Zooplankton Integrated Dataset Metadata Report

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Background

Zooplankton play a key role in pelagic food webs, transferring energy from phytoplankton, detritus, and microbes to higher trophic levels (Runge 1988). Understanding the spatial and temporal distribution of zooplankton species allows us to assess food resources available to fish and to evaluate how those resources vary across ecosystem types. Specifically, the limited availability of certain zooplankton has been widely hypothesized to limit the success of Delta Smelt (Slater and Baxter 2014; Moyle et al. 2016; Hammock et al. 2017), an endangered fish endemic to the upper San Francisco Estuary. These zooplankton communities also support other important fish populations in the estuary, such as Longfin Smelt, Striped Bass, and juvenile Chinook Salmon; understanding these trophic interactions is central to better management of these species (Feyrer et al. 2003; Baxter et al. 2008; Goertler et al. 2018). Considerable resources are being invested in enhancing the production of zooplankton for the benefit of fishes, and many projects collect data on zooplankton as a metric of food availability for fishes in the estuary (Herbold et al. 2014; Brown et al. 2016). However, the methods used to collect zooplankton and calculate zooplankton densities differ for some of these projects, making data sets difficult to compare. Reconciling some of these differences and better integrating existing data would facilitate more robust analysis and synthesis opportunities.

The different gear types and trawling methods used across monitoring programs and special study projects have varying efficiencies for different species and life stages of zooplankton. Many methods exist for comparing zooplankton data sets collected using different field collection techniques (Ohman and Smith 1995; Clark et al. 2001; John et al. 2001), but not all integration techniques are applicable for all analysis questions. Methods for sub-sampling and sample counting in the laboratory may also lead to differences in calculating abundance of certain species or life stages (Harris et al. 2000). For this project, we compiled available data sets and metadata from various Interagency Ecological Program (IEP) sampling programs and identified methods to compare and combine data. Combining datasets required certain adjustments and calculations to be made, which are described in the Integrated Dataset section of this report.

An integrated dataset may be useful for performing comparative analyses that are not possible using data from single surveys. Our goal is that this work will increase the usability of existing IEP datasets and potentially influence future zooplankton work done by IEP programs, academic partners, consultants, and others. By improving comparability of multiple zooplankton datasets from existing sampling programs, we may be able to perform more robust analyses without additional sampling. The synthesis may also identify opportunities to streamline and improve efficiency in zooplankton sampling and sample processing.

The 2020–2024 IEP Science Strategy includes the following recommended action:

> Expanding monitoring of zooplankton during fish trawls — available food is quickly becoming an important management-related habitat attribute of interest. Performance of within-year life stage cohorts has been shown to depend on available food resources — for many native species, including smelts, these food resources contain zooplankton species with complex lifecycle dynamics of their own that respond to management inputs.

Our hope is that any expansion of zooplankton monitoring will (1) account for existing spatial and temporal coverage in order to make efficient use of resources, (2) use comparable methodologies to IEP surveys so as to facilitate data combination, and (3) be documented and made accessible according to the recommendations contained herein in order to facilitate dataset integration.

Goal

Increase the usability and comparability of zooplankton data collected by IEP.

Objectives

- Document existing IEP zooplankton surveys and their methods.
- Create computer code to integrate existing zooplankton data sets and publish those methods in an open-source R package.

- Develop a graphical user interface for the integrated zooplankton dataset to allow it to be used by a wider range of audiences.
- Provide recommendations for data sharing to streamline future data-integration efforts.

Overview of monitoring programs that collect zooplankton

The Interagency Ecological Program (IEP) is a consortium of State and federal agencies that has been conducting cooperative ecological investigations since the 1970s. The IEP runs over 20 long-term monitoring surveys in the Upper San Francisco Estuary (for more information, see https://water.ca.gov/Programs/Environmental-Services/Interagency-Ecological-Program). These surveys monitor phytoplankton, zooplankton, benthic invertebrates, water quality, and many types of fish. Several fish surveys sample zooplankton concurrently, and information on zooplankton species composition and abundance can be coupled with fish diet studies. The IEP long-term surveys that monitor zooplankton are the Environmental Monitoring Program (EMP), 20-mm Survey, Fall Midwater Trawl (FMWT), Summer Townet Survey (STN), the Yolo Bypass Fisheries Monitoring Survey (not included in this report), and the Fish Restoration Program (FRP). An overview of these programs is provided in Table 1 and a map of sampling locations in Figure 1. Detailed metadata, such as sampling and processing methodologies, regions, and environmental variables, are presented in Appendix A.

Zooplankton surveys sample three different size classes of zooplankton using collection nets of different mesh sizes. Two classes (macro- and mesozooplankton) are collected by towing these nets through the water column, while microzooplankton are collected by pumping water directly into the net. Typically, the types of zooplankton found in these size classes are:

1. Macro (500–505 micrometers [µm]): Amphipods and mysids.
2. Meso (150–160 µm): Copepods and cladocerans.
3. Micro (43–50 µm): Copepods (especially larvae) and rotifers.

Every IEP survey collects zooplankton samples with a mesozooplankton net, which targets adult copepods and cladocerans, because these taxa are believed to comprise the majority of zooplankton in juvenile and adult planktivorous fish diets (Meng and Orsi 1991; Nobriga 2002; Hobbs et al. 2006; Slater and Baxter 2014; Slater et al. 2019). Some surveys also sample with micro- or macro-zooplankton nets.

Table 1 Overview of existing long-term ecological monitoring programs of the Interagency Ecological Program illustrating the type of zooplankton samples collected by each survey. This table identifies three size classes of zooplankton sampled by different nets, (1) Macro (500–505 µm): Amphipods and mysids; (2) Meso (150–160 µm): Copepods and cladocerans; and (3) Micro (43–53 µm): Copepods (especially larvae) and rotifers. Programs vary in start years, frequency of sample collection, time of year sampled, and the target habitat sampled. Detailed metadata for other characteristics that vary between these sampling programs is presented in Appendix A. *Not included in the integrated dataset due to issues with taxonomic consistency.

Study Name	Macro	Meso	Micro	Start Year	Frequency	Months sampled	Habitat sampled
Environmental Monitoring Program	X	X	X	1972	Monthly	Year-round	Open-water channels
20mm survey	—	X	—	1995	Twice per month	Mar–Jul	Open-water channels
Fall Midwater Trawl	X	X	—	2007	Monthly	Sep–Dec	Open-water channels
Summer Townet Survey	—	X	—	2005	Twice per month	Jun–Aug	Open-water channels
Fish Restoration Program	X	X	—	2015	1–8 times/ year	Mar–Dec	Wetlands
Yolo Bypass Fish Monitoring*	—	X	X	1998	Twice per month	Year-round	Floodplain & adjacent channel

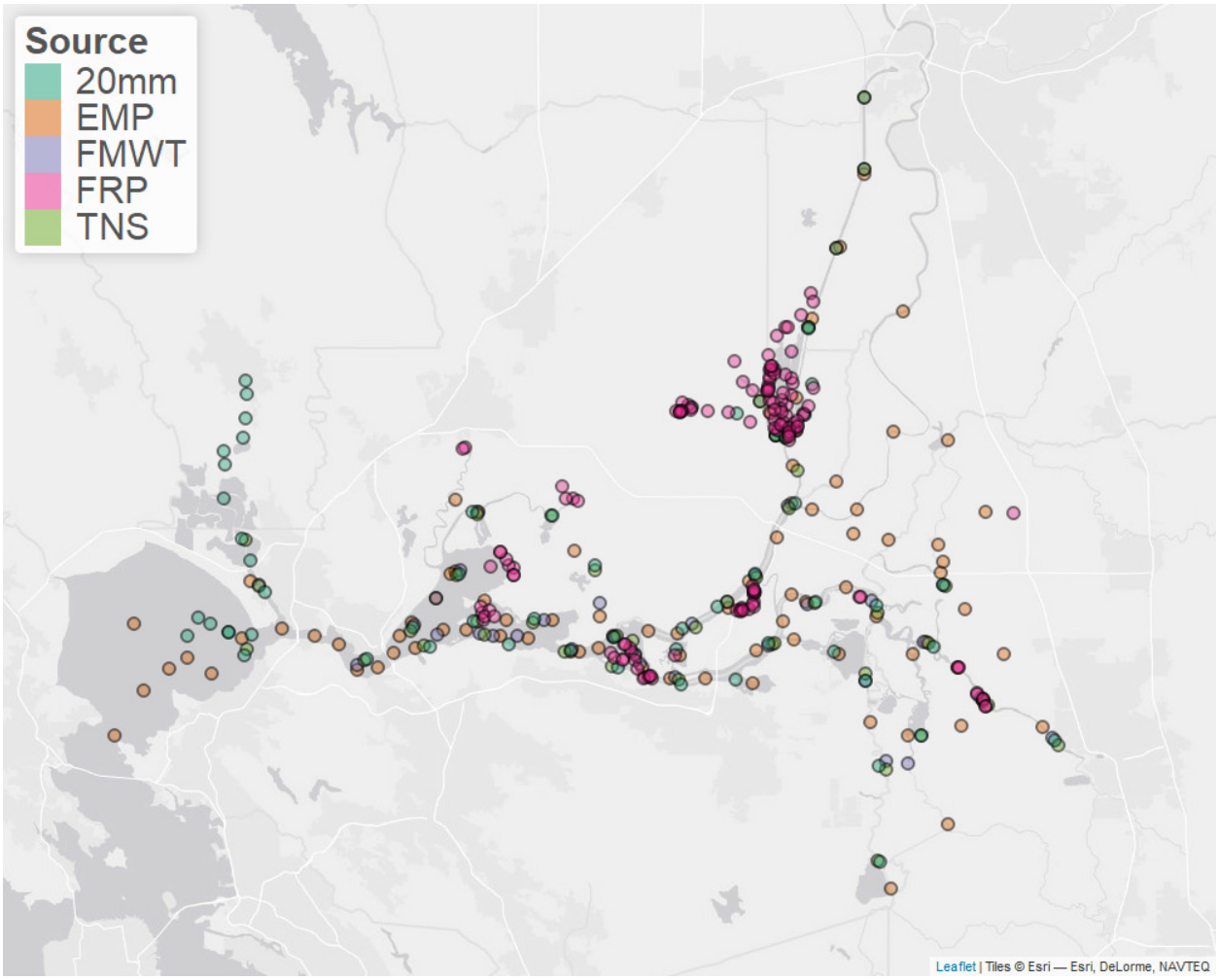
Environmental Monitoring Program

The Environmental Monitoring Program (EMP) Zooplankton Study (also known as the IEP Zooplankton Study) began in 1972 to assess trends in fish food resources ranging from San Pablo Bay to the east Delta. The study also detects and assesses the impacts of recently introduced zooplankton species on native species. The study is mandated by Water Right Decision 1641 for

operation of the State Water Project and Central Valley Project (State Water Resources Control Board 2000). The EMP study is conducted by the California Department of Fish and Wildlife (CDFW), California Department of Water Resources (DWR), and US Bureau of Reclamation (Reclamation) and currently samples 17 fixed stations and two to four floating entrapment zone stations. Entrapment zone stations are locations where the bottom conductivity is between 2 and 6 millisiemens per centimeter (mS/cm). When these points occur upstream of the confluence of the Sacramento and San Joaquin rivers, two stations at each salinity point are taken, one on each river. There are also three additional stations located in Carquinez Strait and San Pablo Bay, which are sampled during periods of high outflow and low salinity (Figure 1). Historically (prior to 1995), the survey sampled at a much larger number of stations.

