Standard operating protocol for measuring crustacean zooplankton body length

This SOP has been created for the **ZooSize[1] project**. References and methods from colleagues[2] have been combined by the ZooSize champions[3] to produce a general guideline for subsampling and measurements of individual zooplankton species body lengths.

Collaborators who have not yet analysed their sample can choose to follow the protocol in this document OR to use the protocol that is routinely used in their laboratory/institute/university. What matters is to specify as clearly as possible the method in the excel template (LakeName_ZooSize.xlsx), in the respective comment cells. To aid in further interpretation and homogenization of datasets, and if you are able to, the protocol should be shared with the ZooSize team champions.

Table of contents

[1] ZooSize project description: https://rosalieb.github.io/rosaliebruelweb/ZooSize.html
[2] Thanks to Mireia Bartrons, Jessica Beyer, Sandra Brucet, Elvira deEyto, Jon Doubek, Fabio Lepori, Valerie McCarthy, and Jim Rusak, for sharing their protocol / answering our questions / submitting the first datasets that allowed us to get a sample of the diversity of methods! Thanks to Luke Ungerrer for compiling the results from the preservative survey
[3] Maria Caldero Pascual, Rosalie Bruel, Lauren Barth, Zeynep Ersoy

1. Some key points

- We are asking for data measured by microscope in a first step, but the data call is also fitted for other methods (e.g., Flowcam, optical plankton counter), as there are many interesting questions we can answer with diverse sampling methods.
- When possible, and especially for low zooplankton densities lakes, **samples >= 30 L are preferable**, to reduce the chances of having single individuals in the sample (our goal is to look at inter and **intra**-specific size variability).
- Our goal is to obtain sample data **with at least 200 total individual length measurements OR at least 20 individual length measurements per dominant taxa.**
- We ask that you differentiate between copepodites and nauplii life stages (only when data are available or if a new campaign is carried out for this project: no need to go back to the sample if it has already been processed). Some data providers have also added rotifers and adult crustaceans' sex (i.e., male or female) and in case of females if they were carrying eggs (e.g., +eggs). Feel free to add this information as an extra column linked with each measurement provided, even though it is not required at this stage.
- Additionally, we ask that, if possible, you provide a photo identifying how each taxa was measured for length, so we are aware of potential protocol differences (e.g., measurements including vs excluding helmets of *Daphnia*).

Guideline to identify different copepodite life stages (from P1 to P5 or adult)

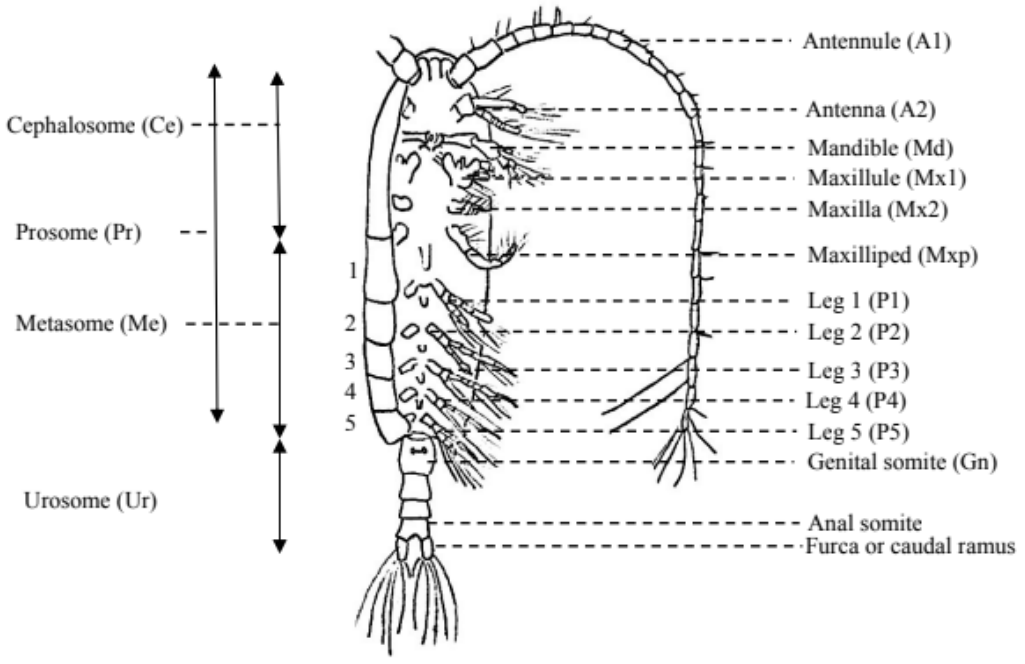


Fig. 1. Diagrammatic illustration of the external morphology and appendages of a CVI♀ calanoid copepod. Abbreviations for parts are shown and the pedigerous segments are numbered (Based on Mauchline, 1998).

