Algorithm for the calculation of Cladocera biomass

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Introduction

The calculation of zooplankton biomass in a lake is of great interest in aquatic ecology. A part of the zooplankton, particularly waterfleas of the genus *Daphnia* effect as a switch between the parameters of the nutrient supply (bottom-up) and the animal stock (top-down).
There may be effects by both sides upon the *Daphnia* population. Therefore, it is very important to assess the actual population. Nobody can tell me how many waterfleas are existing in one cubic metre of lake water. I do not wish to use the word standard. This term should be restricted for accepted measurement units as the first metre in Paris or fundamental constants. Any statement about the number of waterfleas or their biomass in weight can only be an approximation.
Therefore, it is difficult to establish a significant correlation between the size of zooplankton biomass and other parameters. Maier and Stich (2012) show in their paper "Projekt Zooplankton – Länge, Volumen, Masse" on page 30 in figure 21 such attempts for correlations between the zooplankton biomass which is not detailed more and the parameter Secchi depth, total phosphorus, chlorophyll-*a*, and the biological volume of the phytoplankton. The authors renounce the regression computation and only present a supposed regression curve.
However, it is known from the investigation of several limnologists that *Daphnia* species depend on phosphorus as a limiting factor for the growth (Hessen 1992; Schindler et al. 1993; Elser et al. 2001; Plath & Boersma 2001).

I may ask why a significant calculation is not calculated. An exception is the paper by Hanson and Peters (1984), which is cited. But this calculation is restricted to data <200µg l^{-1}-1 TP and I miss further contributions by other ecologists.
From my own results from Lake Buchtzig, I am able to show that for parts of *Daphnia* it is possible to calculate a correlation with the total phosphorus at a significant level.
Immediately, the question will be asked why exceptions resp. assortments are necessary in this context. Such is in the design of future investigations. Before I start to design, I should envision how I may obtain sufficient accuracy in my data. I focuse on the methodology. For this purpose, I repeat some know-how about zooplankton which is already on my homepage www.p-fraktionen.de

Differences concerning the flow speed in the net mouth exist from the centre towards the frame of the net mouth especially in conical nets. Additionally there is a zone of turbulence and reduced velocity in the centre of a net with bridles (Tranter & Heron 1967).
Brander et al. (1983) found different flow velocities in the net mouth. According to Evans and Sell (1985) a netting of 76 µ causes a high resistance against the inflowing water. Stich, Maier and Hoppe (2010) mention the clogging effect of the netting to the phytoplankton. The clogging of the net mashes has an unpredictable impact to the influx of water into the net and thereby to the filtration effectiveness. For such reasons, alternative methods for the net tow are desired. Whereas Nayer (2002) found no differences between plankton sampling by net and pump, Møhlenberg (1987), Riccardi (2010) and Chick et al. (2010) prefer the pump for the plankton sampling. Møhlenberg (1987) reckon the reliable measuring of the filtered water volume in the sampling by pump without clogging of the net meshes and the depth of the sampled water.
Møhlenberg (1987), Riccardi (2010) had sampled in coastal waters, Chick et al. (2010) on a river. Since the investigations of Elster (1958) on a lake for plankton sampling by pump many changes in the pump technology happened. But in Central Europe these technical improvements were neglected and German aquatic ecologists adhered to the net tow.
In the eighties I worked in ichthyoplankon cruises in the ocean and I know the literature which discusses the weakening and potency of net designs. On a lake in Central Europe I need a mesh size of 100 µ for the sampling of zooplankton, particularly *Daphnia*. This means a risk of clogging during a net tow and consequently a high dispersion in the collected data. For which reason I changed to the pumping technology for my zooplankton sampling in 1994.
The calculation of zooplankton biomass is exemplified with data from samplings at Lake Buchzig.
The calculation of zooplankton biomass is carried out in several steps. The algorithm may be explained using the example of waterfleas of the genus *Daphnia* from the sample collected on 12th July 2005 and recorded in the file BuZooJli05.xls. See my blog.
In this place it may be presented in detail. https://pfractions.blog/author/pfractions/
For comprehension I like to notice that in the Excel files the numbering of the tables is preset by Arabic numerals. In order to avoid confusions the tables in this contribution the tables are marked by capital letters. I restrict myself to *Daphnia* since these Cladocera earn an interface. They are exposed to effects from nutrient supply as well to the impact from animal populations (Corethra larvae, predacious Copepoda and planktivorous fish).

Calculations

First of all during the sampling, the volume of the pumped and filtered water must be measured. For this purpose, from the depths of 1 and 3 metres a bucket of ten litres was filled and the time measured in seconds until the marking of ten litres was attained. The measurements are listed in table 1 of the file BuZooJli05.xls and in this contribution in table A. Three single measurements were performed each for the depths of 1 and 3 m and the arithmetic mean calculated..