EMP samples zooplankton in all three size ranges: microzooplankton (< 1.0 millimeters [mm]), mesozooplankton (0.5–3.0 mm), and macrozooplankton (1–20 mm). All EMP zooplankton are collected monthly at fixed stations year-round in open channels at high slack tide and preserved in 10 percent formalin dyed with rose bengal. Macrozooplankton and mesozooplankton are collected using a mysid net and a Clarke-Bumpus net, respectively, during 10-minute oblique tows. The mysid net is 124-cm-long with a 28-cm mouth diameter and 505-µm mesh, while the Clark-Bumpus net is 73-cm-long with a 12-cm mouth diameter and 160-µm mesh. Both nets have a flowmeter mounted in the mouth and cod-ends with the same mesh size as the net. Prior to 1974, macrozooplankton were sampled with a 930-µm-mesh net. Microzooplankton are collected with a Teel marine pump while the intake hose is raised through the water column and pumped into a net with 43-µm mesh. Pump samples collected approximately 1.5–1.9 liters (L) from 1972–2007, and 75 L from 2008–present, measured by a digital flowmeter connected to the hose.

Figure 1 Map of locations sampled by the programs included in the integrated dataset



Microzooplankton samples are passed through a 154-µm-mesh sieve nested on top of a 43-µm-mesh sieve in the lab, and only the smaller size fraction that passes through the larger sieve and is retained on the smaller sieve is counted. Mesozooplankton and microzooplankton are processed by diluting the sample to a standard concentration (200–400 organisms per ml) and counting organisms until a target is reached. From 1972 to 2003, 1-ml subsamples were counted until at least 200 total organisms were counted; from 2004 to 2005, organisms were counted until 6 percent of the dilution volume had been processed; and from 2006 to present, organisms were counted until 6 percent of the dilution volume had been processed, but after at least five and no more than 20 cells were processed. Macrozooplankton are processed using a sorting tray divided into quadrants for subsampling,

targeting at least 220 organisms from 1972 to 1984 and at least 400 organisms from 1984 to present. Lengths are recorded for macrozooplankton, and biomass is estimated by length-weight equations for macrozooplankton and by average values for mesozooplankton and microzooplankton (Appendix B). Recorded environmental variables for all samples include time, depth, surface and bottom conductivity, surface temperature, Secchi depth, and chlorophyll-a.

20-mm Survey

The 20-mm survey (20mm) was initiated in 1995 by the California Department of Fish and Game, now known as the California Department of Fish and Wildlife, to monitor postlarval-juvenile Delta Smelt distribution, abundance, and timing throughout their historical spring range in the Delta. The survey is mandated under the Endangered Species Act Biological Opinion for operation of the State and Central Valley water projects (US Fish and Wildlife Service 2019). *20-mm* refers to the length of the fish targeted by the net. Zooplankton samples are collected concurrently with fish samples to monitor Delta Smelt food supply. Between 41 and 55 stations have been sampled each year since the survey began (Figure 1).

Zooplankton are sampled twice per month between March and July at fixed stations in open channels. Mesozooplankton are sampled using 10-minute stepped-oblique tows with a 73-cm-long 160-µm mesh modified Clarke-Bumpus net. The net is attached to the top of the 20-mm Survey net frame and a flowmeter is mounted in the mouth. Samples are preserved in 10 percent formalin and then processed as in the EMP Zooplankton Study. Lengths are not recorded and biomass is estimated by literature values. Recorded environmental variables include times, tidal stage, depth, surface and bottom conductivity, surface temperature, Secchi depth, and turbidity.

Fall Midwater Trawl

The Fall Midwater Trawl (FMWT) was initiated by the California Department of Fish and Game in 1967 in order to determine the relative abundance and distribution of age-0 Striped Bass (*Morone saxatilis*), but the data has also been used for other upper estuary pelagic fish species, including Delta Smelt (*Hypomesus transpacificus*), Longfin Smelt (*Spirinchus thaleichthys*), American Shad (*Alosa sapidissima*), Splittail (*Pogonichthys macrolepidotus*), and Threadfin Shad (*Dorosoma petenense*). The FMWT is currently

mandated by the 2019 Delta Smelt Biological Opinion for the coordinated operation of the Central Valley Project and State Water Project (US Fish and Wildlife Service 2019). The FMWT samples 122 stations each month from September to December, ranging from San Pablo Bay to Stockton, Hood, and the Sacramento Deep Water Ship Channel. FMWT has sampled both macrozooplankton and mesozooplankton at a subset of these stations since 2011 (Figure 1), with some pilot studies in earlier years.

Zooplankton samples are collected along with the fish trawl at fixed stations in open channels using 10-minute oblique tows. Macrozooplankton are sampled using a 124-cm-long net with 505-µm mesh, while mesozooplankton is sampled using a 73-cm-long modified Clark-Bumpus net with 160-µm mesh. For both zooplankton sizes, samples are preserved in 10 percent formalin dyed with rose bengal, then processed as in EMP. Lengths are recorded for macrozooplankton but not mesozooplankton, biomass is estimated for both as in EMP. Recorded environmental variables include time, tidal stage, depth, surface and bottom conductivity, surface temperature, Secchi depth, *Microcystis* presence, and turbidity.

Summer Townet Survey

The Summer Townet Survey (STN) was initiated by the California Department of Fish and Game in 1959 in order to determine the relative abundance and distribution of upper estuary pelagic species, namely age-0 Striped Bass (*Morone saxatilis*). As with the FMWT, the STN is currently mandated by the 2019 Delta Smelt Biological Opinion (US Fish and Wildlife Service 2019) and began in response to the development of the Central Valley Project pumping plants. The Summer Townet Survey collects mesozooplankton samples from 32 historic stations and eight supplemental stations ranging from San Pablo Bay to Rio Vista, Stockton, Cache Slough, and the Sacramento Deep Water Ship Channel. Zooplankton monitoring began in 2005 with samples collected every two weeks between June and August.

STN samples only mesozooplankton during their fish trawl, using a net attached to the townet frame. Zooplankton samples are collected during one of the fish tows at each fixed station in open channels using 10-minute oblique tows. Mesozooplankton are sampled using a 73-cm-long modified Clark-Bumpus net with 160-µm mesh and preserved in 10 percent formalin dyed with rose bengal, then processed as in EMP. Biomass is estimated and

recorded environmental variables include time, tidal stage, depth, surface and bottom conductivity, surface temperature, Secchi depth, *Microcystis* presence, and turbidity.

Fish Restoration Program

The Fish Restoration Program (FRP) is devoted to restoring 8,000 acres of tidal habitat in the Delta and Suisun Marsh to provide Delta Smelt habitat and 800 acres of low salinity habitat to benefit Longfin Smelt. These restoration projects are pursuant to requirements in the 2019 Biological Opinions for State and federal water project operations (US Fish and Wildlife Service 2019). The FRP Monitoring Team monitors fish and their food resources (including zooplankton) within these restored wetlands in order to better understand the benefits of the restored habitats to native fish species. The FRP Monitoring Team surveys zooplankton in shallow waters, generally near tidal marshes or sites that will soon be converted to tidal marsh. The FRP has worked closely with some other IEP surveys to compare zooplankton communities in shallow water with the open-water channel samples collected by the long-term surveys (Contreras et al. 2018).

Zooplankton are sampled annually to monthly between March and December, beginning in 2015. Samples are taken from randomly selected locations within fixed sites at restored and existing wetlands and adjacent open-water areas across the Delta and Suisun Marsh. Macrozooplankton are collected with 10-minute horizontal surface tows using a 0.4 m x 0.4 m mouth net (500-µm mesh size). Mesozooplankton are collected with five-minute surface tows using a 14.6-cm-diameter net (150-µm mesh size). A flowmeter is attached to the net for both zooplankton size collections. Samples are preserved in 70 percent ethanol with rose bengal. Mesozooplankton catch values are calculated using a minimum of five 1-ml subsamples with a pipet until 400 organisms are counted, or 20 ml total, depending on which occurs first. Lengths are recorded for macrozooplankton but not mesozooplankton, biomass is estimated by literature values for both. Recorded environmental variables include time, tidal stage, surface conductivity, surface temperature, Secchi depth, turbidity, *Microcystis*, pH, chlorophyll, and dissolved oxygen.

Descriptions of common methods used

For this report, we survey and compare some field, laboratory, and analytical methodologies used for zooplankton sampling. One of our goals is to inform people using zooplankton data about the different techniques used by the sampling programs. Another goal is to facilitate aligning techniques between sampling efforts to increase the compatibility of datasets. Additional details on field methodologies, including standard operating procedures (SOPs) for different field sampling methodologies, were previously published in an IEP Report from the Tidal Wetlands Monitoring Project Work Team (IEP Tidal Wetlands Monitoring Project Work Team 2017a).

Habitat types sampled

Most long-term IEP surveys have concentrated on sampling the open-water channels of the Delta. These channels are often deep (> 10 m), surrounded by rip-rapped banks, and sometimes maintained by dredging. These habitats were historically unvegetated, though invasive submerged aquatic vegetation has been expanding in the freshwater areas. More recently, some surveys (such as FMWT, STN, and 20-mm) have expanded their range into some of the smaller sloughs off the main channels of the estuary, such as the Cache Slough Complex. These channels are shallower, narrower, and more likely to contain submerged vegetation.

