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ZOOPLANKTON BIOMASS IN PONDS - DETERMINATION OF BIOVOLUME AND DRY WEIGHT

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Abstract

The aim of this study was to evaluate the possibility of using simple screening methods to determine zooplankton biomass in ponds. Among the applicable methods, we selected sedimentation determination of wet biomass and dry biomass determination. Of the 369 samples analysed, the median volumetric zooplankton biomass was 0.012 $ml.l^{-1}$ and the median dry weight of the samples was 0.44 $mg.l^{-1}$. There was a relatively close relationship between the volumetric biomass determination and the zooplankton dry weight determination, allowing only one of these methods to be used. Due to the variation of results over a wide range of values, it is more appropriate to use a logarithmic expression for the correlation. No statistically conclusive relationship was found between the zooplankton biomass determined and any of the other physico-chemical or production parameters. Nevertheless, it was possible to trace the influence of fish production, altitude and nutrient content (nitrogen and phosphorus) on the size of zooplankton biomass. The use of screening determination methods can be recommended especially for long-term monitoring of sites to get a quick overview of zooplankton biomass in ponds.

Keywords: zooplankton, volumetric zooplankton biomass, fishpond, screening methods

INTRODUCTION

Zooplankton are an important group of organisms for monitoring the status of standing waters. It is permanently present, relatively easy and inexpensive to sample. Its analysis can provide a wealth of information indicating a range of characteristics with varying rates of change in each water body. Zooplankton is a community comprising a range of organisms, from protozoa to multicellular organisms. Zooplankton in ponds generally consist of Cladocera, Copepoda and Rotifera. An important determinant of zooplankton size and species composition is the level of fish stocking. As the size of the zooplanktivorous fish (carp and other cyprinids) increases, the zooplankton size generally decreases, the proportion of cladocerans decreases and the biomass of rotifers increases. The current high eutrophication of ponds leads to nutrient overload, mainly phosphorus and nitrogen. Highly eutrophic ponds have high primary production (usually dominated by cyanobacteria) that is not utilized by the zooplankton species present. Thus, the efficiency of utilization of the huge primary production by zooplankton into fish production is low (Pechar, 1995; Potuzak *et al*., 2007).

Quantification of zooplankton is a key part of understanding ecosystem structure and function, particularly in terms of changes in trophic state, phytoplankton, and fish communities. On the other hand, the actual sampling and processing is burdened by a number of errors in counting methodology, variability in subsampling and the use of different length-weight relationships for biomass estimates. Even when done correctly, subtle differences in laboratory techniques can lead to differences in density and biomass estimates among taxa. Zooplankton are not closed population, but a population undergoing immigration and emigration processes, with a patchy horizontal or

vertical distribution, often with a strong vertical gradient in abundance that is constantly changing due to the diel vertical migration. Zooplankton body size ranges from a few micrometres to approximately 6 mm (up to 18 mm in the predatory cladoceran *Leptodora kindtii*) with varying ability to avoid sampling devices. Thus, there is currently no single standardized methodology that can be used to sample zooplankton in all types of water (Bowen, 2017; De Bernardi, 1987).

There are basically two main types of zooplankton quantification, biomass determination and enumeration methods. The use of a particular method depends on the purpose of the study, cost-effectiveness, feasibility, technical conditions of the laboratories and accuracy requirements. Zooplankton quantification can be expressed in terms of abundance (or density, number of individuals), biomass by volume (biovolume), dry weight, organic carbon and nitrogen or biochemical components such as proteins. Most of these methods require a qualified person, sampling and laboratory equipment. The actual sampling is carried out using a plankton net (with different mesh sizes, typically from 40 to 250 µm), a plankton tube or different types of sampling equipment (Patalas, Friedinger, van Dorn samplers, etc.). For an indicative determination of total zooplankton biomass, biovolume or dry weight determination can also be used (Postel *et al*., 2000).