2. Preserving zooplankton

Problematic

The preservative used influences the real zooplankton dimensions (Jaspers and Carstensen, 2009, https://doi.org/10.4319/lom.2009.7.430). For example, the use of formalin shrinks zooplankton while the use of ethanol bloats animals. There are existing published correction factors to account for this (e.g., Jaspers and Carstensen, 2009). We therefore will need to know your protocol in detail to correct for these differences.

Poll results

In January 2022, we launched a poll to ask the ZooSize participants their method for preserving zooplankton. 32 people responded as of 2022-05-01, and the summary below is based on these people's answers. Representative countries of respondents are quite diverse being United States the one with more representation (4 participants), followed by Spain and Canada (3 participants), Germany, Hungary and Czech Republic (2 participants), and the rest of the countries with 1 participant representation (Ireland, U.K., Denmark, Colombia, Sweden, Switzerland, Turkey, Serbia, Israel, Austria, Brazil, Portugal, Nigeria, and Italy).

Results: Formalin, ethanol, and lugol are the three most commonly used preservatives within the respondent to the poll (see Table below for concentration). Furthermore, 19% (6 out of 32) of participants narcotized animals with Alka Seltzer (3 participants), soda water (2 participants), or bicarbonate (1 participant). Most of the participants use internal protocols from their own labs/institutions.

Sample preservatives most commonly used by the community (32 respondents). Only preservatives used by at least 3 respondents are shown below.

Method	Preservative	Concentration	Percentage (and number) of respondent
1	Ethanol	70-95 %	34 % (11)
2	Lugol	3-5 %	31 % (10)
3	Formalin solution	4 % sucrose	19 % (6)

Recommandation

We advise to narcotise the animals within one hour of collection with soda water/carbonate water (e.g., Great Lakes SOP[4]; Ordonez, 2010; Culver, 1985) or Alka-Seltzer® tablet (Maria Caldero and Jon Doubek use that method for instance), and then use ethanol 70%.

3. Measuring zooplankton

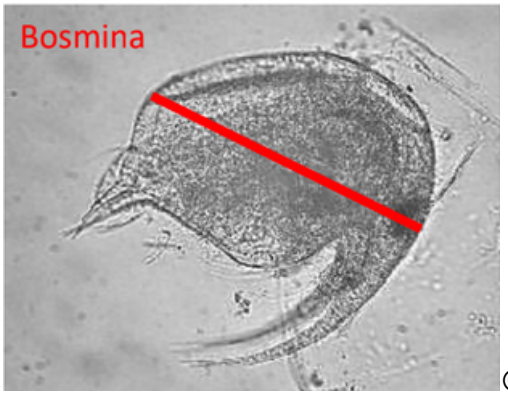
The table below summarises how to measure the different taxa. Refer to the following pages for examples and pictures.

Taxa name	**Recommended measurement (see photos below)**	**References**
Bosmina sp.	Length from the top of the head to the end of the carapace.	Standard Operating Procedure for Zooplankton Analysis, Great Lakes, LG403 (2016)
Bythotrephes sp.	Body length, excluding the caudal process.	Standard Operating Procedure for Zooplankton Analysis, Great Lakes, LG403 (2016)
Calanoids	From top of the head to tip/extremity of caudal rami not including caudal setae	Malley et al. (1989) See measurement '*' in image by Bottrell et al (1976)
Cercopagis	Body length, from the top of the eye to the end of the caudal claws.	Standard Operating Procedure for Zooplankton Analysis, Great Lakes, LG403 (2016)
Cyclopoids	Length from the top of the head to the tip/extremity of caudal rami not including caudal setae	Malley et al. (1989) See measurement '*' in image by Bottrell et al (1976)
Daphnia sp.	Length from top of the head to anterior base of the spine not including the tail.	Malley et al. (1989)
Daphnia sp. with helmet	For the cyclomorphotic forms, if possible measure both from the anterior edge of the eye to the base of the tail spine (defined as standard length = SL in this study) and from the anterior margin of the helmet to the base of the tail spine (defined as total length = TL in this study).	Culver et al. (1985)
Diaphanosoma sp.	Length from the top of the head to the base of the caudal spine.	Malley et al. (1989)