Small marsh channels have been historically under-sampled in this estuary (IEP Tidal Wetlands Monitoring Project Work Team 2017a). Marsh channels are narrow (less than 4-m wide), shallow (often less than 2 m), surrounded by emergent vegetation, and often contain submerged vegetation. FRP is the only long-term IEP study that regularly samples in marsh channels, though multiple special studies have investigated them in the past.

Floodplains border many of the Delta tributaries, though they are only activated during flood events, typically in winter and spring. These areas can have extremely high zooplankton production during ideal conditions (Corline et al. 2017). The Yolo Bypass Monitoring Program is the only long-term IEP monitoring program that regularly samples zooplankton on flood plains. When the flood plain is not activated, they sample the adjacent tidal slough (toe drain) and collect comparative samples from the Sacramento River. The Yolo Bypass zooplankton data is not included in the 2020 version of the

integrated dataset because of issues with consistency of taxonomic identification. These data may be integrated in the future as these issues are addressed.

Mesh sizes

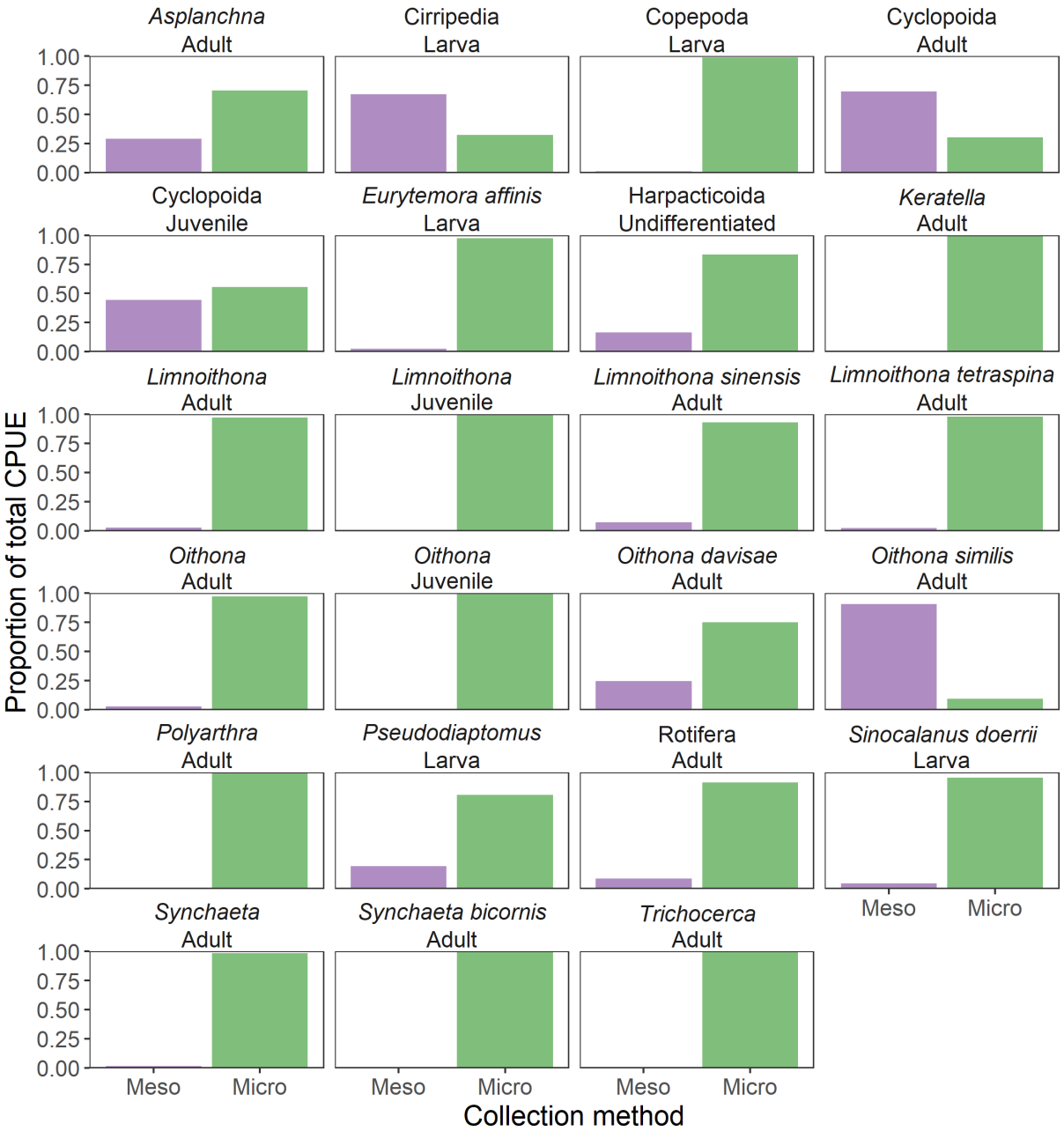
Zooplankton sampling typically involves either towing (by winch) or pulling (by hand) a net through the water or pumping water into a net. The mesh size and mouth diameter of the net will determine the size of organisms collected by the net. However, it can be difficult to accurately predict which organisms will be retained by the net, since organismal dimensions, overall shape, swimming behavior, "spikyness" of the organism, and towing speed will all affect whether an organism will be caught in the net (Evans and Sell 1985; Mack et al. 2012). Other environmental conditions, such as presence of high loads of algae or detritus in the water, will also change net efficiency due to clogging (Harris et al. 2000; Mack et al. 2012).

Nets/sieves typically sample zooplankton species whose smallest dimension is larger than the mesh size but may also capture some organisms smaller than the mesh size (which are under-sampled since some of these smaller plankters are washed through the mesh). Furthermore, organisms significantly larger than the net mesh may be able to avoid the net and thereby evade capture.

Since the meso- and micro-zooplankton data overlap in sampled taxa, we investigated sampling biases of these two mesh sizes by comparing taxa counted in both. We used EMP data filtered to include only stations and dates when both meso- and micro-zooplankton samples were collected. We also applied our approach to converting 0s to NAs for time periods when a taxon was not counted (see the Data collection and compilation methods section for more information). For each taxon (or life stage) represented in both datasets, we plotted the total summed catch per unit effort (CPUE) (individuals per m^3 of water sampled) from the mesozooplankton net (153-µm mesh, net) and the microzooplankton (43-µm mesh, pump) to visually assess where each method may be under-sampling (Figure 2). In almost all cases, the two methods had drastically different CPUEs, with the microzooplankton (pump) sample collecting substantially more individuals in most cases (19 out of 23). The mesozooplankton (net) sample was only better at capturing Cirripedia larvae, Cyclopoida adults, and *Oithona similis* adults. The two catches were very similar for Cyclopoida juveniles

(mesozooplankton/net captured 80 percent of the catch of microzooplankton/pump). Using this information, we developed a list of taxa and life stages undersampled by each method (excluding only Cyclopoida juveniles because the catch was so close) and used this list to flag CPUEs from each method that may represent an undersample of zooplankton density in the field. It is important to note that, prior to counting, the EMP microzooplankton (pump) samples are passed through a 154-µm sieve in the lab, and the larger size fraction is counted separately under the assumption that those individuals are better sampled by the mesozooplankton/net sample. Thus, some of the under sampling of larger taxa by the microzooplankton (pump) samples may be an artifact of this lab methodology rather than an effect of the net mesh size.

Figure 2 Relative catch of 2 different collection methods using data from the Environmental Monitoring Program (EMP), the "Meso" (Mesozooplankton, 153 µm mesh, net) vs "Micro" (Microzooplankton, 43 µm mesh, pump) sampling methods, for taxa counted in both. Higher taxonomic levels represent "UnID" or "other" categories and do not include counts from the lower taxa they contain (e.g., *Synchaeta* Adult does not contain counts of the individuals from *Synchaeta bicornis* Adult).



Tow duration and tow type

The method for collecting the sample can also affect data quality. Larger-bodied, faster-swimming organisms may be able to avoid a slowly towed net or a weak pump. Longer tow duration will result in a larger sample size, reducing sampling errors caused by patchiness in the organisms, but the efficiency of the net at catching different sized organisms will change over the course of the tow as the net begins to clog (Harris et al. 2000; Mack et al. 2012). A fine mesh net may clog completely, causing "backflow" that prevents new organisms from entering the net. Higher towing speeds may result in damage to organisms, and high speed may cause organisms normally retained by the net to be extruded under pressure (Harris et al. 2000). The depth of the net in the water will impact which organisms are captured. An oblique tow or vertical tow will equally sample organisms at all depths, while a horizontal tow will only sample one stratum of the water.

Stepped-Oblique net tow

The most commonly used sampling technique employed by IEP's long-term monitoring surveys is the stepped-oblique net tow. In this method, the zooplankton net is attached to a metal sled. This sled may solely be for meso and macro-zooplankton (as used by EMP and FMWT) (Hennessy 2019), or may be attached to a larger fish sampling net (as used by 20 mm and STN) (Fujimura et al. 2017). The sled is deployed off the back or side of a boat using a winch or A-frame with a cable attached to a winch. The cable is spooled out to a standardized length based on the depth of the water. The boat proceeds at slow speeds while a specified amount of cable is slowly drawn in at specified time intervals following a tow schedule. As the cable is drawn in, the sled rises through the water in a stepwise fashion, sampling each strata of the water column.

Horizontal net tow

In a horizontal net tow, the net is held at a constant depth while the boat proceeds forward at slow speeds. Most frequently, the net is held just below the surface of the water (as used by FRP and UC Davis) (IEP Tidal Wetlands Monitoring Project Work Team 2017b), though it may be attached to a sled or Clark-Bumpus apparatus and towed along the bottom or at a constant depth.

Vertical net tow

In a vertical net tow, the net is dropped to the bottom of the water and then hauled straight up through the water column. This method also samples the entire water column, but generally samples much less volume than is sampled in a stepped-oblique tow.

Stationary sampling

In some stations, FRP samples by holding the zooplankton net in a constant position and allowing the current to flow through the net for a pre-defined period of time (IEP Tidal Wetlands Monitoring Project Work Team 2017b). This works best when sampling from shore or a stable structure to attach the net to, and is most commonly used on ebb tides to sample water flowing out of a wetland.

Pump

Pumps are used by EMP for sampling smaller zooplankton (rotifers, copepod nauplii, etc.) (Hennessy 2019). Pumps are considered advantageous because the filtered volume is easier to measure and net clogging is easier to monitor, so it is helpful when sampling for very small organisms; however, larger organisms can escape the narrow mouth of a pump intake (Harris et al. 2000).

