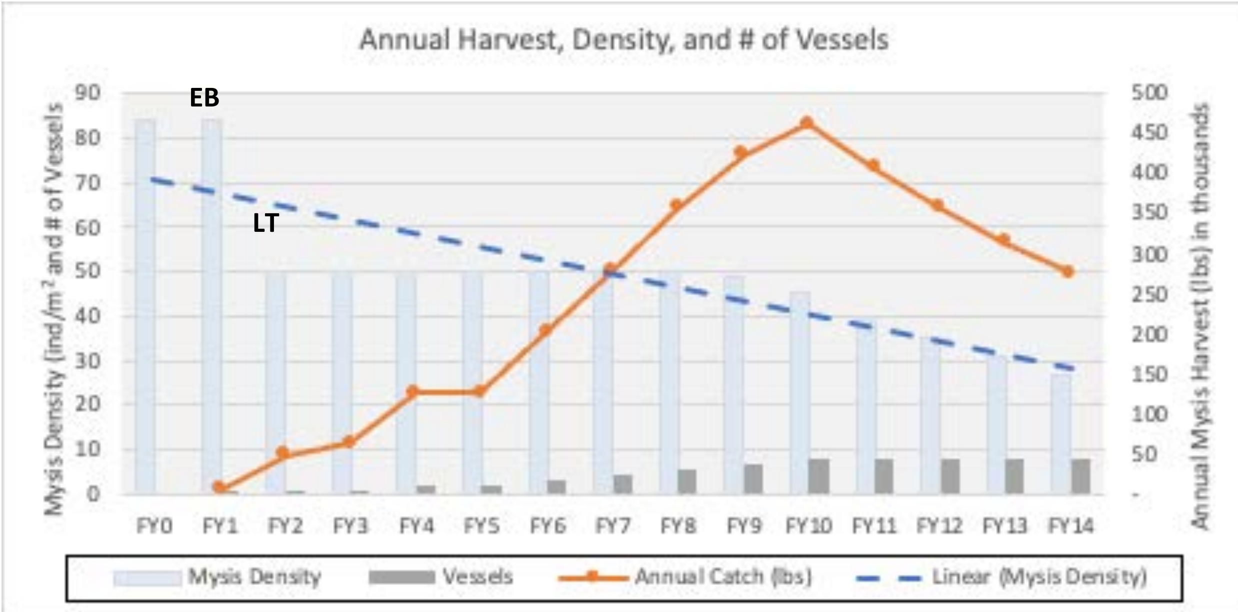


Fig. 9.1: Timeline of attaining *Mysis* control in Lake Tahoe and Emerald Bay. The blue bars indicate the Mysis density that was assumed for the financial model; the gray bars indicate the number of trawl vessels operating; and the orange line indicates the annual Mysis yield (lbs. of *Mysis*, wet weight). The dashed blue line is indicative of a *Mysis* population decline rate, but one that will be adjusted once the resource is better quantified and the population modeling has been completed.

products. Our projected action plan is based on the available data and research conducted to date. Additional research will be required as part of the project to validate these assumptions, refine our projections, and potentially fine-tune the process. All this is directed to achieve a target *Mysis* population density of 27 ind/m^2.

9.1.1 Phase I: Control Emerald Bay *Mysis* Population

Phase I extends from July 2020 through June 2022. FY0 (**Fig. 9.1**) is for the preparatory work toward achieving a commercial scale. Note that our fiscal year runs from July through June in order to best align with the seasonal nature of the *Mysis* harvest. FY0 involves custom net and trawl design, engaging a professional operator, building community engagement and awareness of this project, and setting up a standalone organization for the harvest of *Mysis* and distribution of *Mysis* based products. This standalone nonprofit organization will be comprised of two primary operations which we will refer to as 1) Trawling Operations and 2) Bakery Operations for simplicity. Data collection of *Mysis* and *Daphnia* populations in Emerald Bay (EB) and Lake Tahoe (LT) will also be required to establish a baseline to measure against post-harvest, along with other data including Secchi depth measurements, particle measurements and algal enumeration and identification. Much of this work is already underway.

In FY1, we recommend harvesting in EB to reduce the *Mysis* population to the target 27 ind./m^2 in one season. In addition to reducing the *Mysis* in EB, the commercial trawler will also begin trawling on LT to validate the hypothesis that EB trawl efficiencies carry over to LT. Echosounding data will be collected as part of both operations, along with *Mysis* and zooplankton tows for intercalibration. The *Mysis* harvest will be dehydrated and manufactured into premium dog treats. While the volume of *Mysis* harvested in FY1 will be insufficient to offset Bakery expenses (which includes product development, testing, branding, manufacturing, etc.), the effective sales and distribution of the *Mysis* treats creates an engaged consumer base and validates projected unit economics.

Phase I Goals:

- Reduce Emerald Bay *Mysis* population density to 27 ind./m^2.
- Establish CPUE in both Emerald Bay and Tahoe.
- Collect supporting environmental data to evaluate impacts of operations on ecology and water quality.
- Commence population modeling of *Mysis* and *Daphnia* as part of a broader lake food-web model.
- Establish initial customer base (10-20k customers).
- Validate unit economics.

9.1.2 Phase II: Monitor Emerald Bay and Commence Commercial Trawling in Lake Tahoe

Phase II extends from July 2022 through June 2024. In FY2, we will continue monitoring of EB for the return of *Daphnia* and improvement in water clarity. In 2012, it took about 1 year after the disappearance of Mysis for Daphnia to return and water clarity to begin improving. We expect to see such changes begin in FY2.

Trawling operations could commence on LT in FY2. Having refined our trawling technique and equipment in FY1, we expect to be trawling near maximum efficiency for the entire 3-month period (late August through early November 2022). Experiments will also be conducted into whether the CPUE can be maintained over a longer trawling season. The thicker, high density layer of *Mysis* in LT suggests that may be possible. Over the full harvest season, we expect a ten-fold increase in the *Mysis* harvest over FY1, largely due to the greater size of LT and the benefits of method refinements having already taken place. Bakery operations will scale up in FY2 from distributing a minimum viable product (MVP) to early adopters to establishing recurring revenue streams with a now engaged consumer base. This direct-to-consumer subscription sales and distribution strategy maximizes both gross margins and customer loyalty, while increasing community engagement in order to raise public awareness of LT's ecosystem. The increased harvest volumes from LT provides economies of scale that will allow the Bakery operations to reach cash-flow breakeven by FY2 (note that this does not yet cover the cost of Trawling operations).

FY3 will be similar to FY2, and we will look to continue building on previous successes. By FY3, we expect *Daphnia* in EB to have returned in healthy numbers and water clarity to be improved by 10-20 feet. Periodic trawling of EB may be necessary to keep *Mysis* numbers low. The frequency of this return trawling will be a key piece of information. Trawling on LT will continue, and we will look to increase the length of the harvest season by 30% while maintaining a similar CPUE. During this period, the contract trawl operator will begin training Tahoe-based captains and boat crews in preparation to pass off the trawling operations to the local organization established in Phase I. This will be the beginnings of a new Tahoe-based industry.

Phase II Goals:

- Monitor EB clarity (10-20 ft. improvement expected).
- Scale up LT trawling efforts and validate that CPUE can be maintained for 5 months.
- Transition early adopter consumer base into subscription customers.
- Cash flow break even for Bakery operations.
- Begin transitioning trawling operations to local crews.

9.1.3 Phase III: Reduce Tahoe *Mysis* Population Density to 27 ind./m^2

Phase III extends from July 2024 through June 2034. If all the key variables have been validated and our projections remain on target, FY4 is when operations at Tahoe will be scaled up. Most significantly, this means progressively purchasing additional vessels and trawling equipment, hiring local crews, and taking over all operations in-house. The contract trawlers will be available as consultants on an as needed basis, but the bulk of the harvest operations will be conducted by the stand-alone local organization. This change in operation requires significant upfront capital expenditure, but ultimately reduces the long-term operational cost of harvest as well as provides local training and employment. The reduced cost of Trawling operations paired with the increased scale of Bakery operations allows the harvest of *Mysis* to begin paying for itself.

From here, we expect to add additional vessels each year to scale up the *Mysis* harvest until we reach peak trawling volumes in 2030 (FY10). Our current estimates project peak annual revenue of $18.7M in gross revenues. This equates to roughly ½ of 1% of the estimated 4.5B US dog treat market, or if we take a more targeted approach, 20% of the CA/NV premium dog treat market. Once we reach peak harvest levels, we expect the *Mysis* population density to decrease toward 27 ind./m^2 over the next four years. As *Mysis* densities decrease, our CPUE will naturally decrease as well, thereby increasing the per pound cost of *Mysis*. Assuming no additional efficiencies gained over the 15-year project period, current projections forecast net income to be negative in FY13 and FY14 as we near 27 ind./m^2. That would be the point where trawling operations would transition to other lakes.

Multiple lake ecosystems throughout North America have been disrupted by invasive *Mysis*, and the capital investment on *Mysis* trawlers, vessels, and crew training can be redeployed to harvest other lakes in the west. This potential to harvest other lakes could offset the loss from trawling on Tahoe when densities are at or below 27 ind./m^2. To be conservative, the project financials only show the expected returns of trawling on Tahoe over a 15-year project period. We expect that with improved efficiency, the natural regeneration rates of *Mysis*, the opportunities to work on other lakes, and the very large dog treat market, we believe that clarity maintenance through *Mysis* removal can operate profitably indefinitely. As more information, both scientific and financial, becomes available we will be able to refine this sustainable plan.

9.2 Cost Projections

To demonstrate the respective benefits of our two-pronged operational approach (Trawling + Bakery), we have separated the financial projections to highlight the stand-alone costs of Trawling operations and the potential offset through Bakery operations.

The estimated cost of trawling to get Tahoe *Mysis* population densities down to 27 ind/m^2 is $32.0M over a 15-year project. The costs ramp up significantly in later years as the number of vessels and crew increase over time.

Table 9.1 Annual costs of Mysis trawling in $M.

	Jun-20														Jul-35
(millions)	**FY0**	**FY1**	**FY2**	**FY3**	**FY4**	**FY5**	**FY6**	**FY7**	**FY8**	**FY9**	**FY10**	**FY11**	**FY12**	**FY13**	**FY14**
Trawling Cash Balance:	(0.10)	(0.65)	(1.15)	(1.81)	(3.33)	(4.17)	(5.92)	(8.19)	(10.96)	(14.23)	(18.01)	(21.48)	(24.94)	(28.46)	(32.04)
Trawling FCF:	(0.10)	(0.55)	(0.50)	(0.66)	(1.53)	(0.83)	(1.75)	(2.27)	(2.77)	(3.28)	(3.78)	(3.46)	(3.46)	(3.52)	(3.58)

Bakery operations has the potential to generate 37.7M in pre-tax cash over the 15-year project while creating an engaged community of over 200k annual customers.

Table 9.2 Annual revenues from *Mysis* dog treat sales in $M.