[4] Great Lakes SOP - specifics for sample collection and preservation steps: "The zooplankton samples should be refrigerated as soon as possible after collection. In the shipboard biology lab, 20 mL of soda water is measured with a graduated cylinder, into the sample to narcotize the organisms within 1 hour of sample collection. The sample then stands for 30 minutes in the refrigerator. Under a hood, 20 mL of sucrose formalin solution is added to the sample. The sample storage bottle (500-mL plastic sample bottles) is filled to the top with reagent water and tightly capped, the cap and neck are wrapped with parafilm to prevent leaks, and the sample storage bottle is stowed in a designated cooler in the walk-in refrigerator."

Holopedium sp.	Length from the top of the head to the end of the carapace.	Malley et al. (1989)
Leptodora sp.	Length from the top of the head to the end of the carapace excluding spines.	Standard Operating Procedure for Zooplankton Analysis, Great Lakes, LG403 (2016)
Nauplii	Along the longest linear distance	SOP Lake Ontario shared by James Rusak
Polyphemus sp.	From head to base of the spine	McCauley 1984

Bosmina sp.



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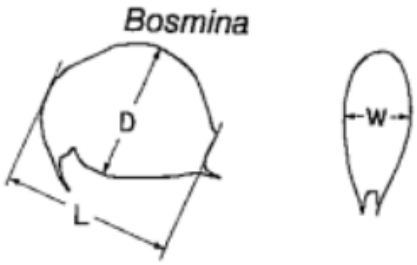
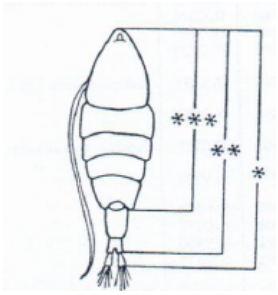
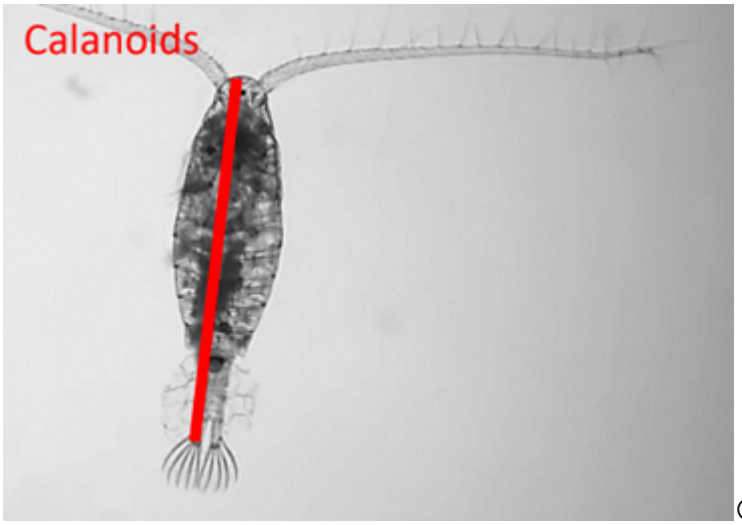


Malley et al. (1989)

Calanoids



Bottrell et al (1976) - measure the length indicated by a single *



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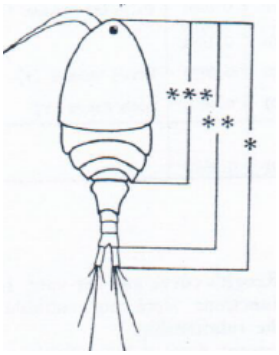
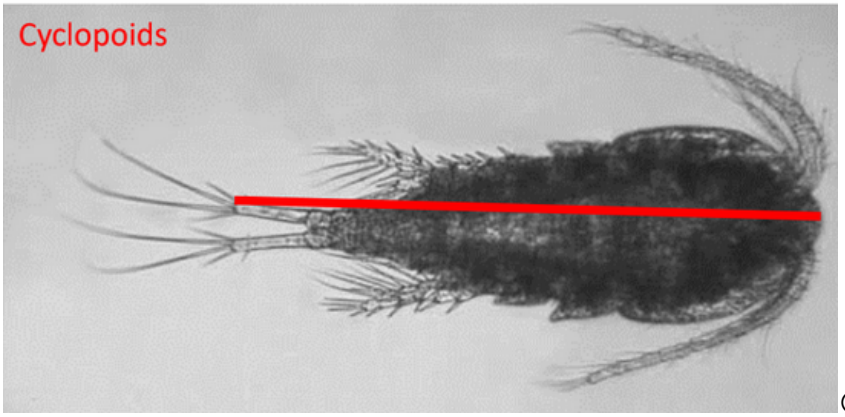
Cyclopoids