Determination of the bulk biomass of zooplankton in ponds is an important part of a comprehensive analysis of pond communities. In contrast to the determination of phytoplankton, where the total biomass can be relatively easily characterized by chlorophyll a content, the determination of zooplankton biomass is more difficult. Our aim was to compare two of the simplest methods of zooplankton biomass determination (determination of bulk biomass and dry weight) and their potential use in assessing the status of pond ecosystems.

MATERIALS AND METHODS

During 2017–2020, 35 fishery-managed ponds were sampled throughout the growing season (April–October). Zooplankton sampling was carried out by oblique pulling of a plankton net from about 0.5 m above the bottom to the surface (to avoid collecting sediment). A plankton net with a 40 µm mesh size, diameter of 20 cm, and an outlet tap was used for sampling. The vertical-horizontal haul was relatively fast and uniform: approximately one meter per two seconds. After the plankton net was pulled above the surface, the net was carefully rinsed. An outlet tap was used to transfer the concentrated seston quantitatively into a wide-mouth plastic collection container of approximately 60 ml and fixed with formaldehyde to the final concentration of 4% in the sample.

The formaldehyde preserved zooplankton sample was poured over a 20 µm mesh plankton sieve to remove excess water (reduction of water is necessary to ensure that the volume of the zooplankton sample does not exceed the volume of the graduated cylinder) and to rinse the formaldehyde from the sample so that all zooplankton was quantitatively transferred to the sieve. The sedimented zooplankton free of excess water was transferred to a calibrated graduated cylinder of the appropriate volume, typically 10 ml or 25 ml according to the sample size. The sieve was rinsed with distilled water and all zooplankton was poured into the graduated cylinder so that no organisms remained on the sieve. A few drops of wetting agent were added to decrease the surface tension and to prevent zooplankton from being attached to the surface. The sample was sedimented for 24 hours and then the volume of sedimented zooplankton was recorded.

If coarser organics, invertebrate larvae, fish fry or macrophytes (e.g. duckweed) were present in the sample, they were rinsed with distilled water and removed before sedimentation using tweezers. Samples with high cyanobacterial biomass were generally problematic. Cyanobacteria accumulated near the surface were carefully removed during the first sample processing. If simple sedimentation failed to separate small zooplankton from cyanobacteria, the determination of zooplankton bulk biomass in these samples was not performed. Samples with low zooplankton abundance, where the zooplankton biovolume was lower than 1 ml, were also not analysed.

Sample dry weight was determined using a freeze dryer (Heto PowerDry LL3000, Thermo Fisher Scientific). The sample was transferred to a suitable container (a low sample layer should be achieved) and stored in a freezer at -18 °C. The frozen sample was placed in a freeze dryer, the lyophilisation time depended on the total amount of samples lyophilized at one time. After lyophilisation, the samples were weighed on a laboratory balance to four decimals places. Then the zooplankton sample was removed from the container and the empty container was weighed again. The weight of the empty container can also be determined before adding the sample. Dry weight of zooplankton was calculated from the weight of the sample after freeze-drying times 100 divided by the weight of the fresh (fixed) sample.

The results obtained were statistically evaluated by simple correlation and analysis of variance. Statistical analyses and graphs were generated using Statistica software (Tibco Software, USA).

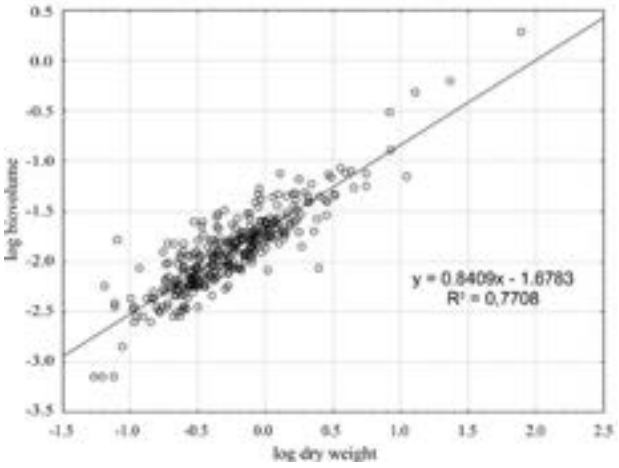
RESULTS

A total of 396 zooplankton samples were analysed. Out of this number, bulk biomass could not be determined for 16 samples due to the