	Jun-20														Jul-35
(millions)	**FY0**	**FY1**	**FY2**	**FY3**	**FY4**	**FY5**	**FY6**	**FY7**	**FY8**	**FY9**	**FY10**	**FY11**	**FY12**	**FY13**	**FY14**
Bakery Cash Balance:	(0.03)	(0.14)	(0.25)	0.24	1.28	2.36	4.52	7.43	11.14	15.61	20.54	25.32	29.77	33.90	37.72
Bakery FCF:	(0.03)	(0.11)	(0.11)	0.49	1.04	1.08	2.16	2.91	3.71	4.47	4.92	4.78	4.46	4.13	3.82

Combined Financials (tax-exempt):

While the nature of the tax exemption status is dependent on IRS approval, we believe the revenue from pet treat sales is core to the nonprofit vision of restoring lake clarity and educating the public. As such, we expect revenues for the combined entity to be tax exempt. The combined cashflow from both operations (harvesting and treat sales) is shown below in **Table 9.3**, where we expect a 20% IRR over the 15-year life of the project. As noted previously, net operating income decreases in later years as *Mysis* densities diminish, but modest efficiency gains in gross margin are sufficient to maintain positive cash flow even at target *Mysis* densities.

Table 9.3 Annual net revenues from combined harvesting and *Mysis* dog treat sales in $M assuming our 501c3 tax-exempt status is approved by the IRS with no Unrelated Business Income Tax requirements.

	Jun-20														Jul-35	
(millions)	FY0	FY1	FY2	FY3	FY4	FY5	FY6	FY7	FY8	FY9	FY10	FY11	FY12	FY13	FY14	
Combined Cash Balance:	(0.13)	(0.79)	(1.39)	(1.57)	(2.05)	(1.80)	(1.39)	(0.75)	0.19	1.38	2.52	3.84	4.84	5.45	5.68	
Combined FCF:	(0.13)	(0.65)	(0.61)	(0.17)	(0.48)	0.25	0.41	0.64	0.94	1.19	1.14	1.32	1.00	0.61	0.24	**IRR**
PV:	(0.13)	(0.65)	(0.61)	(0.17)	(0.48)	0.25	0.41	0.64	0.94	1.19	1.14	1.32	1.00	0.61	0.24	20%

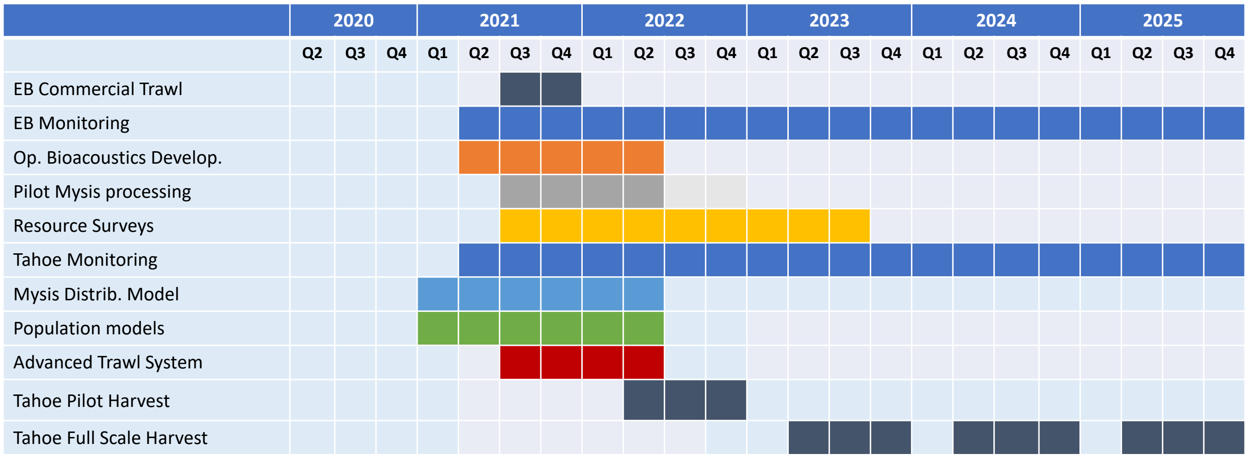
9.4. Concomitant Monitoring and Research Needs

The TSAC commissioned peer-review of this project identified a number of scientific questions surrounding the consequences of *Mysis* trawling in Lake Tahoe. Many of these questions are now included as part of the "Suggested Actions" in Section 8.0. We believe that these questions would best be addressed as part of the *Mysis* removal.

9.5. Science and Monitoring Timeline

While a 15-year timeline for the full control of *Mysis* in Lake Tahoe and Emerald Bay is presented above, a shorter timeline is presented here for the concomitant science and monitoring to be done in conjunction with *Mysis* removal. As the harvesting of *Mysis* is heavily dictated by seasonality, it is important that the timeline be built around that seasonality. For that reason, the third quarter of 2021 is a critical date. If the commercial trawling cannot commence at that time, the entire undertaking is effectively pushed back a year.

While some activities are shown commencing earlier than that, they are not on the critical path (except with the possible exception of the pre-project monitoring which would need to commence at least 3 months ahead of the trawling). These activities, such as resource surveys, modeling, advanced trawling systems are still important to address early. They all impact the efficiency with which *Mysis* removal can be achieved, something that impacts the long-term costs and the timing of when this endeavor may generate net revenues.



2020
2021
2022
2023
2024
2025
Q2 Q3 Q4 Q1 Q2 Q3 Q4 Q1 Q2 Q3 Q4 Q1 Q2 Q3 Q4 Q1 Q2 Q3 Q4 Q1 Q2 Q3 Q4
EB Commercial Trawl
EB Monitoring
Op. Bioacoustics Develop.
Pilot Mysis processing
Resource Surveys
Tahoe Monitoring
Mysis Distrib. Model
Population models
Advanced Trawl System
Tahoe Pilot Harvest
Tahoe Full Scale Harvest

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11.0 Appendix 1

Response to Peer-Review Comments and Introduction to the Draft Report

The project team expresses its thanks to the TSAC Peer Review Committee and to the external reviewers who supplied many valuable comments. These comments served several purposes.

First, they provided different viewpoints and alerted us to some findings that had not been completely addressed. Many of these have now been incorporated in the revised Report. Second, they highlighted areas of the Report where the level of detail wasn't fully adequate. One example was the issue of bycatch (the accidental capture of non-target species). Whereas all reviewers brought this up as a concern, this was an aspect that we monitored closely throughout the project and found conclusively that bycatch rates were low (both for fish and for cladocerans) due primarily to the vertical separation in the water column of the *Mysis* and fish. This information was omitted from the draft report, but it is in the revised Report. Third, the reviewers' overall recommendations confirmed those by the project team, despite some comments to the contrary. The Reviewers recommended that more information was needed prior to the application of lake-wide *Mysis* control (at the scale of Lake Tahoe) was conducted. This is the same conclusion drawn by the Project team, and our recommendation to the CTC and to NDEP (the project sponsors).

Where we may differ is that we believe that the initiation of the Plan that we have put forward and the ecological studies suggested by the reviewers could occur concurrently. There are sound reasons for doing this. At the heart of the matter is that it can be very quickly determined if the CPUE by a commercial trawler is in line with our projections. If they are (or they exceed it) then the project actually earns money, and knowing the ecological consequences are critical. The Plan would also permit the measurements/experiments to be conducted in Emerald Bay which would have been cleared of *Mysis*-domination. At the same time, Lake Tahoe measurements, as suggested by the Reviewers, could go ahead for several years if necessary, with little impact from the Mysis removal activities. If unforeseen negative affects became apparent, Mysis removal could simply stop. As was evident during the 2011-2017 period in Emerald Bay, the absence of Mysis from that system for over 2 years did not produce any untoward impacts.

What we believe that the Reviewers glossed over was the data record since *Mysis* introduction at Tahoe/Emerald Bay (and elsewhere). As the data in the report showed, *Mysis* introduction was the sole reason for the demise of cladocerans within a 4 to 5 year period during the 1960s (see multiple citations in the report). Cladocerans are universally known to be extremely capable of clearing the water column of particles in the clarity sensitive range – they are promoted as a lake restoration treatment. The exact same Cladoceran disappearance and clarity loss as occurred in Lake Tahoe also happened in Emerald Bay in the 1960s, and while we may ruminate on how similar or different the two water bodies are, there are 50 years of data to show that they both responded to *Mysis* introduction in essentially the same way.

By chance, a multi-year crash in the *Mysis* population in Emerald Bay saw both a return of Cladocerans and of clarity over a three-year period. This was followed by a return of the *Mysis* and the repeat of what had happened 50 years earlier *viz a viz* cladocerans and clarity loss. It is important to note, that this repetition occurred after 50 years during which urbanization had been taking place, climate change had been occurring, atmospheric deposition was present, wildfires had occurred, etc. These are factors that the reviewers say would make the return of clarity uncertain. ~~The monitoring that was done during this event was limited as it was an unfunded undertaking, and we simply did not fully appreciate what was happening at the time~~.

The research project just concluded was primarily directed at answering one simple question: If "natural" *Mysis* removal yielded large improvements in clarity and restoration of important elements of the ecosystem, then could the deliberate removal of *Mysis* yield similar results in Emerald Bay and by extension the greater Lake Tahoe system. Despite how little was known about the removal of *Mysis* and their behavior over the seasons, we ~~were optimistic that a large removal could occur, albeit with a part time crew of scientists, with a small boat and no experience in commercial trawling. While the level of optimism for the complete drawdown of the *Mysis* population was proven to be too high, the team was able to determine what was needed for the next steps. We~~ were able to develop a robust methodology for the quantitative location of *Mysis* in the water column, understand their seasonal dynamics (in Emerald Bay), obtain a valid estimate on removal efficiency based on the equipment being used, determined the times of year and appropriate methodologies for efficient *Mysis* drawdown, and commence the quantitative assessment of *Mysis* distribution and behavior in Lake Tahoe itself. All that knowledge has moved us significantly closer to answering the central question – can *Mysis* abundance be reduced to the level at which Cladocerans can co-exist and improve clarity again.

In our report we have recommended that in Phase I of our Plan, a commercial *Mysis* removal effort be undertaken in Emerald Bay, along with appropriate monitoring of that operation and continued exploration of Lake Tahoe. We don't disagree that more research may be needed, but testing whether the removal is possible by commercial trawlers using professional grade equipment, and at what cost, is the real priority. If the removal of *Mysis* is not possible under those conditions, then research into the questions that the reviewers suggest may be interesting, but not a priority for the Lake Tahoe basin at this time.

Reviewers expressed varying degrees of concern about whether a scale-up of catch beyond that of our research endeavor was possible. Like the Reviewers, we have little expertise in this area, beyond what we have learned by working on this project for two years. For that reason, we have been working with a commercial trawling and research company to plan such an operation. They have provided a cost-estimate for such a removal and have made specific recommendations on the boat size required, have already produced a preliminary net design (14 times larger opening than the previous net), and have high speed winches real-time net depth and video equipment available to be used. That team is planning to be at Lake Tahoe in late January for a reconnaissance visit.

We have included some of the scenarios from our financial model for *Mysis* removal. This is an area where we have spent considerable time analyzing a range of removal scenarios (with variable harvesting rates, *Mysis* reproduction rates, annual operation rates, number of trawl vessels, and time to achievement of clarity return in Lake Tahoe etc.) in the belief that the economic cost needs to be part of the consideration. The other factor that we spent a large amount of time exploring was the potential for commercial sale of the *Mysis*, the types of products that could be generated, the market potential etc.

The results show that with a relatively modest initial investment by a combination of public and private funding, a self-sustaining commercial operation could be at break-even point within several years and be profitable thereafter based on a 15-year time to complete *Mysis* control. *Mysis* harvesting would cease to be profitable after the first 15 years until numbers recovered at which time operations could recommence. That temporal gap would be a few years. The unique private-public partnership that this financial model supports represents a new paradigm, both at Lake Tahoe and most other aquatic ecosystems. Based just on Lake Tahoe's experience, where restoration efforts to date have cost on the order of tens of millions of dollars a year, we believe there is a real opportunity to deliver a greater clarity improvement for a few percent of that cost.

