Why do I sample zooplankton from a lake?

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Content

What means plankton?

The term πλαγκτός is a word in ancient Greek and means the wandering or roaming, used of ships in poetry and it started out as an adjective (Hutchinson 1974). Plankton is derived as a noun and in the present-day use by aquatic ecologists its meaning is floating or drifting. It comprehends the collectivity of living organisms levitating and floating free in water. Proper motions are mainly restricted to vertical swimming up and down (Hentschel & Wagner 1990). It labels organisms in the pelagic zone, which are not able to swim against currents. There is animal plankton (zooplankton), plant plankton (phytoplankton), bacterial plankton (bacterioplankton), and planktonic fungi (mycoplankton). The ichthyoplankton comprehends pelagic fish eggs and fish larvae. Living organisms whose proper motion is sufficient to swim against a current, are named nekton, i. e. fish (Sommer 1994).

***Daphnia* and her position in lakes**

Cladocera (water fleas) of the genus *Daphnia* are an important part of zooplankton in inland standing waters. They filter small unicellular algae and bacteria form the surrounding water and prefer particles between 1-30 µ diameter. Therefore, they select for algae > 30 µ size. The filtration efficiency of *Daphnia* depends on their size (Jarvis et al. 1988, Knoechel and Holtby 1986, Peters and Downing 1984). Populations of big-sized species as *Daphnia magna*, *Daphnia pulex* und *Daphnia galeata* are able to clear the water in an eutrophic lake during the spring bloom of the unicellular green algae until a secchi depth of 8 m is attained. This state is called the clear water phase and it presupposes that piscivores are present at sufficient numbers in the waters. Waterfleas may experience the possibility of natural mortality and the mortality by predation is low.

If piscivores are missing and there is a scenario with planktivores, waterfleas are the preferred prey of fish. Planktivores select their prey visually by size. Big-sized water fleas are seen before the smaller ones. They are eaten first and therefore a selection pressure towards small water fleas exists (Brooks und Dodson 1965). However, the filtration efficiency of small water fleas is lower and a change of species from *Daphnia galeata* to *Daphnia cucullata* happens.

Chains of causes and effects

The interactions between piscivores, planktivores and water fleas are not restricted to the biology, but there are further reaching effects.

In the case of a presence of piscivores in a lake, the phosphorus concentration in the epilimnetic water decreases during summer stagnation (Wright & Shapiro 1984, Mazumder et al. 1989, Guy et al. 1994, Larocque et al. 1996, Hudson et al. 2001) that it falls beneath the detection limit of 5 $\mu g\ l^{-1}$ TP whereas in the presence of planktivores the phosphorus concentration in the epilimnetic water increases during summer stagnation (Wright & Shapiro 1984, Lazzaro et al. 1992 und Mazumder & Lean 1994).

Carpenter et al. (1992) express the hypothesis that the trophic cascade is effective from piscivores by small fish down to the water fleas in coherence with the nutrient dynamics in the waters. They found that in the scenario with planktivores phosphorus accumulates in seston, whereas in the scenario with piscivores the dissolved fraction increases. Mazumder und Lean (1994) pointed at the same distribution of dissolved and particulate fractions of phosphorus dependent on fish stock.

Vanni et al. (1997) corroborate this hypothesis with their results that fish can amply impact dynamics, distribution, and ratios of limiting nutrients.

These findings are comprehensible in view of the absorbing capacity of dissolved nutrients by bacteria through the big surface compared to their cell volume. In the scenario with planktivores, a microbial loop exists with a multitude of bacteria. Whereas in the scenario with piscivores, water fleas filter bacteria besides algae and the microbial loop is scaled-down. Consequently, dissolved nutrients abide longer in water before resumed by living organisms. It would be nearby to use such results in the assessment of the actual condition of lakes.

Sampling and processing of plankton to what purpose?