Measurement of environmental variables

Salinity, turbidity, temperature, time of day, and tidal stage affect patterns of zooplankton abundance. However, surveys vary in which environmental variables are measured.

Salinity

Each species of zooplankton has a different tolerance for salinity, so the salinity at which a trawl is taken is one of the primary factors affecting community composition.

Instead of directly measuring salinity, most surveys measure electrical conductivity (corresponding to ionic content of the water) or specific conductance (electrical conductivity at a pressure of 1 atmosphere and at 25 °C), which can be converted to salinity using a formula (United Nations Educational, Scientific and Cultural Organization 1981). Most surveys measure electrical conductivity using water quality probes that measure

conductivity by alternating current (AC) voltage applied to nickel electrodes (e.g., YSI EXO Conductivity/Temperature Smart Sensor, YSI Inc, Yellow Springs, OH, as used by EMP), though equipment has changed over time. Some surveys report this as specific conductance, whereas others convert the values to salinity in Practical Salinity Units (PSU) or parts per thousand (PPT). Conductivity is measured 10–100 cm below the surface of the water. EMP, FMWT, 20 mm, and STN also record the conductivity at the bottom of the water column, either with a long cable on the sonde or using a Van Dorn sampler.

Turbidity

Turbidity is a measurement of water clarity. Lower turbidity is known to reduce rates of zooplankton vertical migrations, thereby limiting distribution of adults and later-stage copepodites to deeper waters during the daytime (Kimmerer and Slaughter 2016), so daytime surface trawls taken in high-turbidity water may have higher catches than those taken in low-turbidity water.

Historically, turbidity was measured by dropping a black-and-white Secchi disk into the water until it disappears. The depth (known as Secchi depth) at which the disk is no longer visible is a rough measurement of turbidity (higher Secchi depth = lower turbidity = clearer water). Higher Secchi depth values equate to lower turbidity and clearer water. Most IEP surveys still measure Secchi depth to retain continuity with historical datasets. More recently, spectrophotometric methods have replaced the Secchi disk as a more accurate measurement of turbidity. Water is placed in a spectrophotometer that measures the incident light scattered at right angles from a sample. This value is reported in Nephelometric Turbidity Units (NTU). This is done with a portable turbidimeter (e.g., Hach 2100Q portable turbidimeter, Hach, Loveland, CO, as used by FMWT, 20mm, and STN) or with a turbidity probe attached to a multi-parameter sonde (e.g., YSI 6600 sonde, YSI Inc, Yellow Springs, OH, as used in some FRP samples). Turbidity is measured at the surface of the water only.

Temperature

Increased temperature will increase invertebrate metabolic rates, decrease dissolved oxygen, and may increase the prevalence of harmful algae. Precise measurement of temperature is necessary to accurately measure dissolved oxygen, conductivity, and other environmental variables. Therefore,

temperature may impact both the zooplankton community composition, zooplankton sampling efficiency, and the accuracy of other environmental variables. Temperature is typically measured 10–100 cm below the surface of the water with a temperature thermistor on a sonde (e.g., YSI EXO Conductivity/Temperature Smart Sensor, YSI Inc, Yellow Springs, OH, as used by EMP).

Time of day/tide

Many zooplankters have vertical migrations based on tidal stage or time of day (Hartman 2019). Samples taken on high slack may have higher abundances than samples taken in the same location on an ebb or flood tide. Some sampling programs target sampling for a particular tidal stage, such as the EMP, which targets sampling at high slack. Other studies are not able to standardize their sampling to a particular tide and may not report the tidal stage when samples are collected. Because of diurnal behavior of some zooplankton species, samples collected at night, or at dawn and dusk, may have different abundances, especially for horizontal surface tows. Most long-term surveys only sample in full daylight.

Target organisms identified

Depending on the goals of the study, some surveys will enumerate different organisms than others and identify them to a different level of taxonomic resolution. For example, EMP's mysid net samples are only processed for mysids and amphipods (Hennessy 2019). Other invertebrates (insects, isopods, etc.) are not counted. FRP's mysid net samples are processed for all macrozooplankton and micronekton; however, insects are only identified to the family level, whereas mysids are identified to species (IEP Tidal Wetlands Monitoring Project Work Team 2017b). Comparing these two data sets requires understanding and accounting for these differences (see the Data collection and compilation methods section) to avoid erroneously believing that EMP's samples had lower diversity than FRP's samples.

Sample preservation techniques

Formalin

Formalin is the most frequently used sample preservation technique for IEP's long-term monitoring surveys. Formalin (diluted to 5 or 10 percent), provides the best preservation of small, soft parts of zooplankton and larval

fish (Markle 1983; Krogmann and Holstein 2010). Also, because it is effective at a low concentration, formalin is better able than ethanol to preserve organisms in samples containing large amounts of detritus. Formalin is toxic, though, and handling it in the lab and field requires rigorous safety precautions. Formalin also degrades DNA, so should not be used for samples used in genetic analyses (but see France and Kocher 1996; Baird et al. 2011).

Ethanol

Ethanol (diluted to 70–90 percent) generally preserves arthropods (Black and Dodson 2003), though it may not be sufficient for larval fish or annelid worms and other soft-bodied organisms. Ethanol is much better at preserving DNA, so is generally preferred for genetic analyses (Krogmann and Holstein 2010). Ethanol is much less toxic than formalin; however, it is flammable, so appropriate precautions are necessary for safe transport and handling. Because it is most effective in high concentrations, it may be difficult to reach an adequate concentration in samples with heavy debris or vegetation.

Freezing/chilling

Chilling the sample (4 °C) will produce the best results for dry weight calculations, stable isotope analyses, and DNA analyses; however, samples must be processed quickly (generally within days) before the sample begins degrading (Feuchtmayr and Grey 2003). Chilling is not recommended for traditional microscopy because the zooplankters may remain alive and begin to move once brought to room temperature under a microscope. Freezing of the sample will also produce good results for DNA analyses and stable isotope analyses; however, it may damage soft structures (Krogmann and Holstein 2010) and does not produce stable isotope analysis results as well as chilling (Feuchtmayr and Grey 2003).

Stain

Most of IEP's long-term surveys stain their zooplankton samples with rose bengal (Hennessy 2019). This helps sample processers extract organisms from surrounding vegetation and detritus and has been shown to improve sample sorting time and efficiency (Williams and Williams 1974). Rose bengal dye can also interfere with some genetic analysis methods (Watanabe et al. 2016).

Sub-sampling methodologies in the lab

Because of the patchy distribution of zooplankton in the water column, most surveys collect relatively large samples and process a randomly selected subsample of the original sample; however, differences in subsample method can affect the precision of an estimate (Guelpen et al. 1982). Within IEP, subsampling is generally conducted through 1-ml pipetted aliquots for micro- mesozooplankton and divider trays for macrozooplankton.

Divider trays

In the "divider tray" method, the macrozooplankton sample is uniformly spread across a plastic tray (using a comb or figure-8 stirring motion) and a 4-quadrant divider is then dropped on top of the tray. Technicians then enumerate only the invertebrates the lower right-hand corner of the tray. For very heavy samples, this procedure may be repeated so that a 1/16th or a 1/64th fraction of the original sample is enumerated (for specifics, see IEP Tidal Wetlands Monitoring Project Work Team 2017b; Hennessy 2019). This technique is simple to conduct; however, it relies on the sample being randomly distributed in the tray. Organisms and detritus may also be stuck under the dividers when they are placed in the tray.

Aliquots

Mesozooplankton samples are typically sampled with a micropipette (for specifics, see Fujimura et al. 2017; Hennessy 2019). The sample is first diluted to achieve a zooplankton concentration of between 200 and 400 organisms per milliliter. The sample is then mixed in a beaker and the taxonomist withdraws a 1-ml subsample with a micropipette and places the subsample on a gridded Sedgewick-Rafter glass slide. The organisms are then identified counted under a microscope. Most surveys process between five and 20 of these subsamples.

Counting techniques

The accuracy of an abundance estimate based on a sample is directly related to the number of organisms counted, assuming they are randomly distributed with a Poisson distribution (Harris et al. 2000). Therefore, the size of the original sample and proportion of the sample enumerated will determine the accuracy of any derived abundance estimates. If one program collects significantly larger volume samples or enumerates a higher number

of individuals in its subsample, comparing abundance estimates between the two surveys could be confounded by their differing accuracies.

Target volumes (percent of sample)

All of the microzooplankton and mesozooplankton samples collected by the CDFW long-term surveys (STN, FMWT, EMP, and 20mm) currently target processing a subsample of approximately 6 percent of the volume of each sample, with a minimum of five and a maximum of 20 1-ml subsamples (Fujimura et al. 2017; Hennessy 2019). If the target organism concentration is reached (200–400 organisms per ml), this results in at least 1,000 organisms and a maximum of 8,000 organisms counted per sample. When samples contain debris or detritus, dilution volume is often increased to enable staff to see all the organisms on a slide clearly, which results in lower total organism counts.

Target counts (overall or by taxon)

Other surveys (FRP, EMP, and FMWT's mysid samples) target a minimum number of organisms counted instead of a minimum percentage of the sample. Currently, these surveys target a minimum of 400 organisms (IEP Tidal Wetlands Monitoring Project Work Team 2017b; Hennessy 2019), which gives a precision of ± 10 percent (Harris et al. 2000).

Calculations

Count per unit effort (CPUE)

CPUE calculations are most frequently based on the volume of water sampled by the sampler. Most IEP surveys estimate volume using a flowmeter in the center of the net mouth (model 2030R, General Oceanics, Inc., Miami Florida). Flowmeter counts are used in conjunction with a meter constant and the net mouth area to estimate volume of water sampled by the net; however, some special studies estimate volume based on net mouth area and distance trawled, which may result in an underestimate of organism abundance (Mack et al. 2012). Use of flowmeters with finer mesh nets (< 100 µm) may also result in underestimates of sample volume resulting from net clogging and backflow (Evans and Sell 1985). For EMP microzooplankton samples, the volume of the water pumped into the net is measured directly using a GPI inline digital flowmeter (Great Plains

Industries, Inc., Sparta, NJ) near the output end of the hose where water enters the net for filtration.

Biomass

Meso- and microzooplankton biomass is most frequently calculated based on average weights derived from literature values. These calculations apply a single value for mg of carbon (C) per individual for all individuals of a given life stage (Culver et al. 1985; Kimmerer et al. 2011). There are no existing biomass values for many species, so related species must be used.