Below we provide the verbatim Peer Review Report, together with our responses (shown in red for clarity). Peer review questions are shown in italics.

Tahoe Science Advisory Council: External Peer Review of "Planning for Removal of Mysis Shrimp from Emerald Bay and Lake Tahoe as a Means of Ecosystem and Clarity Restoration"

Adrian Harpold, Peer-review chair

David Beauchamp, USGS, External reviewer,

Michael Brett, University of Washington, External reviewer

Walter Dodds, Kansas State University, External reviewer

This short summary is meant to give an overview of the three reviews. The individual reviews are given in an appendix below. The review charge sought to address five questions with three reviewers' response summarized here.

1. *Does the project report, appendices and/or associated analyses provide evidence that Mysis can be reduced in Emerald Bay using boat trawling and echosounder methods?*

There is some skepticism that the catch rate can be scaled up using larger nets. The reviewers did not feel that the logistical challenges of using larger nets was sufficiently addressed. Also, the unintended consequences of larger-scale trawling operations, particularly bycatch, were not fully explored. Because *Mysis* catch efficiency will be reduced as *Mysis* populations decline the potential financial offsets are not sustainable.

While there is certainly some uncertainty in our estimates, the claimed future increases in yield were based on our work with Hickey Bros Research, LLC, and the Baileys Harbor Fish Company, LLC, to determine that these increases are indeed feasible. These companies have three generations of trawling experience in the Great Lakes and more recently in the west and northwest. Their preliminary recommendations for obtaining the CPUE we claim are now part of the main report.

Bycatch and other unintended consequences have been explored and quantified, but the results were inadvertently not included in the Draft Report. The potential impacts of bycatch on larger-scale operations were also addressed.

2. *Does the project report and/or associated analyses provide evidence that deliberate Mysis removal via trawling or similar methods could lead to increases in native cladocera populations in Emerald Bay?*

The case could have been made stronger that *Mysis* removal will lead to long-term and sustainable increases in cladocera populations. Evidence that factors other than *Mysis* influence the trophic state of the Lake is well known, such as climate, land use, and atmospheric deposition. Bycatch effects on cladocera from the trawling operations were not discussed.

The historic correlation between *Mysis* and cladoceran abundances has been demonstrated twice, over fifty years apart and under very different conditions in climate, watershed land use, etc. The disappearance of *Mysis* and the re-emergence of cladocerans starting in 2011 and the original introduction of *Mysis* in the 1960s both showed the same connection. Indeed, the entire project was predicated on this connection. Although the reviewers raise good points about uncertainty in a statistical sense, we lack the data necessary to make such unequivocal inferences, and as is often the case with management decisions, make recommendations based on the best available data, which suggests that in the absence of *Mysis*, native cladoceran species abundance increases.

We will endeavor to make this point more strongly in the final report.

3. *Does the project report and/or associated analyses provide evidence that increased cladocera populations can improve clarity in Emerald Bay?*

There is modest reviewer agreement with long-term improvements in clarity based on in situ observations. While previous work suggest that higher caldocera biomass does improve lake clarity, there are multifaceted controls on water clarity that are not considered in the report.

As with the relationship between mysis and cladoceran abundances, we lack the data necessary to unequivocally demonstrate the coincident changes in water clarity following declining mysis and rebounding cladoceran abundances was due to increased grazing rates by cladocerans. However, there is evidence at Tahoe and in dozens of other systems that increased densities of cladocera (particularly *Daphnia*) improve water clarity, and numerous laboratory studies that have demonstrated mechanistic linkages between cladoceran abundance and clearance rates of different sized particles. In the Tahoe/Emerald Bay system the main control on water clarity is known to be scattering of light by particles in the 1-4 micron size range. The basis of investigating *Mysis* removal was to enable caldocera to return and resume their highly efficient grazing of particles in this size range.

4. *Does the project report and/or associated analyses provide evidence that the dynamics between Mysis, cladocera, and clarity in Emerald Bay would hold true for Lake Tahoe?*

A lack of new lake-scape observations, and many uncertainties, do not provide evidence that dynamics in Emerald Bay would hold true for Lake Tahoe. Specifically, positive and negative feedbacks in the fish-invertebrate food web would be important to consider at large scales. Moreover, whether the trawling operations are scalable to the entire Lake are questioned by the reviewers.

The two main periods of intensive investigation of the *Mysis*/Cladocera/Clarity observation were after their introduction (from the 1960s through the mid-1970s) in both Tahoe and Emerald Bay, and our studies from 2011 to the present. Both sets of data show that the two systems behave in a similar manner. This is not to say that there may not be other factors that are different between Emerald Bay and Tahoe, but they clearly do not exert a major influence on the *Mysis*/Cladocera/Clarity nexus.

While the detailed investigation of the food-web was beyond our scope, the work that was done indicated its importance. It is for that reason that we have specifically called out the conduct of such studies as part of Phase I of the Plan we have produced.

5. *Does the project report and/or associated analyses offer evidence that pursuing lake-wide Mysis control is a potentially successful method for improving Lake Tahoe's clarity?*

At this point there are too many ecological unknowns and operational uncertainties to justify a lake-wide *Mysis* control effort. Reviewers were particularly skeptical that commercial scale operations at the Lake were feasible and cost-effective given declining populations over time. Several reviewers raised the issue of unintended consequences to the food web, which would need much more careful consideration. The reviewers agree with many of the next research steps offered in the report, including characterizing the seasonal and areal distribution of *Mysis* and developing population models.

Our conclusions indicate that further work is required before the full-scale effort in Lake Tahoe should commence. However, the Reviewers conclusions do not address the key word in Question 5 that “lake-wide *Mysis* control is a **potentially** successful method”. Their skepticism may in part stem from a lack of expertise in “commercial scale operations”. For that reason we are working with commercial trawling companies, as now described in the Report. The second criticism presented here, regarding unintended consequences for food webs in LT, is addressed above in point 5: We agree that population and food web modeling would be necessary as part of the next phase of exploration. Conducting a population study was not part of our scope, although is a needed input to financial models (we have made simple assumptions in the interim). The unintended consequences largely relate to bycatch which we did study and our data concluded that it was extremely small.

Individual reviews

Review 1: David Beauchamp

Review of Report on efficacy of Mysid suppression on return of native cladocerans and lake clarity in Lake Tahoe:

Schladow, G., Forrest, A., Sadro, S., Allen, B., Senft, K., Cardoso, L., Tanaka, L., Watanabe, S., Daniels, B. and Trommer, S. (UC Davis) and Chandra, S. and Bess, Z. (UNR) 2020. Planning for Removal of *Mysis* Shrimp from Emerald Bay and Lake Tahoe as a Means of Ecosystem and Clarity Restoration. Draft Administrative Report to California Tahoe Conservancy and Nevada Division of Environmental Protection.

Review Questions

1-Does the project report, appendices and/or associated analyses provide evidence that Mysis can be reduced in Emerald Bay using boat trawling and echosounder methods?

Yes, if scaled up to the commercial level in Emerald Bay as described on pages 77-79 should be capable of depleting the mysid densities to <27/m^2 over 2-3 months of intensive commercial-scale effort.

Thank you – we fully agree. This is a critical confirmation, as this is the essence of what this project was about.

Declining catch rate as the population becomes locally depleted by trawling is a very real concern and these responses are often nonlinear due to behavioral responses by population. I suspect such responses would be much less by mysids compared to fish populations, but this is still an important lingering uncertainty. An independent survey boat dedicated to scouting near-term shifts in density and distribution might be needed to focus harvest on the most effective regions within Emerald Bay.

We fully agree. Our financial model of a commercial operation (to be further refined once the catch per unit effort data from a professional trawling operation has been determined) takes into account the expected declining *Mysis* population. Under the present assumptions, peak revenues would be generated with a 15-year project, after which time the reduced *Mysis* population would not be economical to harvest. However, the expected population rebound would allow recommencement within a few years.

We have outlined plans in the Report for the use of an Autonomous Surface Vessels (ASVs) equipped with Bioacoustics to provide real-time *Mysis* density and depth data to the trawl vessels. We are also planning to develop a 3-D modeling capacity to predict the *Mysis* distribution. The ASV data would be important to help validate the *Mysis* predictions. This is particularly important in Lake Tahoe. In Emerald Bay the relatively small size of the embayment means that a trawl vessel could readily cover much of it in an evening.

2-Does the project report and/or associated analyses provide evidence that deliberate Mysis removal via trawling or similar methods could lead to increases in native cladocera populations in Emerald Bay?

Yes, reasonable evidence comes from the *in situ* observations in Emerald Bay, and to a lesser extent in the main basin, over the years of this study. Similar results have been reported in via time series and directed studies in ~similar large western lakes sharing similar zooplankton-mysid-zooplankton communities (e.g., Flathead Lake, Lake Pend Oreille).

We agree that the observations at Tahoe and in other systems provide strong evidence.

3-Does the project report and/or associated analyses provide evidence that increased Cladocera populations can improve clarity in Emerald Bay?

Yes-This is supported by the Emerald Bay mesocosm experiment and somewhat by the *in situ* observations. Increased and persistent transparency associated with the emergence/resurgence of cladocerans, especially *Daphnia* is well documented in other meso-oligotrophic lakes. Perhaps the best example is Lake Washington as it transitioned out of a human induced eutrophic state back to historical transparency (3-m secchi depth) initially in the early 1970s until *Daphnia* became established in 1975 and increased transparency to 6-7 m (Edmondson and Litt 1982) which have now persisted for 45 years.

Thank you for reminding us of the additional and compelling evidence provided by Lake Washington.

4-Does the project report and/or associated analyses provide evidence that the dynamics between Mysis, cladocera, and clarity in Emerald Bay would hold true for Lake Tahoe?

While the existing evidence is suggestive, too many uncertainties remain regarding how these interactions might play out in the main basin. The processes driving these interactions should be the same; however, when scaling up to the main basin, the practical applications of whether significant mysid depletions could be achieved are quite uncertain. Overlaid on this is the complexity of the biotic interactions which include both positive feedbacks and negative feedbacks in the fish-invertebrate food web which can lead to unintended consequences that could extend beyond the focal species to the native fishes like Lahontan redsides, tui chub, and Paiute sculpin.

Non-native lake trout rely very heavily on mysids from juvenile through relatively large adults (Fork Length ≤ 625 mm [25 inches]). Their annual population-level consumption of mysids was estimated at 350-400 metric tons across the lake. This approached the estimated annual production rate of mysids without tapping into the standing stock biomass.

However, as mysids are depleted, the response by lake trout will be a crucial element to investigate with several alternative (but not necessarily mutually exclusive) hypotheses:

- Mysid suppression could be accelerated by depensatory predation mortality from lake trout.

- Mysid suppression might increase self-regulations of lake trout via cannibalism (e.g., as in the reverse of Flathead Lake).

- Mysid suppression might shift predation to kokanee and native fishes with significant increases in mortality

- Mysid suppression might induce an ontogenetic shift to earlier or more extensive predation on cladocerans, thus dampening the potential “predation release” expected from a simple linear decrease in predation risk with declining predator abundance

Most, but not all of these hypotheses have important ramifications for a resurgence in cladocerans or transparency of the main basin.