Bottrell et al (1976) - measure the length indicated by a single *



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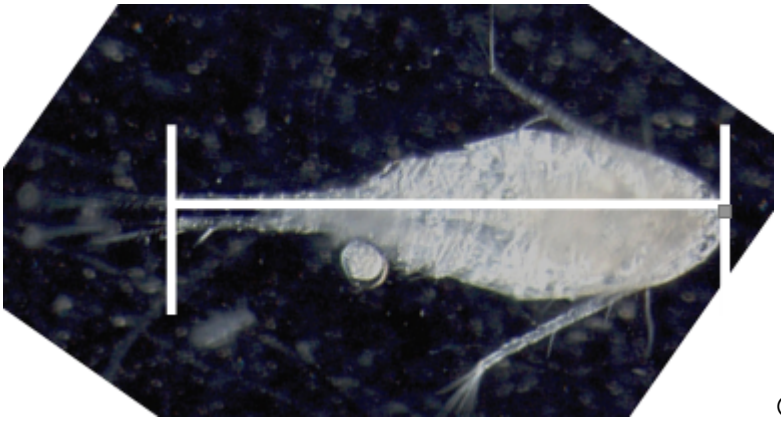
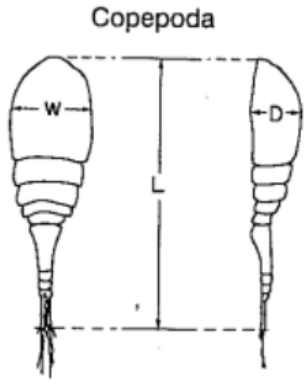
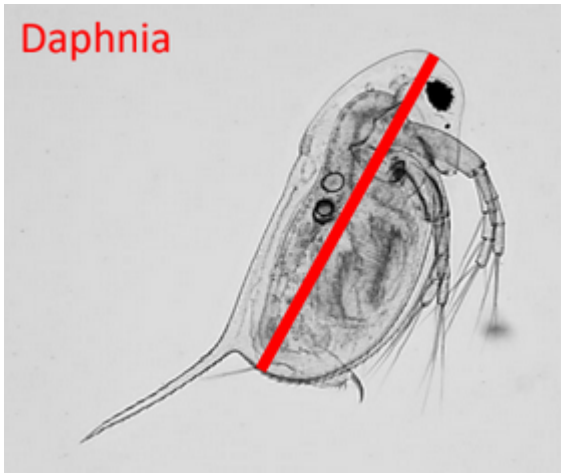

© James Rusak



Malley et al. (1989)

Daphnia sp. (without helmet)



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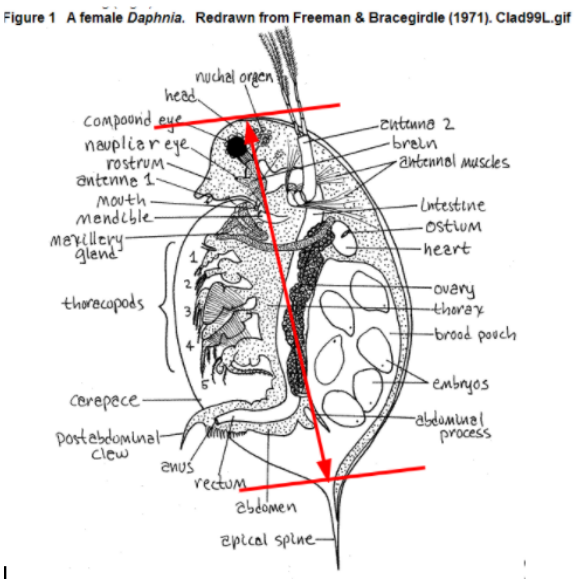