For mysids collected by EMP and FMWT, the first 100 individuals are also measured to the nearest mm. Biomass is than calculated based on length-weight regressions (Burdi et al. In press). Length-weight regressions provide a somewhat more accurate estimate of total biomass, but the extent to which a given individual fits the regression will vary based on sex, reproductive state, health, and time of year.

We have compiled updated biomass conversions from the literature into Appendix B. All species and taxonomic groups are not covered, reflecting gaps in the literature, but these conversion values provide a starting point for researchers interested in estimating zooplankton biomass.

Integrated dataset

Purpose and products

Our ultimate goal for the integrated dataset is to provide users with easily accessible data formatted for their desired use. The full integrated dataset is published in the Environmental Data Initiative (Bashevkin et al. 2020). We also published (1) an R package (zooper) (Bashevkin 2020) and (2) an interactive web application using the R package "shiny" (Chang et al. 2020) (code for the app available here) to enable users with a wide range of skillsets to access the integrated zooplankton datasets. The R package enables users to integrate their preferred zooplankton datasets, filter by environmental variables, and then directly analyze that data with other R packages. The online app makes data available for casual users or those interested in exploratory data analysis by allowing users to integrate, filter, download, and visualize the data using an intuitive, graphical interface. The app visualizes the data using graphs and maps that are customizable according to desired survey, location, taxa, and environmental variables.

Adding datasets from new surveys should be a relatively streamlined process if field and lab methods are similar to EMP, FMWT, STN, 20mm, and FRP. The taxa and sampling stations would first be added to the crosswalk and station tables. The code would then be modified to download the new dataset, rename its variables and taxa, and add the new survey as an additional source dataset option in the function.

Data collection and compilation methods

To consolidate the available data, we first created a comprehensive table that compiled metadata from all available zooplankton surveys (Appendix A). This table contains methodological, geographic, and taxonomic information on individual projects, as well as which environmental variables are recorded. Individuals managing the different programs provided input throughout the process. For datasets which were not yet publicly available online, we contacted project leads to obtain the data and encouraged them to make their data publicly available. We also presented a poster at a regional conference (the 2019 IEP Annual Workshop) to explain this synthesis project and to solicit information on relevant studies which we had not already included.

To combine these disparate datasets into a cohesive package, we applied a series of standardized steps to each dataset (Figure 3):

1. Retrieve the data from online repositories and reformat for consistency.
2. Apply universal taxonomic and environmental variable names.
3. Combine the datasets and resolve differences in taxonomic resolution.
4. Allow user to query data based on dates, locations, or environmental parameters.
5. Resolve taxonomic discrepancies and output a final dataset.

All of these steps were completed using R Version 3.6.3 (R Core Team 2020).

Step 1. Retrieving and reformatting the data

Our code downloads datasets directly from online files into R. If necessary, it then converts datasets from "wide" to "long" form by creating new columns for "CPUE" and "Taxa" and pivoting the CPUE data into those new columns. The result contains one row for each taxon in each sample. All column names are then renamed to a standard set of names to facilitate later dataset integration (Table 2).

Table 2 Column names of the output integrated dataset, along with example values taken from 1 row of data in order to illustrate the format

Column Name	Example Value
Source	20mm
Lifestage	Juvenile
Taxname	Sinocalanus
Taxlifestage	Sinocalanus Juvenile
SampleID	20mm 343 2011-05-11
CPUE	139.5772411
Phylum	Arthropoda
Class	Copepoda
Order	Calanoida
Family	Centropagidae
Genus	Sinocalanus

Column Name	Example Value
Species	NA
Year	2011
Date	2011-05-11T00:00:00Z
Datetime	2011-05-11T16:32:00Z
Tide	High slack
Station	343
Chl	NA
Secchi	27
Temperature	16
Volume	5.253960655
BottomDepth	17
Turbidity	38
Microcystis	NA
pH	NA
DO	NA
SalSurf	4.283370661
SalBott	4.324899358
Latitude	38.18236111
Longitude	-122.3092778

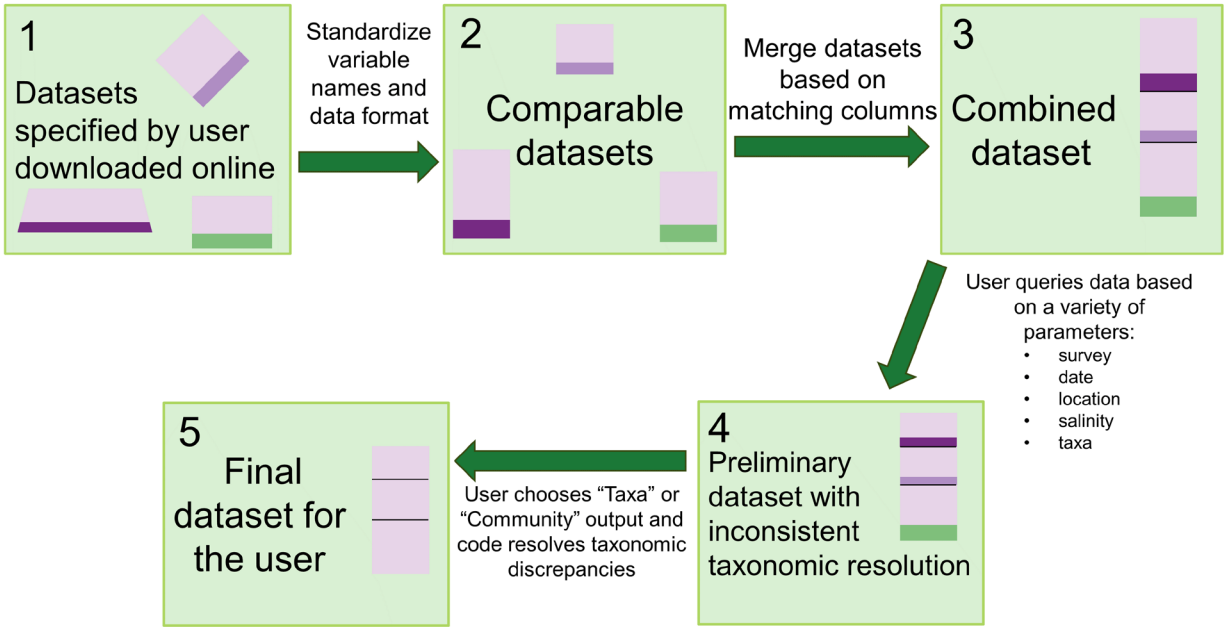
Step 2. Standardize environmental variables and taxonomic names

The environmental variables included with zooplankton datasets differed among studies and within studies over time. Because these data may still be useful, all environmental variables are retained in the combined dataset, using the code "NA" to indicate missing values.

Latitude and longitude

Since not all studies reported coordinates within the CPUE dataset, we compiled a table of all stations along with their latitude and longitude coordinates. The code loads this table into R and adds coordinates to each sample. Some studies also characterized their stations as belonging to a particular region. We decided not to retain regions in the combined dataset because "region" definitions differ among programs, and "regions" of interest to those querying the data may vary from what was provided by the studies.

Figure 3 Overall process for combining and filtering data. (1) Datasets are published with different data structures, names of taxa, and in different file formats. The zooper package standardizes variables and data formats in order to create (2) comparable datasets. Then the package merges these datasets to create a single (3) combined dataset. The user can then query the combined dataset based on a variety of parameters such as salinity, location, or taxa of interest and create (4) a preliminary dataset with inconsistent taxonomic resolution. Lastly, the user specifies a "Taxa" or "Community" output and the code resolves the taxonomic discrepancies to create (5) a user-defined final dataset to download.



Salinity and conductivity

To standardize the way salinity is reported, salinity in parts per thousand (PPT) is calculated from conductivity using the ec2pss function from the wql R package (Jassby et al. 2017), which converts electrical conductivity to salinity using the Practical Salinity Scale 1978 (between 2 and 42) (Fofonoff and Millard Jr 1983), and the extension of the Practical Salinity Scale (Hill et al. 1986) for salinities below 2.

Naming systems and crosswalk table

Taxonomic nomenclature differed among datasets. To resolve these differences, we created a crosswalk table (Table 3), which relates and resolves differences in taxonomic names by specifying a standard scientific name for each taxon. The code loads this table along with the datasets and also replaces taxa names in each dataset with their Latin scientific name and life stage from the crosswalk table.

Table 3 Columns in the crosswalk and hierarchy table for the integrated dataset. "Taxname" is the universal name corresponding to the unique names used by each dataset.

Column Name	Column Description	Example Value
EMP_Micro	Name of taxon in Environmental Monitoring Program (Micro)	LIMNOTET
EMP_Meso	Name of taxon in Environmental Monitoring Program (Meso)	LIMNOTET
EMP_Macro	Name of taxon in Environmental Monitoring Program (Macro)	—
STN_Meso	Name of taxon in Summer Townet Survey (Meso)	LIMNOTET
STN_Macro	Name of taxon in Summer Townet Survey (Macro)	—
FMWT_Meso	Name of taxon in Fall Midwater Trawl (Meso)	LIMNOTET
FMWT_Macro	Name of taxon in Fall Midwater Trawl (Macro)	—
twentymm_Meso	Name of taxon in 20mm Survey (Meso)	*Limnoithona tetraspina*
FRP_Meso	Name of taxon in Fish Restoration Program (Meso)	*Limnoithona tetraspina*
FRP_Macro	Name of taxon in Fish Restoration Program (Macro)	*Limnoithona tetraspina*
Lifestage	Lifestage of this taxon, typically either: Adult, Juvenile, Larvae, Pupae, or Egg	Adult
Taxname	Most specific taxonomic name (e.g. Genus species, Genus, Family)	*Limnoithona tetraspina*
Level	Lowest level of taxonomic specification (can range from Phylum to Species)	Species
Phylum	Phylum	Arthropoda
Class	Class	Copepoda
Order	Order	Cyclopoida

Column Name	Column Description	Example Value
Family	Family	Cyclopettidae
Genus	Genus	*Limnoithona*
Species	Species	*Limnoithona tetraspina*
Intro	If taxon was not naturalized prior to 1968, the year it was first found in the system	1993
EMPstart	Year that taxon was identified to this level of specificity in the Environmental Monitoring Program	2007
EMPend	The last year that taxon was identified to this level of specificity in the Environmental Monitoring Program	—
FMWTstart	Year that taxon was identified to this level of specificity in the Fall Midwater Trawl and Summer Townet surveys	2007
FMWTend	The last year that taxon was identified to this level of specificity in the Fall Midwater Trawl and Summer Townet surveys	—
twentymmstart	Year that taxon was identified to this level of specificity in the 20mm Survey	2006
twentymmend	The last year that taxon was identified to this level of specificity in the 20mm Survey	—
twentymmstart2	The year the 20mm survey restarted identifying that taxon to this level of specificity.	—

Differences through time

There was also some discrepancy in which taxa were identified through time in datasets. In general, studies that date back several decades (as far as 1972) were less specific in taxonomic classification in their earlier years. The metadata for studies usually included a list of taxa with years indicating when each was first identified. There are also several species of non-native zooplankton which have been introduced to the Delta since the initiation of some studies. For some datasets, CPUE was reported as "0" in years before the taxa was identified, which should have been "NA" because if one of these organisms was present it was either not counted or placed under a broader taxon. In order to resolve this issue, a table was compiled with identification start and end dates for all taxa, as well as the introduction year for non-

native zooplankton. For simplicity, these dates are included in the crosswalk table.