We really appreciate the experience and insights of the Reviewer. Yes, there are many unknowns regarding the response of fish, particularly the native fish. Relatively little data exist on Tahoe fish populations at interannual scales. Generally, they are believed to have declined since surveys conducted in pre-*Mysis* days, so a compelling hypothesis could be made that a return of cladocera may indeed help them.

The suggestions that are made here are good, but conducting them would take considerable time and resources. Even then, the main driving factor, the effect of *Mysis* removal, could not be evaluated until such an operation commenced. However, the concept of monitoring some of these potential shifts during a long-term *Mysis* removal operation would be feasible. In fact, part of the potential revenues from *Mysis* product sales are intended to help offset the costs of these scientific studies.

Observing these potential impacts at the scale of Lake Tahoe would be difficult, but including such a measurement program in Emerald Bay, where a large change in *Mysis* is expected in a period of months, would be far more beneficial and achievable.

5-Does the project report and/or associated analyses offer evidence that pursuing lake-wide Mysis control is a potentially successful method for improving Lake Tahoe's clarity?

Not yet. The authors rightfully identify a number of critical knowledge gaps that need to be explored through an adaptive management framework in order to evaluate the feasibility and efficacy of such an endeavor.

Some of the most important considerations relate to the long- term commitment to an effort like this. Assuming that no logistical impediments emerge, that mysid depletion progresses acceptably, and potential ecological impacts are either minor, neutral or beneficial, as the mysids decline and reduce catch rates, the program will require increasing subsidies to sustain it. The nature of such population suppression efforts must be continued into eternity or suffer a rapid rebound by the mysids. The authors are clearly aware of this and have done a good job of keeping this transparent. It'll be up to the policy folks to assess the level of commitment they are willing to shoulder.

While we agree with much in this paragraph, the one area where we would respectfully disagree is "…the program will require increasing subsidies to sustain it. The nature of such population suppression efforts must be continued into eternity or suffer a rapid rebound by the mysids". We have spent a large amount of effort working initially with the UC Davis Graduate School of Management, and more recently with a newly forming non-profit to utilize the harvested *Mysis* as an ingredient in "high end" dog treats. We have conducted market surveys, have researched competing products, looked at production costs, and the sustainability of the *Mysis*. Yes, they will not last forever – that is the goal. But depending on the assumptions made in our financial models, this endeavor becomes profitable after a few years, with revenues estimated at several million dollars per year. Return to investors may be in the realm of 8%. At the end of a 15-year production cycle, *Mysis* numbers will have dropped below a number where commercial viability is lost, but only for a few years. During those off years, there are many other lakes where the operation could be transferred while waiting for Tahoe numbers to build up.

So yes, the Reviewer is correct to say that suppression efforts need to be continued into eternity, but if revenue is being generated, if local employment is being derived, and if part of the proceeds are invested back into clarity-focused science at Tahoe, it then that may be a very good thing.

Specific Review Comments

P15 Section 3.0—The 2011-2017 Emerald Bay Natural Perturbation

The extraordinary Mysid crash and then rebound does indeed provide an unique opportunity to explore relations among Mysids, cladocerans and other bottom-up or top down effects on the food web of Emerald Bay with potential implications for the main Basin of Lake Tahoe.

Any hypotheses about the cause of the mysid crash? Seems like an epizootic is a likely candidate worth investigating.

You are quite possibly right on the cause of the crash. We can't determine it now, but with the increased monitoring we are continuing with we are looking out for repeat occurrences. With the 30-year gap in *Mysis* data collection we cannot be sure that similar occurrences did not happen in the past. If it happens again we may likely see it quickly and determine the cause.

P20- Mysid sampling: Conical "Mysid" net (0.75 m diameter 0.5 mm mesh)

Nocturnal vertical tows: 0-60 m depth-integrated vertical tow in EB; 0-100 m at LTP and 0-200 m tows (MLTP & South Shore)

Zooplankton sampled with conical 0.75 diameter 0.080 mm mesh net. Were zooplankton tows also conducted at night or during daylight? I assume during daylight as is standard practice for lake monitoring, but confirmation would be appreciated.

Yes, we can confirm that the zooplankton tows were conducted during daylight.

CONCERN-Depth-stratified zooplankton sampling would have been very informative, especially for monitoring cladocerans, which frequently exhibit highest densities above the thermocline, about 50% lower density within the thermocline, and very low densities below the thermocline, often 10% or less of epilimnetic densities.

We did some depth stratified sampling for *Mysis* but not for cladocera. It was done for native zooplankton in Tahoe by Hans Burgii in the 1990s (H.-R. Bürgi, J. J. Elser, R. C. Richards & C. R. Goldman (1993) Zooplankton patchiness in Lake Tahoe and Castle Lake USA, Internationale Vereinigung für theoretische und angewandte Limnologie: Verhandlungen, 25:1, 378-382, DOI: 10.1080/03680770.1992.11900140). Other papers have shown the thermocline serves as a barrier to *Mysis* creating a thermal refuge for cladocera. We have observed in Emerald Bay that the temperature stratification in summer and fall is such that the temperature segregates the *Mysis* and the cladocera. It is hypothesized that predation by *Mysis* kept the cladocera numbers low below the thermocline. With *Mysis* control, a lot more of the water column should be available for their range – a testable hypothesis with a follow up project.

P21 Fig 4.2. Use same Y-axis range (0-400) for both mysids (mysids/m2) and cladocerans (#/m3)

We thought that given the units were different, we did not need to use the same quantitative range in the Y-axis.

During 2019, can a plausible cause-effect narrative be developed between trawl removals of mysids (during Sep-Dec 2018 and May-June 2019), depressed mysid density to ~40/m2 during Win-Spr-Sum (resurgence in fall), the very limited increase in cladocerans and increased secchi transparency?

For instance, how do trawl removals in terms of biomass and numbers of mysids compare the population reduction that must have taken place to result in declines from 100-200 mysids/m2 to 40 mysids/m2. What fraction of this reduction can be credited to trawling removals versus natural mortality?

It would have been very easy to claim all of that reduction to our trawling efforts. But we have little information on the magnitude (or causes) of natural inter-annual populations swings. Our analysis of the data suggested that our removal numbers were not sufficient to fully account for the drop. With continued monitoring we can start to understand the seasonal and spatial fluctuations and their causes.

P22-24. Proportions of reproductive female mysids peak in Dec through winter in Emerald Bay at 70-80% whereas only 20-30% of females reproductive in main basin of the lake (similar temporal pattern as in EB).

-Mean brood size ~13 eggs/female, but size-dependent fecundity with no significant mortality from egg through development to juvenile release from brood pouch.

-Where is the evidence that the life span in Emerald Bay is only 1 year? A temporal series of length frequency distributions in the South Shore showed a strong 2-year life span (McCoy 2015).

-Comparable mortality of free-swimming juveniles to annual survival rate reported by McCoy 2015 (17.7%).

We will look again at McCoy's work. Much of the earlier work(1970s) had concluded that Emerald Bay and Tahoe populations were one year and 3-4 year life spans respectively (references were included especially in the Appendix). Our work in both Emerald Bay and Tahoe seemed to support that too. Going forward we can look more closely to see if a shift has occurred.

P33. Hydroacoustic methods

FYI-"hull mounted" implies that the transducer is permanently mounted in a through-hull position. What is displayed is a temporary Pipe mounted configuration.

Our apologies for misrepresentation, this should be clarified as a temporary pole mounted configuration on the side of the boat. The sonar head was lowered beneath the depth of the hull to ensure no interference.

I believe you meant to say the Echosounder resolved 1.8 m (not mm) depth bins. Target resolution with a 200 kHz transducer would be roughly 0.3 m at a standard pulse width of 0.4 milliseconds. Was echo-counting or echo-integration used?

We used both the default configuration for the pulse duration (0.4ms) and sampling rate (41,667 Hz) which generates a depth sample size of 1.72 cm. We had rounded up but have now corrected it to be the exact number although we had made an error that it should have been 'cm' rather than 'mm'. Our apologies for that. Because of the size of the target we were looking for (and a lack of an acoustic model for this organism), we did not use echo-integration as directly provided by the Visual Analyzer tool written by Biosonics and instead took the raw counts and wrote new code following the protocol laid out by Gal et al. (1999a and b) and Rudstam et al. (2008) and conducted our own echo-integration in this process.

Presumably these surveys were conducted at night, but this needs to be stated explicitly. This isn't clarified until page 41.

Will correct, although some of our measurements were continuous for 1-2 days (attached to a surface buoy). Some testing was also conducted during the day to make sure we weren't acquiring false returns from the depths below the thermocline.

More specifications are required regarding how the hydroacoustic data were acquired, specifically, depth range over which targets were accepted, ping rate, pulse duration, were one or both frequencies used? I assume just the 200 kHz was used for mysids. It looks like data acquisition ceased below depths of 150 m based on the degrading signal-to-noise ratio. But this information is buried down on page 37 instead of up front where all these settings should be summarized.

The system that was used was a DT-X Autonomous Portable Scientific Echosounder operating with a 70 / 200 kHz split frequency. As detailed in the text, the detection of the *Mysis* shrimp was conducted with the 200 kHz system. However, the 70 kHz was used to mask the fish returns. All of the settings that were used were the default settings.

Again, clearly state which frequency was used for these surveys (presumably 200 kHz), because the relationship of frequency, target strength, and mysid size vary considerably among frequencies.

We finally learn that data were analyzed via the echo-integration method, again several pages later than expected.

Using a -60 dB maximum target strength threshold to eliminate fish targets might not be low enough to filter out the pelagic larval stages of sculpin (which lack swim bladders). I'm not certain whether the native Paiute sculpin express a pelagic larval stage, but many other westerns species do and can be abundant at some times.

We are not aware of Paiute sculpin having a pelagic larval stage. A review of the Cal. Fish Website hosted by UC Davis (http://calfish.ucdavis.edu/species/?ds=241&uid=63) indicates the larval stages remain benthic. This publication on Tahoe indicates they remain benthic throughout their life. http://www.nativefishlab.net/library/textpdf/15724.pdf with the comment "The Paiute sculpin of Lake Tahoe is closely associated with the substrate throughout its life cycle (Phillip H. Baker, MS)"

We were trying to eliminate the larger fish that were very noticeable in the echograms in the mid-water column. For this, -60 dB worked very well and was a value that came from other work (Rudstam et al. 2008).

- Nocturnal depth distributions of mysids were located much shallower in Emerald Bay during peak thermal stratification in August than was reported in South Lake Tahoe during August (McCoy 2015) where the mysid layer spanned 40-85 m relative to the 20-40 m (top-bottom) thermocline.

Unlike the main body of Lake Tahoe (McCoy, 2015) the shallow band in Emerald Bay is hypothesized to result from shallower depths and a sharper thermocline. In the larger lake body, the greater migration length would simply mean that they would have more room to roam. In order words, the vertical migration distance in Tahoe would likely create a more diffuse and wider band of *Mysis*. How this changes areally across the lake is of great interest to us, as it makes sense to use any natural focusing mechanisms to increase catch efficiencies. Our **Fig 4.21** shows a broad (40-80m) *Mysis* layer in Tahoe.

- The dynamics of nearshore-offshore densities and potential influence of ontogeny could be clarified in the future by simply examining the mean, variance and frequency distribution of target strength as functions of distance from shore, bottom depth, and depth of target among months or seasons.