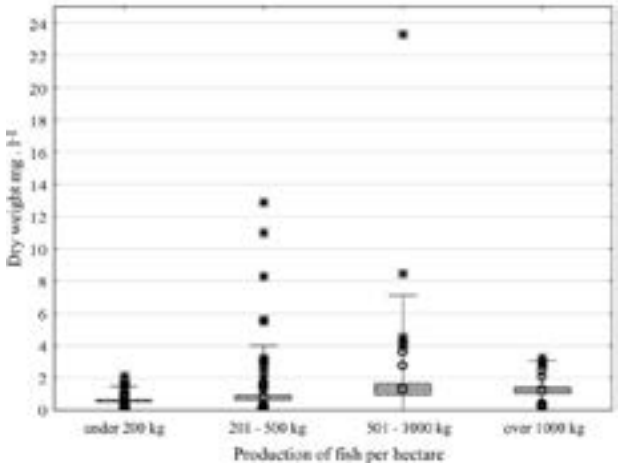
presence of aquatic cyanobacterial blooms and the inability to quantitatively separate zooplankton and phytoplankton, and for 21 samples due to low sample volumes lower than 1 ml, which was set as the detection limit for biovolume determination. For 30 samples, the dry weight of the sample could not be determined due to the low abundance of zooplankton. Zooplankton biomass ranged from thousandths of a $ml.l^{-1}$ to 1.91 $ml.l^{-1}$ (median 0.012 $ml.l^{-1}$), and dry weight ranged from thousandths of a $mg.l^{-1}$ to 78.2 $mg.l^{-1}$ (median 0.44 $mg.l^{-1}$). There was a relatively close relationship between zooplankton dry weight and bulk biomass determination (R^2 = 0.77). The occurrence of samples with high biomass of large cladocerans distorts the correlation and therefore a logarithmic expression is preferable (Fig. 1).

Due to significant variations in phyto- and zooplankton biomass and a number of physicochemical parameters during the growing season, there was no statistically conclusive relationship between zooplankton biomass and any of the physicochemical or production parameters we observed. When converting the determined parameters into ranges of values, certain trends can be observed. When comparing zooplankton biomass with the magnitude of fish production (Fig. 2), the lowest zooplankton biomass was observed in the ponds with the lowest fish production. Conversely, total zooplankton biomass was higher at higher fish stocking rates.

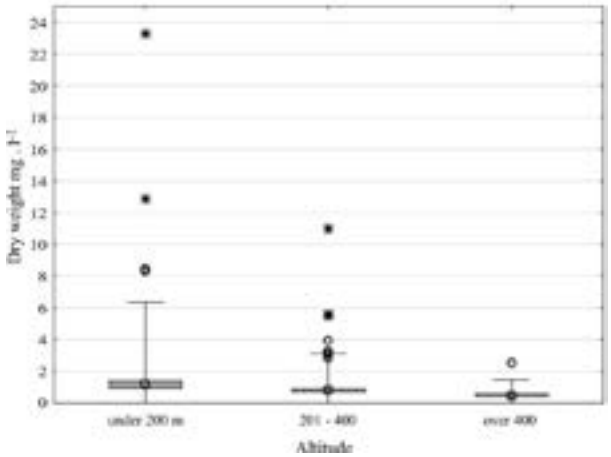
When the studied ponds were divided into groups according to the altitude at which they were located, the mean zooplankton biomass was highest at low altitudes and lowest at higher altitudes (Fig. 3). Similar trends were observed for phosphorus and nitrogen, and consequently nitrate-nitrogen values. As the element concentration in water increases, the zooplankton biomass also increases (Figs. 4 and 5).