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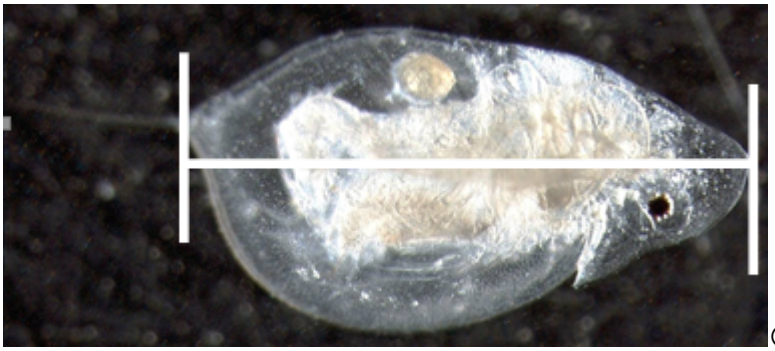
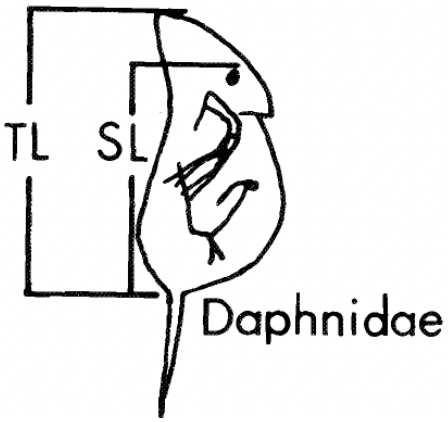


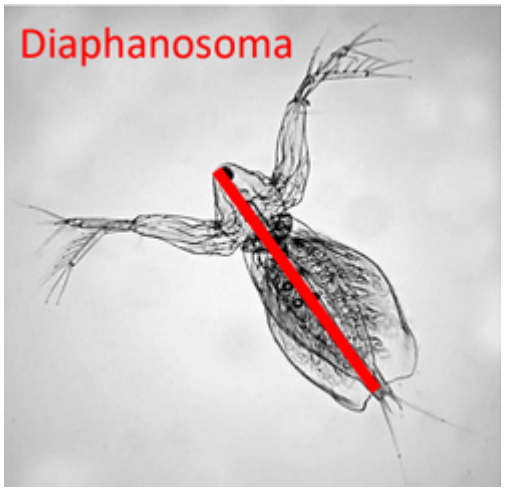
© James Rusak

Daphnia sp. with helmet



© Culver et al. (1985)

Diaphanosoma sp.



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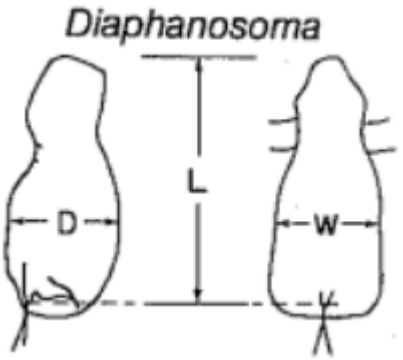
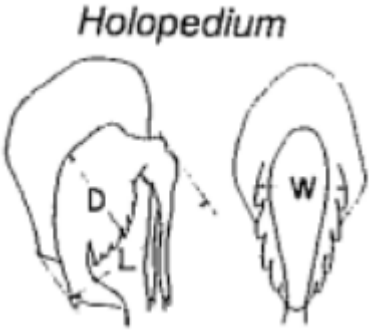


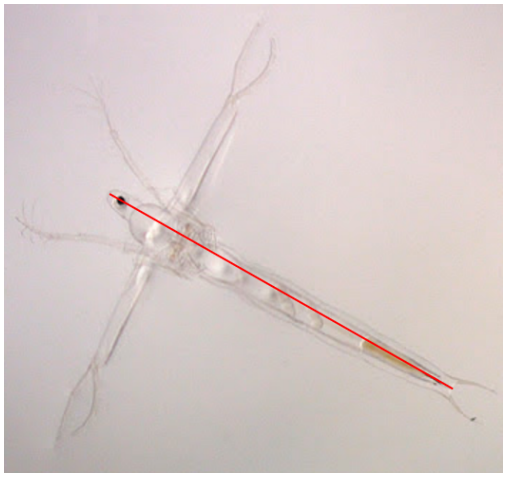
Malley et al. (1989)

Holopedium

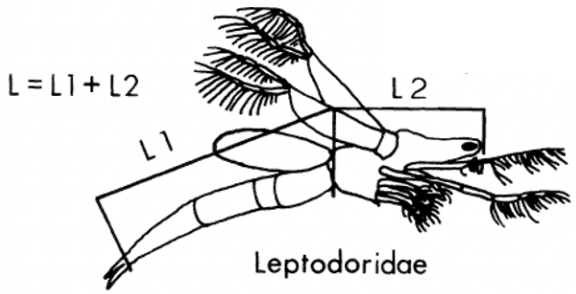


Malley et al. (1989)

Leptodora sp.