P47-49 Hydroacoustic methodological refinements. The authors are on the right track here. It sounds like some initial system configurations during data acquisition precluded some of the normal target strength analysis that would ordinarily address many of the Target Strength questions posed. Evaluating the TS frequency distribution from each survey should provide straightforward data regarding mean target strength values to apply specifically to each survey and whether mono-modal or bi-modal distributions require some modifications to their calculations.

The authors would like to thank the reviewer for the suggestions and recommendations for these analyses have now been included as recommendations for future research and implementation. The main aim of this portion of the work was to identify depths that the populations were at for trawling purposes but it would definitely be good to refine this work further for future implementation.

This should relieve the reliance on concurrent midwater trawl catches of mysids to enable biological calibration. While the trawl samples would become less important for the population assessment element, midwater trawl samples will be essential for providing biological measures for mysids and of course would be the primary focus for the suppression program

P52-Assuming a maximum survey depth of 100 m for nocturnal surveys should be fine, based on data observed from various months and seasons (thermally stratified and destratified).

P70-Emerald Bay Trawling Results:

TERC had a (3 m x 3 m) Isaacs-Kidd midwater trawl built in the early 1990s to sample kokanee and mysids, but was never used seriously-very little time devoted to refining techniques or modifications. If still intact, it would be a good net to try as it is a single-wire hydrodynamic trawl that can be towed much faster than trawls requiring doors.

The Isaacs-Kidd trawl is no longer with TERC. We looked for it when this project started, but could not locate it. Utah State University, who we worked with at the time, could not locate it either. Regardless, the size of that trawl is far smaller than what a commercial trawling operation will require. The company we have been dealing with has the capacity to trawl and recover far faster than we were able to, have real-time cameras and pressure sensors to know precisely and in real-time where the net is and what is entering it, and high speed winches to quickly recover and redeploy the net. The net that is currently being considered for the next stage would have a depth of approximately 10 m.

We thought we were pretty good, but have since learned that research equipment is not sufficient for this task.

P74. We have never witnessed very narrow bands of suspended mysid (e.g., 2-3 m high) as would be desired. Even during summer stratification, the nocturnal layers were 30-45 m high (August 2012; McCoy 2015)

Our survey work in Tahoe was limited, because of the size of the lake and the need to also conduct trawling in Emerald Bay. In future work there are a lot of unanswered limnology questions that may increase our understanding. For example, we know that the prevailing southwest winds impose a long-term tilt on the thermocline, and the consequent “expansion” of the metalimnetic waters in the south west and west. We would expect that compression of isotherms in the east would have the opposite effect. Better understanding this would allow us to take advantage of the lake dynamics. The planned 3-D modeling may help clarify this.

The new net that has been designed for us is closer to 8 m tall, so it should be ideal for Tahoe.

P75. If 80% of adults in Emerald Bay reproduce during peak season, then harvesting them anytime they are vulnerable to efficient capture before reproduction (even many months earlier) would have the most impact on the population.

Conflicts with recreational boating may preclude summer operations. We have been thinking of harvesting adults in the August-November period. The juveniles are in the littoral zone in Spring, so a different approach may be possible to remove them. If we could have two seasons (spring and fall) that would be very fortuitous. We run the risk here of increasing the by-catch of native fish. Just further confirmation that ongoing research is needed.

However, in the main basin of Tahoe, with only ~20% of adults reaching reproductive status, and given the limited harvesting power relative to the potential harvesting area, a strategy to focus on the reproductive adults in time and space could be a strategy for most effectively focusing harvest effort. If reproductive adults don’t segregate from others, then simply target the highest-density regions that can be effectively harvested.

Still need to see if the juvenile shift to the littoral occurs in Tahoe too, or whether the occurrence of buoys and docks prevents harvesting them.

Harvest:

Emerald Bay-Highest CPUE with large trawl in Fall-early winter, very low in spring (adult die-off and juveniles nearshore).

Main Basin Lake Tahoe has limited shallow nearshore habitat relative to deep pelagic, and adults live 2-4 years, so any spring die-offs don’t result in major reductions in abundance. See temporal length frequency series from McCoy 2015 below:

This is understood and one of the reasons we believe a Tahoe harvest could take place year round if *Mysis* present themselves in densities at depth that allows for efficient harvest.

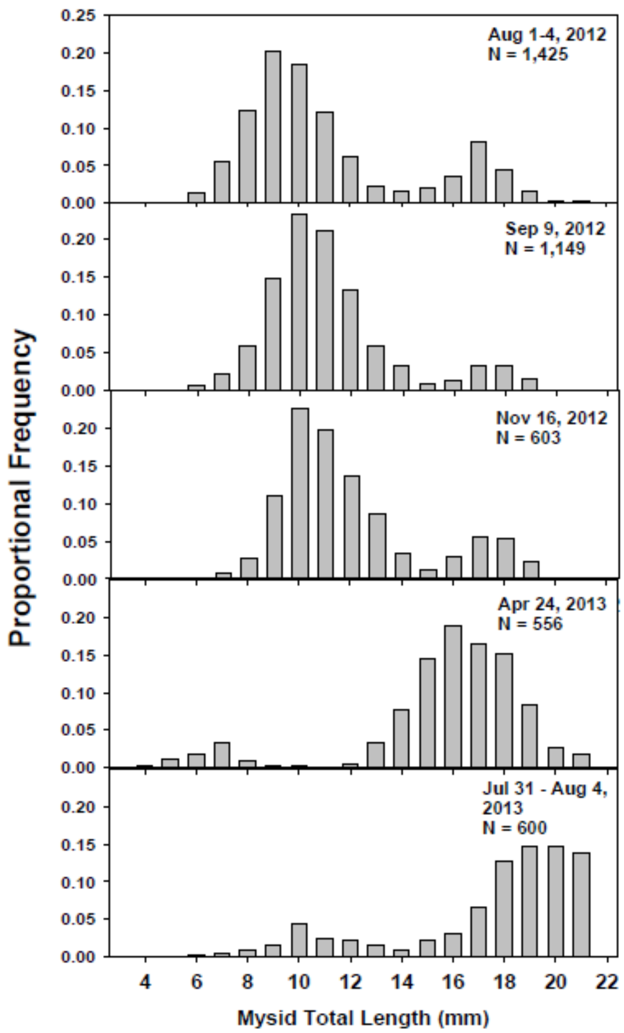

Figure A1. Seasonal length frequencies of mysids in Lake Tahoe sampled during 2012-2013.

P103-Mysid Diets

The diet data (Tables 7.1 & 7.2 [mislabeled as Tahoe, should be Emerald Bay]) should be stratified into large and small mysids, since stable isotopes and other information suggest an ontogenetic shift to more zooplankton predation by larger individuals.

Table 7.2 should, indeed, be labeled "Emerald Bay". Thank you for pointing this out. We have changed the caption for table 7.2 so that it is now accurate. The information in Tables 7.1 and 7.2

otherwise remains the same. The literature shows that mysids switch to predation in the adult life stage, and most publications we are aware of use a cutoff of 8 mm to delineate juvenile mysids from adults. For instance, Lesutiene et al. (2007) used stable isotope analyses to indicate that *Paramysis lacustris* in the Baltic Sea need to reach a threshold size of 8.7 ± 0.7 mm to feed on zooplankton. Additionally, James Rybock's 1978 dissertation examining diet contents of mysids in Lake Tahoe found that mysids switch to predatory feeding at a size of 7 to 8 mm. All mysids included in our diet analyses are 10 mm or larger. Mean length of these mysids was 15.23 mm, and standard deviation was 2.12 mm. We purposely included only adult mysids in this diet analysis. Therefore, all mysids included in the analysis should have predatory capabilities. We have added this information on mysid size distribution to the 7.0.1 Diet Analysis section.

In the main lake basin, the identifiable diet contents indicate a high degree of herbivory or perhaps detritivory with episodic seasonal pulses of zooplankton predation (the predominant copepod *Epischura* and the rotifer *Kellicottia* in spring with cladocerans contributing a minor fraction. Diets in Emerald Bay showed much lower zooplankton predation.

The authors of this section agree with this statement. We are uncertain of what causes the observed difference in diets between Lake Tahoe and Emerald Bay mysids. It is possible that the relatively higher primary productivity of Emerald Bay may lead mysids in the bay to feed more heavily on phytoplankton.

Lessons Learned P111-

Mysid life span: Mysids only exhibit 2 distinct size modes in the main basin of Lake Tahoe based on length frequency distributions. While it's possible older (age 3-4) adults might exist and simply not grow beyond the length achieved by age-2, I would expect a bit messier frequency distribution with more variability around the larger mode if there was significant survival beyond age-2.

I think that either aging via hard parts or some bio-accumulative biomarker would be needed to resolve this issue definitively.

The findings in this study utilized previous life history studies focused on *Mysis* from Lake Tahoe and did not attempt to reevaluate the age structure of the Tahoe population. Our size distribution did not provide strong evidence to suggest a shift has occurred. However, based on changes in primary productivity since earlier studies were conducted, it would be beneficial to use the techniques mentioned to more accurately determine the current age structure of *Mysis* in Tahoe.

Hydroacoustic surveys and observations of behavior and distribution of mysids (and fish) are an absolutely essential element of any effort to suppress mysids, but also to gain invaluable quantitative understanding of temporal-spatial-ontogenetic patterns in abundance, distribution and movement of mysids and pelagic fishes that can influence them.

Exploring the potential applications of AUVs or ASVs is a great idea for this and many other future applications.

We agree too. Tahoe is simply too large to have people out all night doing a survey that is largely electronic. The cost savings are huge, and I would rather have people's time used to analyze the data.

Review 2: Michael Brett

Review of DRAFT ADMINISTRATIVE REPORT "Planning for Removal of *Mysis* Shrimp from Emerald Bay and Lake Tahoe as a Means of Ecosystem and Clarity Restoration" by Schladow et al.

Thank you for asking me to review this draft report. I will respond to this review request by directly addressing the five questions outlined in the review charge. These are:

1. Does the project report, appendices and/or associated analyses provide evidence that Mysis can be reduced in Emerald Bay using boat trawling and Echosounder methods?

No. The *Mysis* removal attempted in the original project failed to meet its objectives for a variety of reasons, but the authors of this report claimed that with a factor 30 higher harvest rate they anticipate they could reduce the *Mysis* population in Emerald Bay. A 2X portion of the hoped-for increased harvest rate would be due to trawling for *Mysis* twice as long each night. That would be easy enough to accomplish. However, the other 15X increase in harvest rate would be achieved by using a trawl net that is 15 times larger than was used in the test trawling. It remains to be seen whether this will be successful. For reasons explained in the original report, trawling a net with a very large opening is difficult and perhaps even dangerous in a system like Emerald bay, and especially in Lake Tahoe. The report I read did not adequately explain how this research team would be able to trawl using a net that was 15 times larger. Presumably this would require a much larger boat, much more rigorous equipment, and an experienced crew. It is always a huge challenge to dramatically scale up from an experimental to an actual full-scale system. I would need more information to be convinced that it will be possible to trawl a 15 times large net effectively and safely in Emerald Bay.

The report did not assert that the research team would be conducting the trawling. On the contrary, we demonstrated that such an activity was beyond the capacity of current research equipment and staff training. We have been working with a professional trawl company (referenced above) and they have the boat, staff and equipment to conduct the trawling at the scale we proposed. They do not see the limitations on capacity that the Reviewer does.

The factor increase that we proposed in the report was an estimate based on the results of our trawling, our measurements of *Mysis* density and what our experiments concluded were the obvious deficiencies of trying to remove *Mysis* at a commercial scale as part of a research effort. The fact that a commercial trawl operator believes that those numbers are achievable speaks volumes to the conclusions of the research.