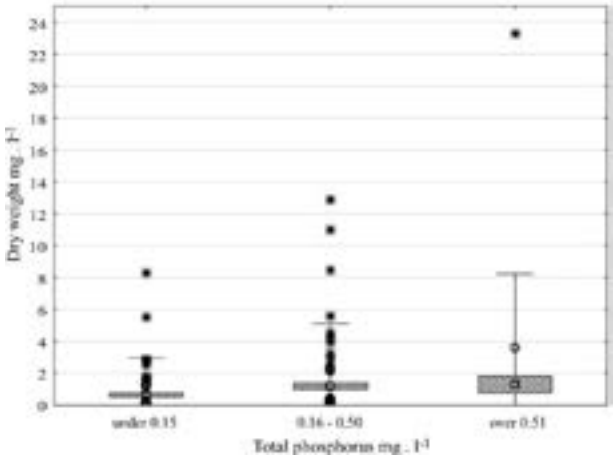
1: *Correlation between zooplankton dry weight and determination of zooplankton biovolume (N = 360)*



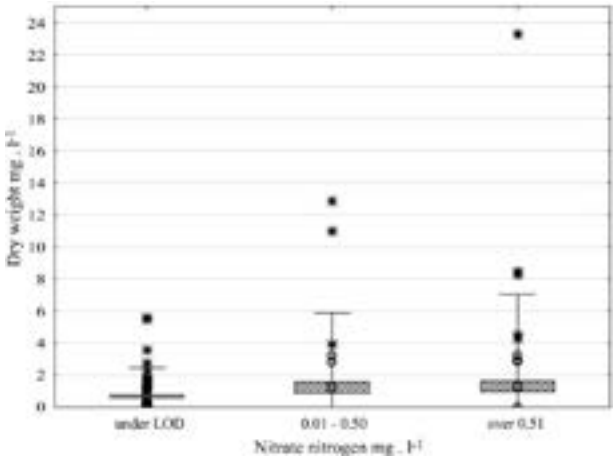
2: *Zooplankton dry weight values as a function of production of fish per hectare. Box includes 25th to 75th percentiles, with the middle point representing the mean, the circles showing the outliers and the asterisks showing the extremes.*



3: *Zooplankton dry weight values as a function of altitude. Box includes 25th to 75th percentiles, with the middle point representing the mean, the circles showing the outliers and the asterisks showing the extremes.*



4: *Zooplankton dry weight values as a function of total phosphorus concentration in water. Box includes 25th to 75th percentiles, with the middle point representing the mean, the circles showing the outliers and the asterisks showing the extremes.*



5: *Zooplankton dry weight values as a function of nitrate nitrogen concentration in water. Box includes 25th to 75th percentiles, with the middle point representing the mean, the circles showing the outliers and the asterisks showing the extremes, LOD - under the detection limit.*

DISCUSSION

Bulk biomass determination is used to express the volume of zooplankton in both fresh and salt waters. It is an aggregate measurement and as such does not allow to distinguish between water content, inorganic and organic fractions of organisms. The simpler procedures are generally relatively time-saving and, in addition, the same sample used for volume measurements can be used for other analyses, including taxonomic and morphological studies. Data on bulk biomass are satisfactory if the shapes of individual plankton species do not differ dramatically, e.g. for example in pure copepod samples. Long pendants, gelatinous organisms, and species with significant buoyancy make this method less accurate (Steedman, 1976).

The space that remains between organisms when they settle depends on factors such as their orientation and the number and length of their spurs. Also, air trapped within an organism or on the surface of its body tends to entrain it. The volumetric biomass of freshwater zooplankton obtained in this way is on average about twice as high as the sum of the individual biomasses of zooplankton individuals, because the gaps between them remain filled with water, i.e. 1 ml of sedimented zooplankton corresponds to approximately 0.5 g of its fresh weight (Steedman, 1976; Prikryl, 2006). If we convert the values of volumetric biomass to fresh weight according to the above relationship, we arrive at an average fresh weight of 13.5 $mg.l^{-1}$. The average percentage of zooplankton dry weight is then 9.7%. Musil (unpublished data) has found an average dry weight value for zooplankton of 8.3% for ponds, with increasing dry weight values for smaller species. The zooplankton of the ponds surveyed in our study was composed mainly of medium and small-size species, which may explain the higher dry weight value of our samples.