© Mireia Bartrons



© Culiver et al. (1985)

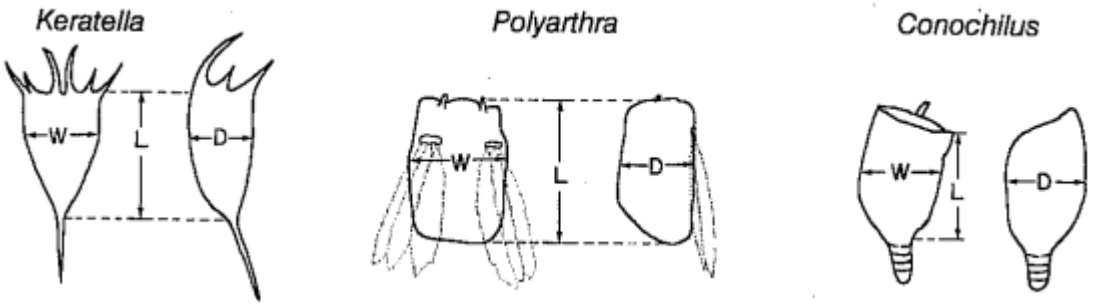
Nauplii

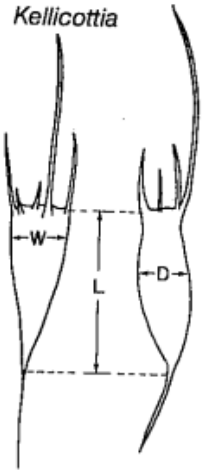
Nauplii © Mireia Bartrons

Polyphemus

© Fernando Chaguaceda

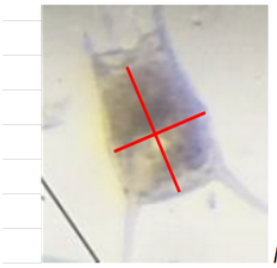
Others / rotifers



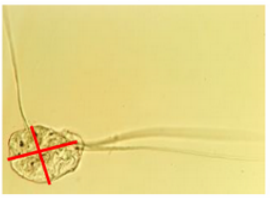


Malley et al. (1989)

Keratella sp. I



Keratella sp. II



Filina sp.

Font: Center for Freshwater Biology (2003-2013)

4. Counting zooplankton - Links to protocols

Below are links to 4 protocols displaying a range in complexity and details. They all provide ways of estimating abundances from zooplankton samples. We accept data collected with any of these protocols. If you already have a protocol within your lab, and you would rather keep using it, it isn't a problem; just document your methods precisely in the data form.

1. *EPA - Great Lakes*
2. *Ontario, Zebra2-Dorset method - James Rusak shared protocol*
3. *Lough Feeagh Long-Term Monitoring Program - Elvira deEyto*
4. *Valerie McCarthy and Maria Caldero protocol*

5. Versions and updates

Log of changes: (yyyy-mm-dd)

- 2021-11-20 - v0.1
- 2021-12-04 - v1.1
  - Added picture + a row in Table 1 for *Polyphemus*. (v1.1)
  - Thomas Jensen (Lake Atnsjøen, Norway) → The protocol is described in his recent paper. They use Lugols as a preservative.
    https://www.jlimnol.it/index.php/jlimnol/article/view/jlimnol.2019.1877/1563
- 2022-06-13 - v2.0: a new version of the SOP with an added section before "Measuring Zooplankton". We added the main results from the poll on preserving zooplankton, and what participants use.

6. References

Some collected pdfs:
https://drive.google.com/drive/folders/1Gq--2ztR35Nv9vstT9oRQaWMJrdRXw5M?usp=sharing

Papers regarding correcting factors depending on the preservative used:
https://www.tandfonline.com/doi/abs/10.1080/17451000.2021.1900576
https://academic.oup.com/plankt/article/18/4/483/1490972?login=true
https://academic.oup.com/plankt/article-abstract/16/12/1601/1461253
https://www.scielo.br/j/ni/a/RbpB6YdFMLZB3tcgx6vVjfL/?lang=en