Given this, we believe the "No" by the Reviewer is extremely subjective. The question was whether we "*provide evidence that Mysis can be reduced in Emerald Bay using boat trawling and Echosounder methods*". Based on the data and analysis, we believe we have provided precisely that evidence (through our detection methods, our seasonality observations, our *Mysis* sampling, our trawling experiments). Without that having taken place, we would not be able to be having this conversation.

2. Does the project report and/or associated analyses provide evidence that deliberate Mysis removal via trawling or similar methods could lead to increases in native cladocera populations in Emerald Bay?

Maybe. I believe the figure that is supposed to make this point is **Fig. 3.1** on page 15. This figure shows that during a 2 ½ year period when *Mysis* abundance was quite low, there was a marked increase in *Daphnia* abundance after about one year and this increased *Daphnia* abundance persisted for about 1 and ½ years until *Mysis* abundance recovered. Based on prior research in Lake Tahoe, and many other lakes, it seems quite plausible that a high abundance of *Mysis* in Emerald Bay could suppress *Daphnia* biomass to low levels. But just because *Daphnia* increased one year after *Mysis* declined to low levels does not mean there will always be a high *Daphnia* abundance when *Mysis* levels are low. Also, as noted in my response to question #1, it is not yet clear whether trawling can reduce *Mysis* abundance enough to allow *Daphnia* to recover. That is still a major point of uncertainty.

The important point that the Reviewer does not address is that the *Mysis* levels that were observed in Emerald Bay were totally unprecedented in their low magnitude (a factor of 100 lower). Similarly, the corresponding high *Daphnia* numbers were equally unprecedented since the time of introduction of *Mysis*. The fact that it took a year for *Daphnia* numbers to build up further demonstrates that it was a population rebound in the absence of grazing that produced this (population growth does not take place overnight). *Daphnia* are native to Tahoe and Emerald Bay and were dominant prior to the introduction of *Mysis*. As there have been no other zooplankton or other grazers introduced to Tahoe since *Mysis* that would feed in this size range, the reviewer's doubts appear to be highly conjectural.

What does happen overnight is the increase in clarity that was observed in Emerald Bay. The return of *Daphnia* allowed for the removal of fine particles, and given the feeding rates of *Daphnia*, the rapid rate of clarity return is fully consistent with the role of *Daphnia*.

3. Does the project report and/or associated analyses provide evidence that increased cladocera populations can improve clarity in Emerald Bay?

During the time when *Daphnia* were more or less abundant in Emerald Bay secchi depth increased from about 14-15 m to 20-21 m. It is plausible that some or even most of this increased clarity was due to *Daphnia* grazing. Elsewhere in the report it was claimed that low *Mysis* abundance and high *Daphnia* abundance were associated with a 11 m improvement in water clarity. It is unclear what this claimed 11 m improvement is based on. I am guessing that is was based on comparing the lowest clarity in the pre-*Daphnia* period to the highest clarity observed during the time when *Daphnia* were abundant. However, the data I summarize above suggest an improved water clarity of about 6 m is more representative for the overall trends for these two time periods. Although it is plausible that *Daphnia* grazing played a substantial role in this improved water clarity, I believe the factors that control water clarity in Emerald Bay and Lake Tahoe are multifaceted and complex. This time series alone does not convince me that increased *Daphnia* abundance will all by itself lead to a consistent 6 m improvement in water clarity. Furthermore, I think the claimed 11 m improvement in water clarity is not supported by the totality of the data presented in this report. Based on the evidence presented in this report, and the general literature lake food webs, I believe a substantially higher *Daphnia* biomass would benefit water clarity in the Lake Tahoe system.

There has been a large body of data collected and published at Tahoe on the causes of clarity decline at Lake Tahoe, that the reviewer may not be familiar with. Yes, the processes are complex and multi-faceted, but it is the presence of particles in the size range of 1-4 microns that control light scattering. Other lake systems are more complex on account of the presence of colored dissolved organic material (CDOM) but at Tahoe CDOM is very low (even in Emerald Bay, which is more colored than Tahoe). The complexity in part arises from the effects of thermal stratification and basin-scale lake motions, which can cause large fluctuations in clarity in Tahoe on account of its size and the effects of stratification on controlling the depth at which light scattering particles are present. Emerald Bay is far more sheltered from large, basin-scale motions so these large fluctuations do not affect its clarity. Rather, we believe that spatial heterogeneity is responsible for the fluctuations we observed in secchi depth measurements in Emerald Bay.

The insinuation that the 11 m clarity improvement is not supported by the data is refuted. The Reviewer assertion that Emerald Bay clarity was 14.5m prior to the improvement by *Daphnia* is incorrect. The reviewer failed to notice that when the monitoring started (December 2011) the secchi depth was increasing from an unknown value. Looking at secchi depth values at the end of the period (2018) shows that after the re-establishment of *Mysis* and the loss of *Daphnia*, the annual average clarity was in the range of 10-12 m, the range it has been observed to be at for decades.

Regardless of the value of clarity increase, 6 m or 11 m or anything in between, the only way such a prolonged increase in clarity could take place is the removal of light scattering particulates. The most likely way that could happen so quickly would be through intensive grazing.

4. Does the project report and/or associated analyses provide evidence that the dynamics between Mysis, cladocera, and clarity in Emerald Bay would hold true for Lake Tahoe?

This report did not present new evidence that high *Mysis* abundance can suppress cladoceran abundance in Lake Tahoe. However, this was already established in previous research from Lake Tahoe done in the late 1970s and earlier 1980s. The report does not present any original evidence that low *Mysis* and high cladoceran biomass is associated with great water clarity in Lake Tahoe. It seems likely that high *Mysis* abundance is associated with low cladoceran biomass based on previous Tahoe research. Additionally, based on the Trophic Cascade Hypothesis and the substantial research done on that topic in many lakes, it is also plausible that high *Daphnia* biomass would also be associated with greater water clarity in Lake Tahoe. However, on page 151 of the report, the authors claimed that without the loss of cladocerans "Lake Tahoe's clarity could conceivably have been largely similar today to what it was historically." This same paragraph suggests that the expected improvement in Lake Tahoe clarity if cladoceran biomass was at pre-*Mysis* levels would likely be 11 m greater. I think these conclusions go well beyond the supporting data presented in the report! High *Daphnia* biomass might improve Lake Tahoe water clarity by up to 6 m, but the claim of an 11 m improvement seems to be greatly over-stated.

As the Reviewer points out, we rely on the evidence gathered at Tahoe in the 1970s and 1980s (and the 1960s before *Mysis* introduction). Gathering further evidence on this was not part of the project scope.

Again, the mechanistic cause of clarity decline is largely due to particle scattering of light. Prior to *Mysis*, the lake was clear and dominated by cladocera, which are known to be very efficient grazers of particles (organic and inorganic). The fact that the TMDL program specifically targets particles shows that this is consistent with current management priorities and the science that underpins them. The recommendation of controlling *Mysis* to allow cladocera to return and remove the scattering particles is simply a logical extension of that previous work. Arguing about whether it could produce 6 m or 11 m of clarity improvement is beside the point at the present time – achieving 6 m of clarity improvement would be a tremendous achievement. It would seem that efforts to date to reduce the external load of fine particles or to control the growth of fine algae within the lake are not keeping up with the need, albeit it at a very high cost. The evidence suggests that both approaches combined may provide clarity (and ecosystem) gains.

5. Does the project report and/or associated analyses offer evidence that pursuing lake- wide Mysis control is a potentially successful method for improving Lake Tahoe's clarity?

No. As previously noted, a successful *Mysis* control trawl effort in Emerald Bay would require a 30X improvement in *Mysis* harvest. However, the surface area of Lake Tahoe is 250 times greater than that of Emerald Bay! So successful *Mysis* control in Lake Tahoe would require a » 8,000 times greater *Mysis* removal effectiveness than was previously demonstrated in field sampling at Emerald Bay. Based on this alone, I am guessing *Mysis* control in Lake Tahoe would require a fleet of ocean sized trawlers at a huge cost.

The report also claimed in several places that *Mysis* trawling could pay for itself. I think this claim is a logical-fallacy. Any *Mysis* harvested from Lake Tahoe would likely have a high Omega-3 fatty acid content, and could be easily sold for a variety of purposes. However, in order for the trawling to have a limnologically meaningful impact on water clarity in Lake Tahoe it would have to reduce *Mysis* abundance to quite low levels. If this happened the trawling program would no longer be economically viable because the *Mysis* CPUE would plummet. The trawling program would only be economically viable when the *Mysis* population is high and it is possible to harvest a large biomass with a modest effort. I will also note, that under the best of circumstances, the test trawling carried out in Emerald Bay only yielded about 3 kg *Mysis* per hour of trawling. Assuming *Mysis* could be sold for a few dollars per kg, a massive improvement CPUE would be needed to recoup the expenses associated with trawling (i.e., purchasing a suitable boat, fuel, hourly wages for the crew, nets and other materials, insurance, etc.) under the best of circumstances.

The Reviewer overlooks the point that the proposed next action is for commercial-scale trawling, and that commercial trawlers believe that the required increase in removal rate by a factor of 30 (based on our results) is well within their capacity. Our financial model (not part of the scope of this project) does factor in an increase in the number of trawl vessels over time (not ocean-sized, but significantly larger and more powerful that our research vessel), as revenues earned allow these to be financed. We do not propose to simply market the *Mysis* themselves. That clearly does not take advantage of the gains achievable through producing value-added products that only contain a small percentage of *Mysis*.

There is no logical fallacy. The financial model (not part of this project) shows that *Mysis* trawling can become profitable (not a "huge cost") in 3-4 years, with quarterly revenues in the order of several million dollars. We have conducted an extensive research in partnership with a newly formed non-profit (Blue Tahoe Harvest) that has invested time in exploring the financing, marketing, and production technology to show that end products that contain a small fraction of *Mysis* can be commercially viable. The expectation is that within a prescribed time period, say 15 years, the *Mysis* numbers would drop to a level where they would no longer be commercially profitable to remove. At that point the operation would temporarily relocate to another western lake where *Mysis* also present a problem (Donner Lake, Fallen Leaf Lake, Flathead Lake etc.). With 2-3 years *Mysis* numbers would have rebounded and Tahoe operations could resume. In the long term we foresee management evolving to a combination of *Mysis* trawling and active measures to control loading to the lake.

<u>Review 3: Walter Dodds</u>

Evaluation of the Draft Administrative Report: Planning for Removal of *Mysis* Shrimp from Emerald Bay and Lake Tahoe as a Means of Ecosystem and Clarity Restoration

27 Sept 2020 Summary of Review:

The report is a good synthesis of past research and ongoing efforts to assess the role of *Mysis* in the food web of Lake Tahoe and the potential for *Mysis* removal as a tool to improve lake clarity that has been lost over historical levels.

This report is a continuation of exploration of feasibility of removal of *Mysis* shrimp as a method to increase clarity of Lake Tahoe and Emerald Bay. The idea is based on several lines of evidence.

1. In lakes where *Mysis* has been introduced, there have been large decreases in other large zooplankton populations (cladocera).
2. In Emerald Bay there was a drastic decline of *Mysis*, followed by an increase in cladocerans and water clarity
3. In Lake Tahoe, *Daphnia* and *Bosmina* all but disappeared after *Mysis* introduction
4. Clarity has continued to decline in Lake Tahoe.