Table A: Measurments of the time in seconds for the filling of a bucket with a ten litre marking.

	1 m depth		3 m depth
	11,66 s		11,1 s
	12,08 s		11,32 s
	11,44 s		11,82 s
arithmetic mean:	11,727 s	Arithmetic mean :	11,413 s

For zooplankton sampling water was sucked by a pump via a pipe system. The pumped water was passed via a tube into a net with 100 µ mesh size. The pipes were purchased in a hardware store. Each pipe was one metre long if they were stuck together. For each metre depth one pipe was used.

The net mouth was situated above the water surface, the main part of the net was held below the water surface. The water jet from the pump flushed the net walls and the clogging of the net was avoided.

Each pumping process lasted five minutes measured with a stopwatch. The net walls were washed up outside by the water jet from the pump. The zooplankton was filled into bottles and put in a cool box.

Table B: Calculation of the filtered water volume

10 litres * 300 seconds/11,727 = 255,82 litres;	10 litres * 300 seconds/11,413 = 262,86 litres

Since this mixed sample was taken with one metre distance from the depth range 1-4 m, both volumina from filling the bucket, I had to double them and afterwards sum it up:

255,82*2= 511,64 and 262,86*2=525,72.

The sum of both measurements related to the time yields 1037,36 litres filtered water. The sample was preserved with 4 % formol in the laboratory the same day.

For processing, the preserved sample was watered in order to remove the formol and filled into a glass cylinder for 100 ml. By means of a piston pipette one millilitre was twice pulled and counted below under the binocular at tenfold magnification. Both counts resulted in 180 and 187 zooplankton specimens. On average 183,5 specimens existed in one millilitre and extrapolated to 18350 specimens for the whole sample.
From the well mixed sample drops were put on a slide provided with a coverglass and I identified the animals at hundredfold or fourhundredfold magnification, the length was measured using an eyepiece and count them as tally list. From these results a table was drawn up in table 1 in BuZooJli05.xls and copied to here as table C.

Table C: species list of the zooplankton with data about the numbers below the microscope and their conversion to percentages.

Zooplankton	**Jul 05**	**1-4 m**			
Cycl.	133	100	285	46,66666667	46,7
Naupl.	4	100	285	1,403508772	1,4
Diapt.	6	100	285	2,105263158	2,1
D. galeata	84	100	285	29,47368421	29,5
D. cucullata	37	100	285	12,98245614	13
Keratell qua.	3	100	285	1,052631579	1
Kellicottia l.	13	100	285	4,561403509	4,6
Polyarthra m.	5	100	285	1,754385965	1,7
	285			100	100

On the left, the names of the identified and counted zooplankton organisms stand. The process of the identification, measuring, and count was repeated several times until more than two hundred plankton specimens were counted. In this example, the total count was 285 specimens. Using the rule of three the percentages of each plankton organism were calculated. The rounded percentages are on the right.
The measurements found with the eyepiece were transferred to an Excel-file in the table 2. The lengths were determined with the factor for the eyepiece as micron (µ). From these length data arithmetic mean, median, and standard deviation were calculated.
The calculation of the biomass as dry weight was done for *Daphnia galeata* in table 7 and for *Daphnia cucullata* in table 8 of BuZooJli05.xls. In the middle is the calculation of the dry weight by the formula and on the right the calculated dry weights are listed for each zooplankton specimen. The number and the sum of the calculated dry weights are listed below. The formula for the calculation is according to Dumont et al. (1975):

Weight (W) = a*Length^{b} $W=9.5*10^{-8}\ L^{2.56}$

The results are 75 as number, 463,98 µg as sum, and 6,18 µg as average dry weight for *Daphnia galeata*. *Daphnia cucullata* is in some way smaller with 34 in numbers, 145,77 µg as sum, and 4,287 µg dry weight on average. The percentage of the waterfleas is 29,47 % for D. galeata and 12,98 % according to table C.

The total number of *D. galeata* in the sample is calculated as:

18350*29.47/100 = 5407.745 specimens for *D. galeata*

Mean*number = biomass as dry weight:

6.1864*5407.745 = 33454.47367 µg dry weight in total sample.

Dry weight in one litre referred to ***Daphnia galeata*** 33454.47367/1037.36 = **32.25 µg l^{-1}.**

The calculations for *D. cucullata* are done in the same way. The total number of *D. cucullata* in the sample is calculated as

18350*12.98/100 = 2381.83 specimens *D. cucullata*

Dumont et al. (1975) do not provide a formula for *D. cucullata*. Therefore I used for this species the same formula as for *D. galeata.*

Mean*number = biomass as dry weight:

Weight (W) = a*Length^{b} $W=9.5*10^{-8}\ L^{2.56}$

Mean*number = B biomass as dry weight *D. cucullata* in total sample.