Step 3. Combining the datasets

Once the data has been reformatted and differences in taxonomy resolved, the datasets are merged based on matching columns.

Step 4. filtering the data

The user can filter the data based on methodological, environmental, or taxonomic variables. Methodologically, the user can filter based on sampling program or net mesh size. Environmentally, the user can filter based on latitude, longitude, date of collection, month of collection, year of collection, surface salinity, bottom salinity, or temperature. When the user chooses the "Taxa" output option, they also can filter based on particular taxa or groups of taxa.

Step 5. Resolve differences in taxonomic resolution with two options: taxa vs. community

The user has the option to choose which type of taxonomic output they prefer, depending on their intended use for the data. For example, one dataset may have identified organisms to a given genus level, while others identified one or more species within that genus. In order to resolve this, we added taxonomic classifications up to phylum to the crosswalk table. The code loads the table and sums CPUEs for the least common denominator (LCD) level of taxonomic resolution among the included datasets. The user's two options for output format of the data are "Taxa" and "Community" (Figure 4):

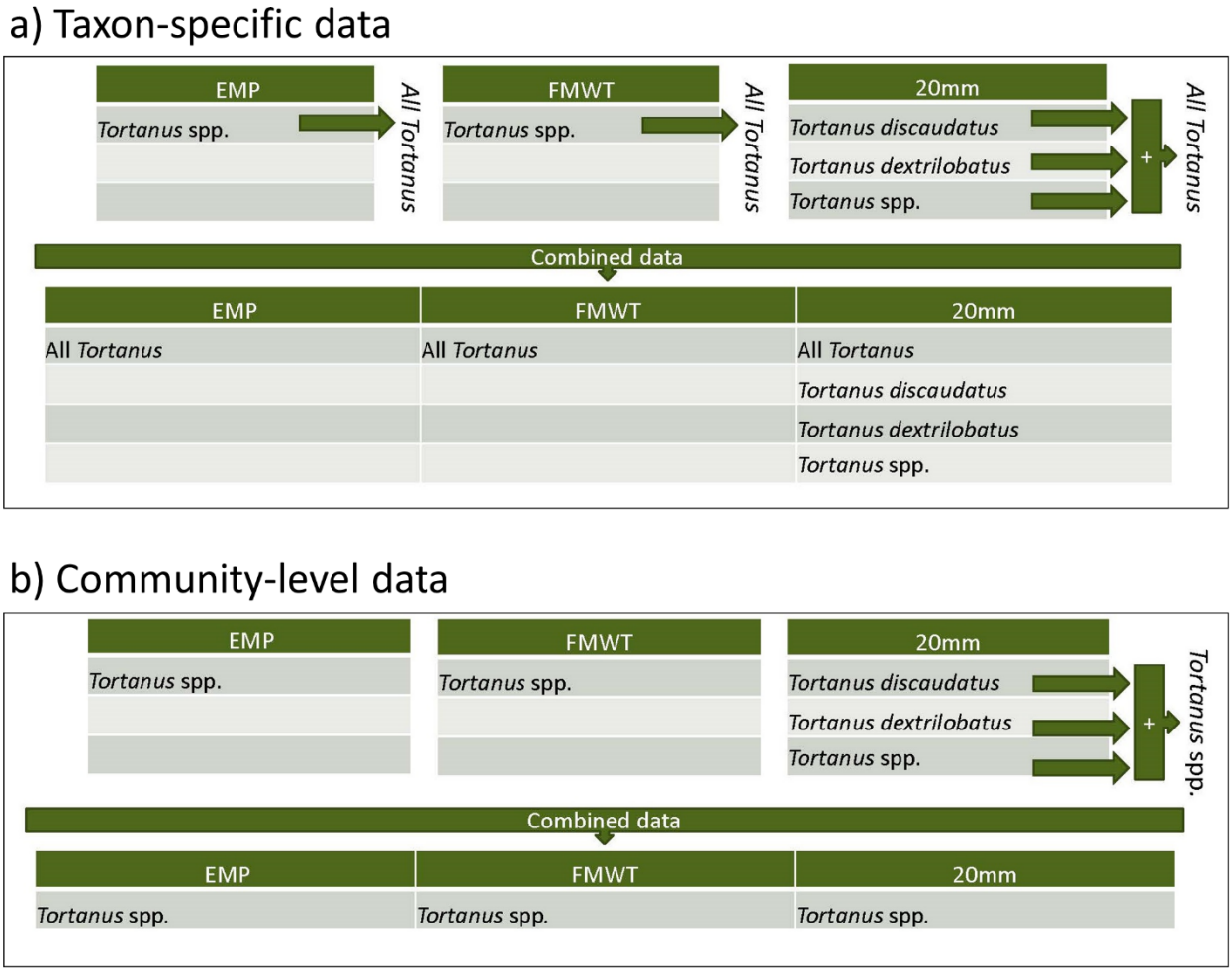
A. The "Taxa" option is designed for users who are interested in all available data on one specific taxon. This option preserves all the original taxonomic resolution and sums taxa into larger groups (genera, families, orders, etc.) to allow comparisons between datasets with differing levels of taxonomic resolution (Figure 4a). For example, one data set identified all copepods of the genus *Tortanus* as "*Tortanus* spp.," whereas another data set identified the species *Tortanus discaudatus*, *Tortanus dextrilobatus*, and *Tortanus* spp. The combined dataset retains all taxonomic resolution, but identifies Genus as the lowest level of taxonomic resolution used by both datasets (the LCD).

Therefore, it also includes a summed "All *Tortanus*" entry for each dataset. These would also be counted within higher summed groups, such as the family and order containing *Tortanus*. This new "All *Tortanus*" category is now comparable across all datasets, but the individual species counts in the second dataset are retained in the integrated dataset.

B. The "Community" option is designed for users who would like to compare entire communities. In this option, all taxa and life stages that are not measured in every input dataset are summed up taxonomic levels to the lowest taxonomic level that is covered by all datasets (LCDs). Remaining taxa and life stage that are not covered in all datasets up to the phylum level (usually less common categories such as Annelida, Nematoda or Insect Pupae) are removed from the final dataset (Figure 4b). For the example described above, the combined dataset would include just *Tortanus* spp., summing all species within the genus for the dataset that identified them. The species-level *Tortanus* data would be removed from the integrated dataset and summed into the Genus level to prevent any double-counting.

If users select the "Community" option, they may also choose to correct for changes in taxonomic resolution over time. Many studies have increased their taxonomic resolution over time, so this feature was added to correct for these changes and avoid data patterns induced by these changes in methodology. If selected, the code will identify all taxa that were not counted every year across the date range of the dataset (but considering the years non-native species were introduced and each survey first started sampling). These taxa are then summed to higher taxonomic levels (LCDs), as is done for taxa that were not counted across all datasets.

Figure 4 An example of LCD taxa calculation based on user preference for (a) taxa-specific data or (b) community-level data. The "Taxa" option retains all taxonomic specificity while also including sums for higher groups in the combined data set. The "Community" option sums to the lowest common taxa present with no taxa being counted more than once.



Warnings and caveats

Each method of taxonomic output comes with caveats which are expressed to the user through warning outputs in the code and Shiny app. For the "Taxa" option, CPUEs for taxa in the original datasets are counted multiple times within each of the higher taxonomic groups they fall under. Thus, additional higher-level taxonomic summations (above species) or analysis combining different levels of taxa should not be done with this combined dataset. For the "Community" option, taxonomic resolution is lost because only LCD taxa are included for comparable datasets. Warning messages

communicate the higher-level taxa that were removed because they have no relatives in other datasets.

Recommendations for zooplankton data

Our work on dataset integration has led us to develop several recommendations for zooplankton monitoring programs. We hope that these recommendations will help streamline data collection and comparability among future zooplankton monitoring programs in the estuary.

Collect and process samples using similar methods across surveys

All programs should strive to use similar methods. New programs should particularly work to model their methods off those used by existing programs so data are comparable.

Publish data online, preferably in open-source tables (e.g., CSV) rather than database formats (e.g., Microsoft Access)

We recommend that monitoring program leads publish their data online in tables (flat files) with open-source formats (e.g., a Comma Separated Value table [CSV]) to ensure data is easily accessible for researchers to download and use. Database formats, including Access, rely on proprietary software and are more difficult to load into R and other statistical software programs, adding additional steps to data utilization and integration projects. Publishing data in a single table, or a small number of related tables, streamlines usability for researchers not familiar with the dataset. Publishing to a data repository that can issue DOIs, like the Environmental Data Initiative, ensures a stable, long-term, and citable home for the data.

Document taxonomic classifications (i.e., the list of taxa and their life stages searched for in every sample) and any changes to identification methods over time. Identify each species to life stage if possible.

We recommend monitoring programs that use outside contractors provide their contractors with a list of taxa to identify, rather than the other way around. Programs that identify zooplankton internally should also work from a list of taxa to identify. This list should be based on those from similar monitoring programs in the San Francisco Estuary. We also recommend plankton be separated into different life stages for taxa with distinct list stages (e.g., copepods). For taxa without distinct life stages (e.g., rotifers),

studies should explicitly state in metadata what is counted in each category. Programs should refer to the Invertebrates of the San Francisco Estuary guide to standardize taxonomic identification methods.

Distinguish between zero-catch (0) and non-counted (NA), and include all 0s in published data

We recommend that monitoring programs record "0" for samples in which the taxon was targeted and not caught, and record "NA" for samples in which the taxon was not counted, regardless of whether it was caught. A "0" recorded for a taxon that was not counted but was present is not accurate. Similarly, recording an "NA" for organisms that were looked for, but not found, is also inaccurate. It is not always possible to track down changes in methods to determine when counting of certain taxa was initiated, and these changes in taxonomic identification may not always be recorded. Cells should not be left blank for zero-catch taxa and 0s should not be recorded for taxa that were not searched for in the sample. This is mostly applicable for cases where taxonomic resolution has changed over time. If taxa were never searched for, they may be left out of the data set entirely.