The idea relies upon the idea of trophic cascades in lakes being able to reduce phytoplankton abundance if large grazing zooplankton (primarily cladocera) can be increased upon removal of their predators. We need to keep in mind that the idea of abating eutrophication with a trophic cascade is controversial in evolutionary terms (e.g. Wetzel 2001) as well as in very oligotrophic lakes, where grazing may not limit phytoplankton production, as food density is so low for zooplankton that they are limited by food production rather than limiting algal production.

More specifically, the idea of successful *Mysis* control that could be followed by increases in water quality is based heavily on the idea that Emerald Bay is an analog for the entire Lake Tahoe. There are a number of reasons to suspect that this may not be true, or at least that we do not have enough data to establish confidence in the similar nature of both systems. In this report alone the authors find different *Mysis* behavior in both lakes, different responses in the mesocosms, and different levels of chlorophyll with maxima at different depths. The data I found in the report were not sufficient to evaluate if the phytoplankton communities were similar.

The observation that the establishment and the consequences of *Mysis* in both Tahoe and Emerald Bay would suggest to us that any differences between the two systems are not significant enough to have influenced that well documented event. The recent (2011-2017) sudden decline and return of *Mysis* in Emerald Bay followed a very similar pattern to the initial introduction in that system, with the same patterns with regard to cladocera and clarity. The phytoplankton assemblages in the two systems are very similar (we have now added data on this to the main report). Differences in chlorophyll maxima are totally consistent with the physical forcing and the light climate in each system. Emerald Bay being fetch limited and relatively sheltered from the wind, is less energetic and processes such as upwelling and induced shear do not produce a deep mixed layer. The light climate in Emerald Bay is also more influenced by a relatively higher watershed area to volume ratio than Tahoe. For this reason, clarity has always been lower than clarity in Tahoe, except for the time period when *Mysis* were absent from Emerald Bay. For those reasons alone the deep chlorophyll maxima are different in each system.

The second general area that casts doubt upon the potential for *Mysis* control leading to increases in lake clarity is the fact that development has continued in the watershed of the lake throughout the monitoring period, the lake is seeing continued increases in recreational pressure, the lake is experiencing climate change that influences temperatures and stratification, and particulate inputs as well as nutrient inputs could be increasing via atmospheric deposition (e.g. greater upwind urban activity, more frequent fires). The fact that periphyton in the littoral zone of the lake appears to continue to increase also suggests that factors in addition to alteration of the zooplankton community by *Mysis* could be influencing trophic state of the lake. All these suggest that there are a number of other potential explanations for decreased lake clarity that do not involve, or are acting in concert with, disruption of the food web by *Mysis* introduction.

We humbly disagree with these assertions. Both Emerald Bay and Lake Tahoe have been subjected to the same development, recreation, climate change, wildfires etc. Yet, when *Mysis* disappeared in 2011 Emerald Bay responded very strongly in the same way it had when *Mysis* were introduced (in the absence of *Mysis*, *Daphnia* were present in large numbers and clarity quickly improved, and when *Mysis* returned the *Daphnia* and clarity gains disappeared). The factors that the reviewer cites have all impacted clarity, and the way they have done so is through the introduction of fine particulates (both inorganic and very small diatoms). Cladocera are known to have very high removal rates of particulates and the fact that in 2 years Emerald Bay saw a large increase in secchi depth can only be explained by the removal of these particulates (in the 1-4 micron range). We agree that clarity is impacted by a range of processes, but the net effect of those processes is to add fine particles to the upper water column. The net effect of *Mysis* is to remove the cladocera that have the ability to remove those particles, even at the elevated concentrations that they exist at today.

Periphyton at Lake Tahoe are not increasing. We have been monitoring this since the mid-1980s and there has been no statistically significant increase. A recent manuscript detailing this research is currently undergoing the minor changes the journal editor has requested.

One area where the report does not provide enough information is with respect to the unintended consequences of the trawling operations. I saw no data on bycatch. Thus, fishes could have been impacted. Additionally, the nets could have caused considerable cladoceran mortality. It is possible that the trawling could remove enough cladocera that it would preclude their bouncing back even if *Mysis* abundance was lowered to a level where they had modest impact as predators on the cladocera.

Regrettably we inadvertently left the bycatch data out of the report. It is now present. For fish the bycatch was very small. For the cladocera it was virtually zero, as the net size was too large to retain them. The reasons for the low fish bycatch are due to the spatial separation of the various populations. The *Mysis* are strictly below the thermocline during the stratified season (because of their temperature tolerances). cladocerans could exist throughout the water column, but below the thermocline they are controlled by the *Mysis*. As we know the depth at which the *Mysis* are present (through bioacoustics surveys) we do not encounter the cladocera.

Similarly with the fish, lake trout tend to occupy deeper water than we are proposing to trawl for *Mysis*. Kokanee occur in shallower water and what bycatch took place we believe occurred as the net was being lowered or raised. The smaller native fish are primarily littoral, and our operations are focused on the pelagic waters. Our apologies for not including that in the Draft Report.

I just get the sense that there could be the potential to make a large ecological mistake here. The initial introduction of *Mysis* was well intentioned, and that led to bad results. Large-scale manipulation in response may not be appropriate. What if someone suggested getting blue whales conditioned to freshwater and letting them do the filtering? This is a “baleen in cheek” comment, but essentially the current proposal involves adding the function of a very large filter feeder into the food web to produce high dollar dog treats. Putting the light comments aside, I do not get the sense that there are enough data yet to be certain that 1) the trawling would be effective at the scale needed to see a strong effect, and 2) what exactly the effect would be even if you could use the trawls to substantially reduce the *Mysis* populations.

We agree with you that the effect of trawling needs to be large to see a strong effect. That is precisely why we are recommending that a commercial trawling operation be tested in Emerald Bay. It is small enough that a month or of trawling would show a very strong impact. Could this impact be negative and irreversible? We doubt it, as the "natural" disappearance of *Mysis* that occurred after 2011 had very positive effects but when *Mysis* returned the ecosystem returned to its previous state in 2-3 years. This research is seeking to repeat that in a controlled and well monitored manner.

So while introductions of foreign species, such as *Mysis* or baleen whales, may have irreversible consequences our actions are readily reversible primarily because we can never fully remove the target species (just reduce them). So in our opinion the possibility of a "large ecological mistake" is not a good analog. If negative effects do become apparent, simply stopping the trawling combined with the *Mysis* reproduction rates would quickly restore the system to its current perturbed state.

A piece of information that might be the most important of all is why exactly did *Mysis* abruptly decline in Emerald Bay? If this could be figured out, it might point to an alternative method of *Mysis* control.

As we were not monitoring when that event was initiated, I doubt we will ever know the cause. It has been suggested by *Mysis* experts that it likely was an epizootic, which is not a practical control measure for reasons the reviewer mentions.

In reading this report I do not find completely compelling evidence for ultimate success of a *Mysis* removal project on Lake Tahoe, but based on the evidence so far I do support many of the recommendations of the report. My comments on each of the suggested actions follow the suggestions from the report in italics. After my comments on suggested actions, I provide more detailed comments on specific points in the report.

Thank you for that support of our recommendations. We agree that much still needs to be learned, and probably the best way to learn that is to start with further work in Emerald Bay. The key question is whether this can be commercially viable, and only by using a professional trawling operation for Emerald Bay will the catch rate be known. The costs of such an operation are known. Our assumptions on catch rates indicate it is profitable. But we need to confirm the actual catch rate to be sure of how much *Mysis* needs to be sold.

Comments on Suggested Actions

- As Emerald Bay could be used as an analog for Tahoe for future management and scientific questions, a baseline monitoring program should be established in order to identify deviations and synchronies between the two. Emerald Bay will be quicker to respond to changes than the main lake and could serve as a sentinel for Lake Tahoe.

This will be necessary before results can be extrapolated to Lake Tahoe. However, I am somewhat skeptical that the bay is an adequate analog.

As noted above, we respectfully disagree on this point.

- The measurement of annual PP in Emerald Bay should be considered as part of future monitoring, including the relative contributions of benthic and pelagic sources of production to ecosystem totals.
This is important because prior research suggests that Emerald Bay is substantially more productive than Lake Tahoe. As mentioned already, trophic cascades may be ineffective in ultra-oligotrophic lakes.

No argument here. We have actually started PP measurements, albeit without funding for it.

- More thoroughly characterize the seasonal areal distribution and age distribution of *Mysis* within Lake Tahoe. This will enable the determination of whether *Mysis* can be efficiently harvested in the spring in Lake Tahoe.

This is a huge data gap so far.
- Develop a population model for *Mysis*, particularly for Lake Tahoe, where the 3-4 year life cycle introduces greater complexity.

This will also be important in understanding effective methods of Mysis population control.

- Develop population models for *Daphnia* and *Bosmina* to better allow planning in a future condition where *Mysis*, *Daphnia* and *Bosmina* could coexist. These models would need to take into account future temperature changes (as water temperature provides different barriers to each species).
This is important as well.

- ASV and/or AUV surveys of *Mysis* distribution in Lake Tahoe are a critical priority. The size of the resource needs to be better quantified, and in particular its areal distribution and how this changes seasonally. The detailed planning, optimizing and costing of *Mysis* removal depends directly on knowing this.

Agreed, although this is a verily high-tech endeavor and covering a substantial portion of the lake would require a large effort.

We are fortunate to have that expertise in autonomous vehicles in our group. It is precisely for the reasons you suggest ("a large effort") that we believe autonomous vessels are the way to go. When you have a crew of two driving a vessel for 8 hours at night for 200 nights a year, it gets very expensive and a waste of people-time as the data are being collected by the echosounder for later analysis.

- Develop real-time, operational bioacoustics to optimize harvest efficiency.

This would increase the probability of success.

Agreed. Being able to tell a trawler where the *Mysis* are can only help increase efficiency and lower costs.

- Archiving bioacoustic data for calibration and validation of three-dimensional particle tracking models, along with other physical data needed for modeling studies.
This would be necessary for retrospective analysis of success

Agreed. It also has the potential, once sufficient data have been collected and modeling veracity has been established, to reduce the time on the water and rely more on the models to guide trawling operations.

- Conduct bioacoustic surveys of Lake Tahoe focusing on littoral – pelagic differences seasonality, and differentiating between *Mysis* age classes.

Agreed, this is probably the largest information gap currently, as we simply do not know where Mysis congregates in Lake Tahoe at different times and as a function of life stage.

- Determine whether littoral *Mysis* populations are evenly distributed around the lake *Agreed*

- A high priority will be to monitor the abundance of *Cyclotella* and fine inorganic particles both before and after future *Mysis* removal efforts.
All phytoplankton should be monitored

We monitor all phytoplankton currently at monthly intervals and at 13 depths, but given that the fine particles are the target insofar as clarity is concerned, a higher frequency of particulate monitoring may be advisable.

-A "full scale", commercial trawling experiment in Emerald Bay is the only way to evaluate the economics and benefits of *Mysis* removal, and necessary steps for up-scaling to Lake Tahoe. It had originally been anticipated that a "research-scale" approach could provide that information, but that proved to be insufficient effort. Based on the experience of this project, the actual trawling for Emerald Bay may take less than one month if the appropriately designed equipment is used.

This might be a bit premature if we do not know if Emerald Bay is indeed an analog for Lake Tahoe

As we said above, knowing the catch rate is critical, as it will determine the financial feasibility. If it turns out that it is not feasible, then knowing that at the earliest possible time seems important.