Determination of zooplankton biomass based on length measurements of individual taxa or other length parameters with subsequent conversion of measured values to volume or mass units is often used. The length of individuals is commonly measured using a light microscope, which is time consuming and usually not feasible in routine sampling. Limnologists have therefore developed species-specific length-dry weight relationships for individual zooplankton species in order to calculate biomass (e.g. McCauley, 1984). It is not sufficient to simply assign size and mass distributions to individual zooplankton species. A relationship between size classes and their corresponding masses should be constructed for each species. Some individuals in the spring are larger than those included in the winter survey. Limnetic species generally weigh relatively less than littoral, periphytic or benthic species. Even within a species, populations with a more pronounced limnetic life history weigh less than littoral populations (Culver *et al.*, 1985; Dumont and Van De Velde, 1975). These methods of determining bulk biomass or dry weight require skilled personnel and are time-consuming and thus unsuitable for routine monitoring of pond zooplankton by the fishery manager.

Our results show that the total zooplankton biomass of the studied ponds is highly variable, with no apparent statistically conclusive trend. This corresponds with results of other authors showing that an increase in plankton-eating fish stocking usually leads to an increase in the abundance of smaller-sized species and a reduction in the abundance of larger-sized zooplankton species (Williams and Moss, 2002; Potužák *et al.*, 2007). The finding that changes in the bulk biomass of zooplankton do not increase or decrease in proportion to the size of fish recruitment was described from Lednice Ponds as early as 1923–1924 by Bayer and Bajkov (1929). The average value of zooplankton biomass in their study ranged from 0.015 to 0.041 $ml.l^{-1}$ in the ponds with a fish production of 140–190 $kg.ha^{-1}$. These values are consistent with our results from the same ponds, where we determined an average zooplankton biomass ranging from 0.014 to 0.051 $ml.l^{-1}$ with a fish production of 30–650 $kg.ha^{-1}$. Thus, the effect of fish stocking on zooplankton is reflected in the structure but not in the total zooplankton biomass (Fott *et al.*, 1980).

The trends we found, although not statistically conclusive, are consistent with the results of other authors. The low zooplankton biomass at sites with higher elevation is mainly due to lower mean water temperatures and lower nutrient content, which is consistent with our results (Moreira *et al.*, 2016). Higher water temperatures, enhanced by ongoing climate change, significantly affect the zooplankton community with varying differential effects on different groups (Shurin *et al.*, 2012). However, zooplankton biomass may not increase with increasing temperature due to increased predation pressure from fish and the production of smaller size individuals that copepods, cladocerans and rotifers produce at high water temperatures (Moore *et al.*, 1996).

An important factor influencing zooplankton development is the level of eutrophication, i.e. the amount of available nutrients. Our results show an increase in zooplankton biomass with increasing nutrient content. This is confirmed by the results of other authors who describe an increase in zooplankton abundance when phosphate and nitrate levels increase (Aubakirova *et al.*, 2021; Karmakar *et al.*, 2022). On the other hand, higher zooplankton abundance does not necessarily mean an increase in zooplankton biomass. In an environment of increased eutrophication, representatives of the main groups of zooplankton in ponds (Cladocera, Copepoda, Rotatoria) are smaller in size, and the large Cladocera *Daphnia magna* is also limited in abundance (Alimov, 2010; Aubakirova *et al.*, 2021).

CONCLUSION

It is clear from the above data that predicting the development of the zooplankton community in ponds is highly problematic. Standard determination of zooplankton biomass at regular intervals during the growing season is usually not realistic outside scientific studies due to its technical and time-consuming nature. Therefore, for long-term monitoring of the zooplankton community at a given site, it is advisable to use relatively quick and inexpensive methods of determining zooplankton biomass, such as bulk biomass or dry weight. Although these methods do not give a detailed picture of the species structure of zooplankton, they do give information on the size of the total zooplankton biomass. Together with the determination of basic physico-chemical parameters and especially chlorophyll-a, a fairly good inference can then be made on the development of plankton communities.

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