Since the beginning of scientific work on plankton different technical possibilities were used, in order to sample plankton. On every occasion it was changed for the use of the newest state of the art. On the one hand, there is the delight in the beauty and richness of living nature. This may be expressed in the inventory of ecosystems and in the investigations of the life cycle of particular organisms. On the other hand, the applied work must be done. Here, the researchers of ichthyoplankton should be mentioned first. If the abundance of pelagic fish eggs as number of eggs per area is known, it can be used to assess the population of the parent stock. The demand by the national fisheries administrations to investigate the plankton of the sea, has given strong stimulations for technical applications in order to record pelagic fish eggs quantitatively.

A further scope of application is the system analysis as research at ecosystems concerning the causal relationships particularly the possibilities for manipulations by mankind. A system analysis may emerge from pure descriptive work or from the applied question and requires an interdisciplinary approach and processing.

Sampling by net

The evaluation of a sampling method resp. of a sampling device must focus on the technical preconditions. McGowan and Fraundorf (1966) compared nets of same mesh size, but of different net mouth and they found that in the small nets the number of species, the abundance of certain species and the diversity was lower. These results can be evaluated as net avoidance. Nets with different mesh sizes were examined by Tseng et al. (2011) for sampling of Copepods. They found a high selection by mesh sizes which had an effect on the qualitative and quantitative composition of the samples. The authors see the problem that results of the research are difficult to compare. Surely, the proportion of the small zooplankters may be estimated which are squeezed through the hooks, but the accuracy of such computations is questioned.

Tranter (1967) gives a formula for the calculation of the filtration efficiency of plankton nets considering the passage of the water, the free area in the netting expressed as porosity and the viscosity of the flowing water. Additionally, a net factor must be determined because of the shape of the net mouth and of the angle which the netting presents to the passing water in the conical part. Unfortunately, I only find theoretical reflections in this paper. During sampling, the practical experience is decisive.

Tranter and Heron (1967) made elaborate investigations with net designs. The conventional conical net has three bridles above the net mouth, fixed with a ring to the towing line. Behind the ring, turbulences are created and a narrow range of diminished flow exists. A flowmeter fixed in the center of the net mouth records only a part of the water volume filtered by the net.

Additionally, Tranter and Heron (1967) examined nets with different lengths and structures in the laboratory and in the open sea with a twin construction, the bongo. The bongo is a frame for two nets. The towing line is fixed in the middle of the frame. Both net mouths are free from bridles. The results showed that the filtration efficiency quickly decreases, if the net length is less than the double of the mouth diameter. Clogging can be diminished by a cylindric net part in the anterior part of the net. This part is cleaned by continuous oscillations. In the laboratory, the desired filtration efficiency was obtained, but during towing in the sea the filtration efficiency was diminished by clogging of the hooks. Evans and Sell (1985) compared nets with different mesh sizes. They mention the difficulty to place the flowmeter at the correct position in the net mouth and calculate for the net with 156 µ mesh size a filtration efficiency of 98 % at a filtration area to mouth opening of 3,06. On contrary, a net with 76 µ mesh size and a filtration area to mouth opening of 1,86 showed a filtration efficiency between 64,7 – 79,6 %.

Smith et al. (1968) carried out more tests about clogging, net pattern, and net length. Their results indicate that important features in relation to the filtration efficiency are mesh size and filter area.

Table 1 as exerpt from Smith et al. (1968): Beschreibung der Netze, die wegen der Verstopfung der Netzmaschen geprüft wurden.