- A monitoring program to determine the changes to physical, chemical, biological and ecological components should be integrated with the above experiment, with monitoring both before and after trawling. The monitoring should include the entire food web (phytoplankton to fish), and the harvested *Mysis* should be used toward developing a marketable product that can either offset or fully cover *Mysis* control costs in the future.

This would be necessary to test feasibility of moving the harvest to the whole lake

Develop a three-dimensional *Mysis* distribution model to be operated both in conjunction with the operational bioacoustics, and to permit longer term planning.

This would be necessary.

Specific comments on document (page numbers refer to numbers on the bottom of the pages, as they do not always correspond to the page number of the Adobe PDF I received)

Page 11. There is a good bit of data suggesting that inert particles are mostly rejected by *Daphnia*, and they can survive in environments with relatively high inorganic turbidity.

The literature we consulted is very clear that *Daphnia* efficiently remove particles in the size range that is important for clarity. The fact that clarity markedly improved when *Daphnia* were present in large numbers in Emerald Bay would seem to confirm that. We have the ability to measure particle concentration and size distribution, so hopefully we can add to the literature if we have the opportunity to bring back the *Daphnia*.

Page 12. Acknowledgement that nutrient increases could be important Page 12. It looks like Secchi has flattened since the late 1990's

We have been monitoring nutrients in great detail in Lake Tahoe and do not believe that they are exerting a significant impact on this issue. The causes of secchi flattening are still related to particles, more than nutrients. But we will add a note to the role of nutrients.

Page 13. Why specifically did Carney and Elser (1990) and Elser and Goldman (1991) reject the importance of grazing in Lake Tahoe?

They investigated the role of copepods (*Epischura* and *Diaptomus*), not cladocera. Copepods have remained present in Lake Tahoe while clarity has gone down. Hence their conclusion was, in our opinion, too general. It should have been that copepod grazing is not important.

Page 21. The figure 4.2 does not allow good comparison of *Mysis* and zooplankton numbers as they are presented per unit area and volume respectively. More important the link between decreases in *Mysis* and increases in *Daphnia* and *Bosmina* is not very clear in this figure. Also, scales that would allow seeing the Cladoceran numbers more clearly would be helpful.

Two issues are raised here. As far as the different units, we chose to use the units that are "traditionally" used for *Mysis* (ind/sq.m) and Zoops (ind/cu.m). We thought it was more important to follow those conventions and better permit comparisons with past measurements. For *Mysis* we essentially divide the total number of individuals caught by the cross-sectional area of the net. Dividing by the depth of the vertical tow would produce a *Mysis* number as individuals per m^3. We will add that conversion methodology to the captions so that a reader can easily do the conversion and get comparable values if they wish.

The reason we used the scale range we did for the cladocera was to permit comparison with **Fig. 3.1**. In **Fig 3.1**, when cladocera returned to Emerald bay their numbers were in the 3000-6000 range. In **Fig 4.1**, where the values are difficult to see on the same scale, the numbers were below 0-300. The point we were trying to make is that cladocera numbers were 2-3 orders of magnitude lower, so the precise values are really secondary. By keeping the scale as is, the message is very visual.

Page 22. The diferring life history patterns of *Mysis* in Emerald Bay and Lake Tahoe suggest that Emerald Bay may not be such a great analog for Lake Tahoe.

The fact that the disappearance of *Mysis* and re-emergence of cladocera (and the subsequent reversal) in 2011-2017 followed the same pattern as what happened with the original introduction suggests otherwise. Also, Emerald Bay and Tahoe have always been slightly different yet the impacts and onset of *Mysis* introduction in both were found to be the same in the 1970s. Basically nothing related to *Mysis* has found to be different to in the two water bodies other than the longevity of the populations.

Page 39. First – we should full paragraph. Evidence of what?

Evidence of a discrete layer formation. Sorry – should have been clearer.

Page 47. While there is a distinct layering, the proportion of the population is not clear.

Good point – we can calculate that.

Page 53. Figure 4.31. The chlorophyll maximum is at a substantially different level of the main lake. This is another piece of data that suggests that Emerald Bay is not an analog.

Tahoe is clearer than Emerald Bay so it stands to reason that the chlorophyll maximum is deeper. We are not sure how that fact impacts the removal of *Mysis*, particularly when we are proposing to locate the *Mysis* using echo-location. The fact that *Mysis* come up at night and form concentrated bands would seem to be the pertinent point.

Page 56. Figure 4.35. Here is a figure that suggests there are intense feeding zones where pulling a very fine mesh net through areas that have high biomass density will capture many organisms that are not necessarily targets.

The depth of the chlorophyll maximum and the depth of the nightly *Mysis* maximum are not related. The *Mysis* are controlled by the thermal stratification, and the thermocline is spatially separated from the chlorophyll maximum. The *Mysis* net size is such that phytoplankton and other zooplankton capture was not an issue. We did not explicitly state that, but in hindsight we should. It is now stated in the Report

Figure 4.42 The y-axis is labeled as biomass, but this is relative abundance.

Sorry – our mistake

Page 63. The idea that *Mysis* could be a grazer of cryptomonads casts doubt on the potential of control of primary production via a trophic cascade. Omnivory is one of the main arguments against the trophic cascade.

Possibly – but our primary goal is the restoration of clarity by removal of fine particles (1-4 microns) through a return of cladocera. The potential impacts on primary production clearly is still at a hypothetical stage.

Figure 4.45 and 4.46. I think something is wrong with y-axis labels

I think you are right!

Page 67. The argument that Emerald bay is an analog to the main lake would be buttressed if the phytoplankton communities were similar.

We have now added this data to the report.

Page 76. This is an important point, and totally agree that understanding differences in life cycles between the main lake and Emerald Bay is key.

Page 76. This would be an important place to note any bycatch of the different mesh sizes.

We only started trawling seriously once we had established the appropriate mesh size (the finest one). We have now reported on bycatch, and as you will see it is very small (and zero for cladocera)

Page 84. The DIN/ SRP ratios are often useless because they do not say anything about flux rates (Dodds 2003).

We agree that is better to use the flux rate of nutrients into an aquatic ecosystem to understand the potential limiting nutrient for algal growth. The experiments were all conducted and we could determine the rates of each nutrient per unit time (per day). This will not change the ratio of nutrients in this study.

Page 85. The mesocosm experiment suggests *Mysis* control will not cause a trophic cascade that goes through to phytoplankton in Lake Tahoe.

The authors of this section of the document agree with this statement. *Mysis* should not cause a trophic cascade that goes through to the phytoplankton in Lake Tahoe. Research concerning Lake Tahoe has indicated that, during times of high primary productivity, Daphnia densities increase as a result of increased birth rates (Byron et al. 1986). However, there isn't any indication that *Daphnia* can effectively control phytoplankton densities in Lake Tahoe, and this is revealed in the Lake Tahoe mesocosm experiment presented in this section of the study. However, *Daphnia* do lead to decreased phytoplankton densities have mesotrophic systems, and this may explain why we observed an influence of Daphnia on Emerald Bay phytoplankton concentrations (Lampert et al. 1986).

Because mysids can control *Daphnia* densities in Lake Tahoe (Richards et al. 1975; Goldman et al. 1979), it is likely that mysids will only effectuate a decrease in Lake Tahoe's phytoplankton density if primary productivity levels continue to increase with cultural eutrophication and enhance the reproduction and populations of Daphnia.

Page 86. If N is excreted as ammonium, and P as particulate materials, then the stoichiometry is incomplete, and the dissolved inorganic ratios could only reflect this fact.

We agree. We do not have a complete understanding of the nutrient concentrations whether particulate or organic. We use the inorganic concentrations as an initial proxy for the influence of zooplankton nutrient stoichiometry but a more complete assessment, even with these lower concentrations is warranted for future work.

Page 89. There are paradoxical effects of *Daphnia* P excretion on P availability that have not been resolved as far as I know (Dodds et al. 1991).

We recognize that not all phosphorous excreted by *Daphnia* will be available to phytoplankton. Further analysis could help to resolve the paradoxical effects of *Daphnia* P excretion on availability to the phytoplankton community. We were not aware of these effects prior to conducting the experiment.

Figure 6.1 Legend. Do not understand what "removals are" or how PPR can be <1 in the figure.

Thank you for pointing this out. This is a typo and has been fixed in the caption of Figure 6.1. The "removals" are the Controls.

Figure 6.5 Not sure the utility of the NMDS plots

The NMDS plots show the difference between the treatments (Control, *Daphnia*, etc.) in the dependent variables. It is a visual complement to the ANOSIM. These analyses show that the differences between the treatments in the Emerald Bay experiment are significant. In contrast, the differences between the treatments in the Lake Tahoe experiment are insignificant.

Page 103. While proponents of the amino acid stable isotope method have argued that you do not need food sources from a specific area to apply the method, I am highly skeptical of that claim. I simply do not believe that some isotope ratios from the amino acids of a few terrestrial plants or cultures of algae or fungi can tell you what those ratios will be in nature. We know as a fact that bulk isotope ratios vary widely among habitats, and that factors such as diffusive flux rates can alter 13C composition of algal producers.

The end members used in the mixing model were reported in Thorp & Bowes 2017. These values were determined from 3 samples of cyanobacteria, 3 samples of green algae, 6 samples of fungi, 12 samples of C3 plants, and 3 samples of C4 plants. Larsen et al. 2013 similarly found that signatures were distinct between groups of algae, macrophytes, terrestrial plants, bacteria, and fungi for organisms collected from the field and reared in the laboratory. Future amino acid stable isotope analyses should consider sampling the primary producers to identify any differences in the signatures of these taxonomic groups.

Page 103. Gut contents suggest few zooplankton are consumed by *Mysis*, indicating they may not be playing the ecological role previously thought. However, the pollen and *Kellekottia* are pretty recalcitrant and may simply not be digested.

The authors of this section agree with this statement. We are conducting further analysis concerning the energetic quality of pollen in the mysid diet. Threlkeld (1983) suggested that the rotifers observed in the foreguts of mysids collected from Lake Tahoe may have been ingested after the senescence of these rotifers. We did not quantify the number of empty loricas in these diets, but doing so may help to understand the role of rotifers in the bioenergetics of these mysids in the future.

Page 109. I think the food source conclusions are tenuous at best without analysis of isotopic composition of potential food sources.

We agree that food source conclusions could be improved with isotopic analyses of the food sources. Further analysis of the signatures of the diet items could lead to clearer interpretations of the mysid diet.

Page 110. ended abruptly in my copy.

The authors do not understand this review comment. It looks light there may have been a typo with this comment.

Page 111. This is still a correlation between *Mysis* increase, *Daphnia* decrease, and clarity decrease.

Page 112. Much of your data suggest that Emerald Bay is not a great analog for Tahoe. Also, I did not see direct comparisons of phytoplankton community structure in this report. Finally, the mesocosm experiments suggested substantial differences.

The phytoplankton data comparing Emerald Bay to Lake Tahoe has now been added to the report. The differences are small.

While there are small (and significant) differences between Emerald Bay and Tahoe, they are from the point of view of the responses to *Mysis*, very similar systems.

Page 121. "These hypotheses are highly relevant for and applicable to Lake Tahoe, but can be tested far faster and more easily in Emerald Bay," true, but this assumes Emerald Bay is a reliable analog.

Yes, it does. Compared to the costs associated with current clarity control measures being funded at Lake Tahoe (that are not yielding the desired impact), the cost to determine the impact of *Mysis* is relatively small.

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