4.2874*2381.83 = 10211.85794 µg dry weight in total sample.

Dry weight in one litre referred to ***Daphnia cucullata***: 10211.85794/1037.36 = **9.84 µg l^{-1}.**

Discussion

The eyepiece in my microscope provides me with the possibilities to measure in steps of 22 µ. Or should I measure in steps of 100, 200, or 300 µ as some people prefer in order to save time (Mischke et al. 1015, Maier & Stich 2012).

I remember a contribution by Nümann after Elster (1958) had given his lecture about his experiments in pumping for zooplankton: “Wie haben wir überhaupt ein absolutes Maß für die Richtigkeit der Ergebnisse? Die Methode, die zu dem größten Wert führt, braucht nicht unbedingt die beste zu sein.” „How do we have an absolute measure for the correctness of the results at all? The method which leads to the highest value, must not necessarily be the best.” Translated from German to English by me, Albert Keim.

Given the case that someone wants to sample zooplankton by vertical tow using a conical net, the quantity of the recorded filtered water depends on the place where the flowmeter is suspended in the net mouth since the velocity of the inflowing water decreases from the centre to the rim of the net mouth. Placing the flowmeter in the centre will give an overestimation of the filtered water and consequently an underestimation of the zooplankton numbers. The contrary will happen, if the flowmeter is put near to the rim. Evans and Sell (1985) discuss such a matter as the position of the flowmeter in the net mouth and the length of the net extensively.

Nevertheless, a majority of researchers at lakes do not use a flowmeter during the net tow (McQueen & Yan 1993) and calculate the filtered water volume with the simple formula area of the net mouth*height of the tow (Stich, Maier and Hoppe 2010). Such a procedure also underestimates the volume of the filtered water, particularly if a mesh size of 60 µ is used. Additionally, the use of conical net suffers from the clogging, the constipation of the net meshes by planktonic organisms which cannot be calculated. There is consequently a high dispersion in samples from tows with conical nets. Researchers involved in oceanic pelagic ichthyoplankton surveys use cylindrical-conical nets for this reason on a twin frame without bridles in front of the net mouth, the so-called Bongo net. Inspired by the Bongo, Bürgi (1983) designed a double cylindrical-conical net device for the collection of zooplankton in lakes. Indeed, such a construction enables to sample a higher number, but people fear the size of the gear may be unwieldy on a small boat which is used to sample on a lake with 5-50 ha surface which are frequent in the Upper Rhine Valley. I do not understand such a reservation. Regarding the sampling on a lake using a boat, I am obliged to work as a team of at least two persons because of reasons for the labour security.

One person keeps the net above the water surface and the other person washes the net walls from outside using a bucket or the pump. In comparison with the Bongo for oceanic ichthyoplankton surveys, the double net device by Bürgy is much smaller and confront us with the difficulty to find the best location where to place the flowmeter in the net mouth. Also the diameter of the flowmeter must be subtracted from the area of the net mouth.

I think that the best solution for these problems in the quantitative sampling at small lakes is the use of a pump. The problem is enhanced for sampling at shallow lakes.

Easily, I can arrange for subsamples above and below the thermocline and the volume of the filtered water can be measured by filling a bucket with known volume and recording the seconds of filtration.

The next problem arises in the laboratory during the processing of the sample. The splitting of the sample should not be difficult. Then a drop from the sample is put on a slide covered by a thin glass and people tell me that they are in a need to save time. Therefore they count and measure in steps of 100, 200, or 300 µ (Mischke et al. 2015).
I have samples from which I counted and measured all the *Daphnia spp.* on the slide regardless of steps limited only by my eyepiece which provides me to use steps of 22 µ.
For checking the effect of steps, I subdivided the measurements for steps of 300 µ, calculated new means and processed these data for the calculation of the dry weight. These new results were used in a regression of TP against dry weight Cladocera. To my surprise, the correlation coefficient in the regression analysis was better than before.
I should think about the multimodal length distribution of the *Daphnia spp.* I do not know, if the obtained mean will hit a peak, a valley or a slope in the multimodal length distribution. Therefore, I stay further with the counting and measuring all specimens on the slide.

Addendum

The methods outlined in this contribution were developed and used ad hoc on the job as a freelancer aquatic ecologist. I do not exclude that some parts may be improved. A possible source of error is the separation in the counting with tenfold and hundredfold magnification.
For now, I do not sample zooplankton for the lack of contracts. In future work, I would take the specimens after counting with tenfold magnification directly drop by drop on the slide in order to measure and identify them. This includes also a counting. At the end, no difference must be between both countings.
I would be grateful for comments and further suggestions in order to improve the work on zooplankton.

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