Nr	Net-material	Net form	Mesh size	Porosity	Filtering area	R = ratio	Length of net
			(µ)		(m^2)		(m)
1	nylon	con	333	0,46	2,50	3,2	3,1
2	nylon	con	333	0,46	3,75	4,8	4,7
3	nylon	cyl-con	333	0,46	2,50	3,2	2,5
4	nylon	cyl-con	333	0,46	3,75	4,8	3,8
5	nylon	cyl-con	333	0,46	5,00	6,4	5,1
6	nylon	cyl-con	571	0,49	3,75	4,8	3,6
7	silk	cyl-con	550	0,36	2,50	3,2	3,3
8	silk	cyl-con	550	0,36	3,75	4,8	4,9
9	silk	cyl-con	450	0,34	3,75	4,8	5,2
10	nylon	cyl	333	0,46	1,25	1,6	1,4
11	nylon	cyl	333	0,46	2,50	3,2	2,2
12	nylon	cyl	333	0,46	3,75	4,8	3,1
13	nylon	cyl	333	0,46	5,00	6,4	4,0
14	nylon	cyl	201	0,43	5,00	6,4	4,2
15	nylon	cyl	101	0,36	5,00	6,4	4,9
16	silk	cyl	550	0,36	5,00	6,4	4,9

Table 2: Explanations to Table 1 and 3

Ratio	R is he ratio of filtering area to mouth area
cone	Conical net
cyl	Cylindric net
cyl-con	Cylindric-conical net
ms	Mesh size

Table 3 as excerpt from Smith et al. (1968) with comparison of two sampling sites with different trophical states. Volumes and time were measured until the efficiency decreased beneath 85 %.

Nr	R	Mesh size	Mesh material	Net form	Initial efficiency percentage	Catalina Volume	Catalina Time	San Pedro II Volume	San Pedro II Time
		(µ)			(%)	(m^3)	(min)	(m^3)	(min)
1	3,2	333	nylon	con	92	112	2	-	-
2	4,8	333	nylon	con	91	542	10	-	-
3	3,2	333	nylon	cyl-con	91	390	7	-	-
4	4,8	333	nylon	cyl-con	89	1172	25	557	10
5	6,4	333	nylon	cyl-con	92	2564	50	-	-
6	4,8	571	nylon	cyl-con	94	3270	60	1137	20
7	3,2	550	silk	cyl-con	87	3210	60	786	16
8	4,8	550	silk	cyl-con	93	3316	60	1461	30
9	4,8	450	silk	cyl-con	94	3069	60	961	18
10	1,6	333	nylon	cyl	71	61	2	-	-
11	3,2	333	nylon	cyl	85	-	-	-	-
12	4,8	333	nylon	cyl	92	2048	45	-	-
13	6,4	333	nylon	cyl	93	30330	60	1194	20
14	6,4	201	nylon	cyl	92	1554	28	654	12
15	6,4	101	nylon	cyl	92	1073	19	95	2
16	6,4	550	silk	cyl	94	-	-	3294	60

Smith et al. (1968) carried out more tests about clogging, net pattern, and net length. Their results indicate that important features in relation to the filtration efficiency are mesh size and filter area. Flowmeters attached in the net mouth and outside were used to test the clogging of the hooks during towing. The ratio of the revolutions of internal to external flowmeters is a measure for the reduction of the flow through the net mouth. The ratio of both flow speeds is called filtration efficiency. The initial filtration efficiency should be 85 % and can attain 94 % under favourouble conditions. There is a dependency from the plankton abundance in the waters under investigation (Smith et al. 1968). Volume and time are listed before the efficiency decreased below 85 %. The difference between the sample sites Catalina and San Pedro I and II is visible and the effects of mesh size and the ratio of filtration area to area of net mouth.

An example for the successful net tow is given by Smith et al. (1968) by reference to sampling the eggs of the Northern Anchovy (*Engraulis mordax*). At an average width of the oval shaped eggs of 0.65 mm, the mesh size must be smaller for not squeezing the eggs through the hooks. Therefore, a mesh size of 0.333 mm was chosen whose diagonal retain the eggs. With a diameter of the net mouth of 0,5 m (0.2 m^2 area net mouth) it is possible to filter a sample with a sufficient number of eggs from 125 m^3 water. The constructed net had 7.8 times more filtering net area than area of net mouth.

Brander et al. (1993) carried out experiments for the measurement of the filtrated water volume and the clogging effect. They used electronic flowmeters mounted one in the net mouth and another one in a tube with the same diameter but without net. A third flowmeter at a stick in the test tank measured the current speed in the tank. They found that there are different flow velocities in the net mouth depending on the distance from the center.