Provide GPS coordinates for sampling locations

We recommend that monitoring programs provide GPS coordinates for fixed and "floating" (or unfixed) sampling locations. Coordinates can be included in a separate table relating the station name to decimal degree latitude and longitude coordinates. Unfixed or "floating" station (e.g., those tied to specific conditions, such as the location where the bottom specific conductance is 2 mS/cm) locations are often not reported with zooplankton data, but these locations are important for spatial analyses and should be included for each sampling date.

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Appendix A: Table comparing all monitoring programs

For the most up-to-date version of this table, please see the version published on the Environmental Data Initiative (Bashevkin et al. 2020).

Table A1 Description of column headers in following tables

Tables	Column Header	Description
All	Study Name and zooplankton type	Name of study and size class of zooplankton
Table A2	Contact person	PI or contact person for the study
Table A2	Contact email	Email for contact person
Table A2	Link to data	Link to the data, if online, or other way to get the data
Table A2	Link to info on study	Link to study website, if available
Table A2	Start year	Year study started
Table A2	Frequency	How frequently samples are collected
Table A2	Time of year	Months in which sampling occur
Table A3	Geographic scope	Regions of estuary where sampling occurs
Table A3	Tidal stage sampled	When on the tidal stage sampling occurs, if relevant
Table A3	Sampling scheme	Are stations randomly selected, or fixed stations?
Table A3	Gear type	Are samples collected with a net or a pump?
Table A3	Sample duration (minutes)	How long are the tows?
Table A3	Tow method (horizontal, oblique, vertical)	Where in the water column are the samples collected?
Table A3	Length of net (cm)	Net specifications, if relevant
Table A3	Mesh size (µm)	Net specifications, if relevant
Table A3	Habitat sampled	Habitat where samples are collected (channels, shoals, shallow water, deep water, wetlands, etc.)
Table A4	Taxa	Broad categories of taxa that are targeted/identified by the study (e.g., Insects, Copepods, Mysids, etc.).

Tables	Column Header	Description
Table A4	Subsampling method	How are samples divided for counting? What parameters are used to decide how much of the sample to count?
Table A4	Magnification	Microscope settings
Table A4	Preservative	How are samples preserved? Usually either formalin or ethanol
Table A4	Sample archived	Are the samples kept after processing?
Table A5	Density estimate/CPUE calculation	How is Catch-Per-Unit-Effort (CPUE) calculated?
Table A5	Biomass	Is biomass estimated? By what method?
Table A5	Lengths	Are lengths measured? yes/no
Table A6	Time	Is time of day recorded?
Table A6	Tidal stage	Is tidal stage recorded?
Table A6	Bottom depth	Is the total depth of the water recorded?
Table A6	Surface conductivity	Is conductivity at the surface recorded? All conductivity measurements are normalized at 25 °C.
Table A6	Bottom conductivity	Is conductivity at the bottom recorded? All conductivity measurements are normalized at 25 °C.
Table A6	Temperature	Is water temperature recorded?
Table A6	Secchi	Is the secchi disk distance recorded?
Table A6	Turbidity	Is water turbidity recorded?
Table A6	*Microcystis*	Is *Microcystsis* presence or absence recorded?
Table A6	Chl-a	Is chlorophyll-a concentration recorded?
Table A6	pH	Is water pH recorded?
Table A6	DO	Is dissolved oxygen concentration recorded?
Table A6	Volume	Is the total volume of water filtered through the net recorded?

Table A2 Contact information, links, and basic information

Study Name	Contact person	Contact email	Link to data	Link to info on study	Start year	Frequency	Time of year
EMP (Macro)	Arthur Barros	Arthur.Barros@wildlife.ca.gov	ftp://ftp.wildlife.ca.gov/IEP_Zooplankton/	https://www.wildlife.ca.gov/Conservation/Delta/Zooplankton-Study	1968	Monthly	All year
EMP (Meso)	Arthur Barros	Arthur.Barros@wildlife.ca.gov	ftp://ftp.wildlife.ca.gov/IEP_Zooplankton/	https://www.wildlife.ca.gov/Conservation/Delta/Zooplankton-Study	1972	Monthly	All year
EMP (Micro)	Arthur Barros	Arthur.Barros@wildlife.ca.gov	ftp://ftp.wildlife.ca.gov/IEP_Zooplankton/	https://www.wildlife.ca.gov/Conservation/Delta/Zooplankton-Study	1972	Monthly	All year
20mm (Meso)	Trishelle Tempel	trishelle.tempel@wildlife.ca.gov	ftp://ftp.wildlife.ca.gov/Delta%20Smelt/	https://www.wildlife.ca.gov/Conservation/Delta/20mm-Survey	1995	Twice monthly	Mar-Jul
FMWT (Macro)	Christina Burdi	Christina.Burdi@wildlife.ca.gov	ftp://ftp.wildlife.ca.gov/TownetFallMidwaterTrawl/FMWT%20Data/	https://www.wildlife.ca.gov/Conservation/Delta/Fall-Midwater-Trawl	2007	Monthly	Sep–Dec
FMWT (Meso)	Christina Burdi	Christina.Burdi@wildlife.ca.gov	ftp://ftp.wildlife.ca.gov/TownetFallMidwaterTrawl/FMWT%20Data/	https://www.wildlife.ca.gov/Conservation/Delta/Fall-Midwater-Trawl	2007	Monthly	Sep–Dec
STN (Meso)	Christina Burdi	Christina.Burdi@wildlife.ca.gov	ftp://ftp.wildlife.ca.gov/TownetFallMidwaterTrawl/	https://www.wildlife.ca.gov/Conservation/Delta/Townet-Survey	2005	Twice monthly	Jun–Aug
FRP (Macro)	Christy Bowles	Christy,Bowles@wildlife.ca.gov	doi:10.6073/pasta/ab6a5e42df9a3bbc0dba13c1a4f9bd74	https://water.ca.gov/Programs/Environmental-Services/Restoration-Mitigation-Compliance	2015	Monthly	Mar–Dec

Study Name	Contact person	Contact email	Link to data	Link to info on study	Start year	Frequency	Time of year
FRP (Meso)	Christy Bowles	Christy.Bowles@wildlife.ca.gov	doi:10.6073/pasta/ab6a5e42df9a3bbc0dba13c1a4f9bd74	https://water.ca.gov/Programs/Environmental-Services/Restoration-Mitigation-Compliance	2015	Monthly	Mar–Dec

Table A3 Field methodology

Study Name	Geographic scope	Tidal stage sampled	Sampling scheme	Gear type	Sample duration (minutes)	Tow method	Length of net (cm)	Mesh size (µm)	Habitat sampled
EMP (Macro)	San Pablo Bay, Suisun, Sacramento River, San Joaquin River	High slack	Fixed Stations	Net	10	Oblique tow	124	505	Open-water channels
EMP (Meso)	San Pablo Bay, Suisun, Sacramento River, San Joaquin River	High slack	Fixed Stations	Net	10	Oblique tow	73	160	Open-water channels
EMP (Micro)	San Pablo Bay, Suisun, Sacramento River, San Joaquin River	High slack	Fixed Stations	Pump	—	Vertical pump sample ~0.075 m^3	—	43	Open-water channels
20mm (Meso)	San Pablo Bay, Suisun, Sacramento River, San Joaquin River, Cache Slough Complex, Napa River	—	Fixed Stations	Net	10	Oblique tow	73	160	Open-water channels
FMWT (Macro)	Suisun, Sacramento River, San Joaquin River, Cache Slough Complex	—	Fixed Stations	Net	10	Oblique tow	124	505	Open-water channels

Study Name	Geographic scope	Tidal stage sampled	Sampling scheme	Gear type	Sample duration (minutes)	Tow method	Length of net (cm)	Mesh size (µm)	Habitat sampled
FMWT (Meso)	Suisun, Sacramento River, San Joaquin River, Cache Slough Complex	—	Fixed Stations	Net	10	Oblique tow	73	160	Open-water channels
STN (Meso)	San Pablo Bay, Suisun, Sacramento River, San Joaquin River, Cache Slough Complex, Napa River	—	Fixed Stations	Net	10	Oblique tow	73	160	Open-water channels
FRP (Macro)	Suisun, Sacramento River, San Joaquin River, Cache Slough Complex	—	Randomly selected within fixed sites	Net	10	Horizontal tow	200	500	Wetlands
FRP (Meso)	Suisun, Sacramento River, San Joaquin River, Cache Slough Complex	—	Randomly selected within fixed sites	Net	5	Horizontal tow	100	150	Wetlands

Table A4 Lab methodology

Study Name	Taxa	Subsampling method	Magnification	Preservative	Sample archived
EMP (Macro)	Mysids, Amphipods	Sorting tray, divided into quadrants for subsampling	6.3X–63X	Formalin-10%	Yes
EMP (Meso)	Copepods, Rotifers, Cladocera	1 ml subsamples, targeting 6% of sample or 5-20 slides	25X	Formalin-10%	Yes
EMP (Micro)	Copepods, Rotifers, Cladocera	1 ml subsamples, targeting 6% of sample or 5-20 slides	40X–63X	Formalin-10%	Yes
20mm (Meso)	Copepods, Rotifers, Cladocera, Other	1 ml subsamples, targeting 6% of sample or 5-20 slides		Formalin-10%	Yes
FMWT (Macro)	Mysids, Amphipods	Sorting tray, divided into quadrants for subsampling	8X–80X	Formalin-10%	Yes
FMWT (Meso)	Copepods, Rotifers, Cladocera, Other	1 ml subsamples, targeting 6% of sample or 5-20 slides	25X	Formalin-10%	Yes
STN (Meso)	Copepods, Rotifers, Cladocera, Other	1 ml subsamples, targeting 6% of sample or 5-20 slides	25X	Formalin-10%	Yes
FRP (Macro)	Mysids, Amphipods, Other	Grid tray	30X–80X	Ethanol 70%	So far, but no plans to keep in perpetuity
FRP (Meso)	Copepods, Rotifers, Cladocera, Mysids, Amphipods, Other	1 ml subsamples with pipet until 400 organisms are counted, or 20 ml, whichever comes first	30X–80X	Ethanol 70%	So far, but no plans to keep in perpetuity