Today, the Bongo is used as a standard for sampling fish eggs and fish larvae in the sea (Smith and Richardson 1977).

A double net was constructed by Bürgi (1983) in Switzerland for sampling in lakes. It has a frame with two nets. The towing line is fixed in the middle of the frame and off the net mouths which are free. Both nets have short cylindric parts behind the net mouth and a long conical part in the posterior range. The frame has a tilting device. A shake of the towing line tilts the frame and closes both nets under water. Samples from different depths may be taken. Bürgi`s net design is an altered shape of the Bongo for the application as closure net. The length of the gear is unfavourable at shallow waters and its use is restricted to deep lakes. It is the best net construction for deep lakes until now. The long conical part provides a high ratio of filtering net area to net mouth. Therefore, the clogging is diminished and the efficiency of the filtration is increased.

Stehle et al. (2007) compared the Longhurst-Hardy Plankton Recorder (LHPR) and a Bongo net und recorded big differences in the number of certain zooplankton organisms. Both nets differ in the shape of the initial part, in the towing speed, in the mesh size, in the diameter of the net mouth. The results show that the net avoidance is species specific. Therefore, for such investigations, only one design pattern must be different for the comparison of the filtration efficiency.

Sampling of plankton by bottle

The use of bottles for sampling admits the calculation of the catch from a defined water volume, but shows the disadvantage that with a volume of ten liters and more the gear becomes cumbersome especially on a small boat. I used in early nineties a bottle with flap valves self-made which had a volume of 20 l. The comparison with plankton samples from pumping shows great differences. The movements of the valves cause turbulences. Seuront et al. (2004) found that some zooplankton organisms avoid turbulences.

De Bernardi (1984) reviews some of these devices. For shallow waters, a plankton trap as proposed by Stich et al. (2000) may be well suited, but with the mentioned disadvantage. In shallow lakes , water fleas stay between macrophytes during day time as a refuge because of predation by fish. One possibility may be to sample during night time when the waterfleas are in the open water. A further disadvantage is the small volume of the water that can be filtered.

Sampling of plankton by pump

Reading the literature on plankton sampling by pump, I learn about Henson (1985) who performed early experiments with a pump at the end of 19th century. Since then further tests were made by several researchers.

Table 4 gives a short incomplete look about the used devices.

*First author	Year	Pump mechanism	Power supply	Capacity	Weight	Mouth inner Ø
				(l s^{-1})	(kg)	(cm)
Chick	2010	Membrane	?	?	?	?
Riccardi	2010	?	petrol	?	?	?
Nayar	2002	Membrane	battery	0,24	2,5	?
Keim	1997	centrifugal	petrol	1,5	6	4,5
MØhlenberg	1987	centrifugal	electric	7	20-60 ?	?
Solemdal	1984 (1977)	centrifugal	hydraulic	167	50	20
Solemdal	1984 (1980)	centrifugal	electric	50	90	15
Solemdal	1984 (1983)	Propeller-	electric	1000 ?	460	100 ?
Taggart	1984	centrifugal	petrol	13,3	300	7,62
Elster	1958	Hand wing	hand	?	?	2,5-4

I recall the papers by Elster in the fifties (Elster 1952 and Elster 1958) where he discussed the questions for a comparison of methods and a further reworking was requested.

In the fifties, there were only hand wing pumps available for the use on boats and they had the disadvantage that differences in pressure occurred which caused an avoidance behavior by zooplankters. Prefixing a funnel ahead of the mouth could handle the avoidance (Elster 1952). With further experiments, Elster (1958) showed that with a diameter between 2.5-4 cm mouth the percentage of the escaping copepods turned down and became insignificant. Prefixing of a funnel was superfluous.

Unfortunately, this spadework was not followed up by other research on pumping methods in Germany for a long period. Abroad from Germany, further experiments for zooplankton sampling by pumps were conducted for comparison.

Taggart and Leggert (1984) enumerate the disadvantages of pump systems for sampling ichthyoplankton:

1) Pumps damage or destroy the organisms they sample,
2) Volumes sampled per unit time are too small, and
3) Pump systems capable of sampling sufficient volume are too cumbersome to operate, particularly from small boats.

MØhlenberg (1987) expressed similar objections and opposed the reliable measurement of the filtered water volume irrespective of the clogging, the current speed, the ship speed, the sampling depth or the towing speed.

The experiments by Solemdal and Ellertsen (1984) and by Taggart and Leggett (1984) showed that for purposes of the work on ichthyoplankton the available pump systems are not sufficient in order to filter the requested water volume of more than hundred cubic meters for one sample. Therefore, fisheries biologist relied further on the Bongo frame and twin nets as catching device.

At inland and coastal waters, the situation is different, particularly if small plankton organisms like copepods or rotifers should be included. Nets with a mesh size of 60 µ that filter such organisms induce a high resistance against the inflowing water und diminish the filtration efficiency of a net substantially. MØhlenberg (1987) and Nayar et al. (2002) compared sampling by net with sampling by pump. Whereas Nayar et al. (2002) view both methods as comparable, MØhlenberg (1987) recorded great differences between net sampling with 100 µ mesh size and pump sampling. Damaging of the sampled organisms was restricted to the Appendicularia. The latter are organisms of the chordates which exist only in the sea.

Riccardi (2010) used in the shallow Lagoon of Venice a pump in order to sample the small crustacean plankton, particularly the copepods and filtered 1200 l water through a net with 80 µ mesh size. In comparison to the results of other authors who sampled in the Lagoon of Venice, she found more species and significantly more biomass in her samples. Chick et al. (2010) refrained from netting for sampling rotifers in a river and pumped water through a net with 20 µ mesh size.

Josef Hönig seized on sampling by pump for zooplankton. He used a garden pump with a two-stroke engine. At first, the objective was sampling zooplankton in sufficient numbers, in order to determine the wet weight by a micro scales and to achieve a notion about the appearance of planktic prey for the fish stocks in gravel pits.

During these field trials for the determination of fish prey, I assisted and learned the system. The results were printed three years later in the silver jubilee publication of the IG Bruhrain (Keim et al. 1997).

Under the impression of this sampling method, I assembled myself such a sampling system by pump after the design of Josef Hönig. The crucial distinction from former pumping trials of other researchers is the use of a motor pump on the boat and of intermateable pipes instead of flexible tubes. The pipes are 1 m long exactly if plugged together. Such a system is ideal for the generation of a profil at the sampling of a standing water. During work, the pumped up water is recorded for temperature, oxygen concentration, pH-value and conductivity, water samples for nutrient measurements in the lab are bottled and at each meter the lifted water was filtered through a plankton net. This procedure avoids unease about disturbing bridles at the net mouth and possible clogging of the hooks. Additionally, it is possible to measure accurately the filtered water volume. It has passed the practical test at Lake Buchtzig (12 m deep), Lake Neureuth (40 m deep), and at the shallow Wilhelmswörthweiher (3 m deep).

Assuming that the epilimnetic layer is in the range between 0-4 m depth, the sampling will made four times at each meter depth for five minutes and at a delivery rate of 1 l s^{-1} more than 1000 l water will be filtered. Even at oligotrophic states in lakes of our county Karlsruhe in the Upper Rhine valley, such a volume is sufficient to count and measure more than hundred water fleas from one sample. The dimension of the equipment can be kept considerably smaller than that of ichthyoplankton sampling devices in the sea.

During this time of testing, I could realize more corrections. The pump with a two-stroke motor was replace by one with a four-stroke motor. Since 1996, the delivery rate of the pump was accurately recorded. For this purpose, the time to fill a bucket with 10 resp. 20 l content was measured by a stopwatch. It was found that after more than one hour pumping the delivery rate of the pump slightly altered. These measurements were converted to l s^{-1} and the accurate computation of the filtered water volume was possible. This pump-pipe system has proved to be successful on several fisherman boats in the county of Karlsruhe.