Table A5 Data presentation

Study Name	Density estimate/CPUE calculation	Biomass	Lengths
EMP (Macro)	(count/subsample)/Flowmeter volume	Estimated by length-weight equations developed by CDFW	Yes
EMP (Meso)	((count/subsample)*dilution volume)/Flowmeter volume	Estimated by literature values	No
EMP (Micro)	((count/subsample)*dilution volume)/Flowmeter volume	Estimated by literature values	No
20mm (Meso)	count/subsample/Flowmeter volume	Estimated by literature values	No
FMWT (Macro)	count/subsample/Flowmeter volume	Estimated by length-weight equations developed by CDFW	Yes
FMWT (Meso)	count/subsample/Flowmeter volume	Estimated by literature values	No
STN (Meso)	count/subsample/Flowmeter volume	Estimated by literature values	No
FRP (Macro)	count/subsample/Flowmeter volume	Estimated by length-weight equations developed by CDFW	Yes
FRP (Meso)	count/subsample/Flowmeter volume	Estimated by literature values	No

Table A6 Environmental variables

Study Name	Time	Tidal stage	Bottom depth	Surface conductivity	Bottom conductivity	Temperature	Secchi	Turbidity	*Microcystis*	Chl-a	pH	DO	Volume
EMP (Macroz-Net)	No	No — all high slack	No	Yes	Yes	Yes	Yes	No	No	Yes	No	No	Yes
EMP (Meso)	No	No — all high slack	No	Yes	Yes	Yes	Yes	No	No	Yes	No	No	Yes
EMP (Micro)	No	No — all high slack	No	Yes	Yes	Yes	Yes	No	No	Yes	No	No	Yes
20mm (Meso)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes
FMWT (Macro)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes
FMWT (Meso)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes
STN (Meso)	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	Yes
FRP (Macro)	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes
FRP (Meso)	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes

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Bashevkin SM, Hartman R, Thomas M, Barros A, Burdi C, Hennessy A, Tempel T, Kayfetz K. 2020. *Interagency Ecological Program: Zooplankton abundance in the Upper San Francisco Estuary from 1972-2018, an integration of 5 long-term monitoring programs. Environmental Data Initiative*. doi:10.6073/PASTA/0C400C670830E4C8F7FD45C187EFDCB9. [accessed 2020 Jun 10]. https://portal.edirepository.org/nis/mapbrowse?packageid=edi.539.1.

Appendix B: Biomass conversions

The Zooplankton Biomass Lookup Table is a collection of carbon weight averages for micro-zooplankton (Table B1; copepods, Cladocera, and rotifers) as well as length wet-weight relations for several macro-zooplankton species (Table B2; mysids and amphipods). These estimates and relations have been accumulated from several published and unpublished studies. Sources for the data are listed within the lookup table, and details for the unpublished studies are detailed here.

Much of the calanoid and cyclopoid copepod weight estimates are derived from a study by Wim Kimmerer, Toni Ignoffo, and Lindsay Sullivan at the Romberg Tiburon Center at San Francisco State University (Kimmerer et al. 2011). The study used 171 samples to determine dry weights and carbon and nitrogen mass as well as mean length. Samples were collected opportunistically and either transported live to the lab or preserved in glutaraldehyde. Individuals of a species were separated from samples and grouped in a sample cup to measure mass in a Sartorius SE-2 ultramicrobalance. All sample cups were dried at 60 °C for two days before being weighed. Sample cups were then analyzed for carbon and nitrogen using a Costech ECS 4010 elemental analyzer. A subset of samples was sent to the UC Davis Isotope Facility for analysis of isotopes and total mass of carbon and nitrogen. Results are presented as micrograms of carbon.

Most of the mysid and amphipod length-weight relations were calculated by the CDFW Fish Diet and Condition Study (Burdi et al, 2020). Individuals were taken from EMP mysid samples preserved in 10 percent formalin and from the stomachs of fish preserved in 10 percent formalin and 95 percent ethanol collected by various IEP Long Term Monitoring Surveys. Mysid and amphipod lengths were measured from the base of the telson to the tip of the rostrum using a dissecting scope. Individual mysids and amphipods were blotted dry and total wet weight was measured to the nearest 0.0001 grams using a Metler Toledo analytical balance. Length-weight equations are in a $W=aL^b$ format, where W is the weight of the individual in grams and L is the length in mm. Separate equations were derived for formalin and ethanol preservation.

For the most up-to-date version of the biomass conversions, see the version published on the Environmental Data Initiative (Bashevkin et al. 2020).

Table B1 Mean carbon mass for micro and meso zooplankton from the literature

Taxon	Level	Life stage	Carbon mass (µg)	Reference
Limnoithona	Genus	Juvenile	0.04	Kimmerer et al. 2011
Acartia	Genus	Juvenile	1.30	Kimmerer et al. 2011
Acartia	Genus	Adult	2.98	Kimmerer et al. 2011
Acartiella sinensis	Species	Juvenile	1.16	Kimmerer et al. 2011
Acartiella sinensis	Species	Adult	2.67	Kimmerer et al. 2011
Bosmina longirostris	Species	Adult	0.60	Dumont et al. 1975
Cirripedia	Infraclass	Larva	3.80	Turner et al. 2001
Daphnia	Genus	Adult	4.00	Dumont et al. 1975
Diaphanosoma	Genus	Adult	1.00	Dumont et al. 1975
Diaptomidae	Family	Adult	4.00	Culver et al. 1985
Eurytemora affinis	Species	Juvenile	1.44	Kimmerer et al. 2011
Eurytemora affinis	Species	Adult	3.55	Ambler et al. 1985.
Harpacticoida	Order	Undifferentiated	1.00	Dumont et al. 1975
Limnoithona	Genus	Adult	0.13	Kimmerer et al. 2011
Limnoithona sinensis	Species	Adult	0.13	Kimmerer et al. 2011
Limnoithona tetraspina	Species	Adult	0.09	Kimmerer et al. 2011
Limnoithona tetraspina	Species	Juvenile	0.05	Gould and Kimmerer 2010
Oithona	Genus	Juvenile	0.07	Uye and Sano 1995
Oithona	Genus	Adult	0.20	Kimmerer et al. 2011
Oithona davisae	Species	Adult	0.23	Kiorboe and Sabatini 1994
Oithona similis	Species	Adult	0.58	Kiorboe and Sabatini 1994
Pseudodiaptomus	Genus	Adult	0.10	Uye et al. 1983
Pseudodiaptomus forbesi	Species	Juvenile	1.24	Kimmerer et al. 2018
Pseudodiaptomus forbesi	Species	Adult	3.27	Kimmerer et al. 2018
Pseudodiaptomus marinus	Species	Adult	4.90	Uye et al. 1983

Taxon	Level	Life stage	Carbon mass (µg)	Reference
Sinocalanus doerrii	Species	Juvenile	1.81	Kimmerer et al. 2011
Sinocalanus doerrii	Species	Adult	3.41	Kimmerer et al. 2011
Tortanus	Genus	Adult	18.69	Hooff and Bollens, 2004

Table B2 Length-weight conversions for macro-zooplankton, from the literature and unpublished California Department of Fish and Wildlife (CDFW) data. Type indicates whether the equation is for a wet or dry weight, the sample size is indicated by "N," Min and Max lengths indicate the range in the data used to estimate the equations (in mm), and a and b refer to the coefficients in the equation Weight (g) = a * Length (mm) ^ b.

Taxon	Level	Preservative	Type	N	Min length	Max length	a	b	Reference
Hyperacanthomysis longirostris	Species	Formalin	Dry	200	2	9	0.0103	2.2593	CDFW unpublished
Neomysis mercedis	Species	Formalin	Dry	700	2	16	0.0012	3.2533	CDFW unpublished
Neomysis mercedis	Species	None	Dry	63	7.4	16.4	0.006604527	2.57	Chigbu and Sibley 1996
Americorophium spinicorne	Species	Ethanol	Wet	108	2.0	6.5	0.0000307	2.646	Burdi et al 2020
Americorophium stimpsoni	Species	Ethanol	Wet	25	2.1	5.9	0.0000317	2.47	Burdi et al 2020
Amphipoda	Order	Ethanol	Wet	367	1.9	10.2	0.0000210	2.896	Burdi et al 2020
Corophiidae	Family	Ethanol	Wet	156	2.0	6.5	0.00003107	2.631	Burdi et al 2020
Crangonyx	Genus	Ethanol	Wet	37	2.3	5.5	0.0000093	3.284	Burdi et al 2020
Gammaridae	Genus	Ethanol	Wet	209	1.9	10.2	0.0000163	3.049	Burdi et al 2020
Gammarus daiberi	Species	Ethanol	Wet	84	2.0	10.2	0.0000120	3.225	Burdi et al 2020
Hyalella	Genus	Ethanol	Wet	39	1.9	10.0	0.0000334	2.594	Burdi et al 2020
Hyperacanthomysis longirostris	Species	Ethanol	Wet	50	3.3	9.5	0.0000116	3.060	Burdi et al 2020
Sinocorophium alienense	Species	Ethanol	Wet	19	2.0	5.1	0.0000250	2.64	Burdi et al 2020
Americorophium spinicorne	Species	Formalin	Wet	113	2.1	7.5	0.0000220	2.826	Burdi et al 2020

Taxon	Level	Preservative	Type	N	Min length	Max length	a	b	Reference
Americorophium stimpsoni	Species	Formalin	Wet	57	2.2	7.8	0.0000443	2.03	Burdi et al 2020
Ampelisca abdita	Species	Formalin	Wet	196	2.1	6.2	0.0000239	2.739	Burdi et al 2020
Amphipoda	Order	Formalin	Wet	599	2.1	9.7	0.0000225	2.744	Burdi et al 2020
Corophiidae	Family	Formalin	Wet	292	2.1	7.8	0.0000199	2.844	Burdi et al 2020
Gammaridae	Genus	Formalin	Wet	307	2.1	9.7	0.0000251	2.672	Burdi et al 2020
Gammarus daiberi	Species	Formalin	Wet	106	3.3	9.7	0.0000074	3.275	Burdi et al 2020
Hyperacanthomysis longirostris	Species	Formalin	Wet	107	2.9	11.0	0.0000054	3.232	Burdi et al 2020
Monocorophium	Genus	Formalin	Wet	109	2.1	4.3	0.00001974	2.871	Burdi et al 2020
Neomysis mercedis	Species	None	Wet	63	7.4	16.4	0.002288177	3.45	Chigbu and Sibley 1996

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