Further problems as task

We all want to use better methods for the comparison of results. With pleasure, I agree that the resulting method may not be an “universal method zooplankton” (Stich et al. 2010), but there is a need for more modified and adjusted methods. The results from Wilhelmswörthweiher indicate that the carp had ingested sand grains together with zooplankton. It is assumed that the plankters stood fairly near the bottom at time of being eaten by the fish. Additionally, it is known that in shallow waters, zooplankton performs diurnal migrations in horizontal direction; waterfleas may find a refugium where they are not exposed to predation by fish (Burks et al. 2001). At such conditions with waterfleas near the bottom or between densely growing macrophytes, it is quite difficult to collect those populations in a quantitative way.

Therefore, comparative investigations should be made at day and night time in shallow lakes.

I see one more advantage by the use of a pump system. During pumping with a delivery rate of 1 l s^{-1}, the stratification of the water is broken after three minutes and the boat must be moved during pumping. This movement may compensate for the clould-like distribution of zooplankton.

Particularly the sampling of zooplankton in shallow lakes needs further investigations. For an evaluation of the ecological state in a lake, quantitative data are needed. This is a big-sized field open for treatment in order to embrace “bottom:up” and “top:down” effects. However, the systematic default at net towing by the clogging and the conjoined varying filtration efficiency of a net must be avoided. Moreover, there are uncertainties at the suspension of the flowmeter in the net mouth, since the velocity of the inflowing water is not evenly distributed. An alternative would be the use of suitable pumps, which are not commented by Stich et al. (2010). That is a pity. Since some of the papers cited by the authors deal with the use of pumps for plankton sampling (Elster 1958; Chick et al. 2010).

What does mean accuracy and precision?

In the discussion to the discourse by Elster (1958), Nümann announced principal objections:

"How do we have an absolute measure for the correctness of the results at all? The method which bring about the highest figure, must not necessarily be the best."

This statement is true until today. Nobody is able to determine the absolute numbers of plankters in a given volume of water, since there is not any standard. The land surveyor has the measuring tape, the chemist weighs out his standard for a reference solution. Thus correct measurements in terms of "accuracy" can be achieved. This is not yet possible for zooplankton in a lake until today. A possible approximation to absolute figures would require elaborate tests in experimental tanks like those performed by Brander et al. (1993). As long as such results are missing, I have to rely on other criterions.

The collection of ichthyoplankton serves to assess certain fish stocks. It is a matter of the magnitude. The aim of zooplankton sampling has more ambitious goals. An aim is the correlation of determinants with variables in the lake and the development of causal relationships. For such a purpose I need to collect results with a high accuracy. Samples taken with a 98 % efficiency of the equipment are beyond threshold. The systematic error is oversized. As far as the data facilitate to calculate regressions at a level of 1 % significance or better, I may accept them preliminary as sufficient. The confirmation by further research is pending.

The English language makes a difference between accuracy und precision on contrary to other languages i. e. German. For good luck, I include here some links with explanations.

https://www.boundless.com/chemistry/introduction-to-chemistry/measurement-uncertainty--7/accuracy-precision-and-error/

http://www.gmat.unsw.edu.au/currentstudents/ug/projects/Mui/precision_and_accuracy.htm

http://conflict.lshtm.ac.uk/page_49.htm

In the laboratory, a sample can be counted entirely or I take aliquots and it is possible to work with accuracy and precision. Van Guelpen et al. (1982) processed samples in the lab and evaluated different procedures and equipment for the enumeration of zooplankton. But there is not yet a measuring tape in the lake for sampling zooplankton. All the known sampling devices for zooplankton are an attempt to approach to 100 % in different extent as approximation to the truth.

Why System analysis?

Nature is not considerate of artificial established borders between disciplines. For the sake of system analysis at an ecosystem, I have to transcend the borders. This starts with the physics of the water body, is continued with nutrient dynamics, the growth of plants, the grazing upon the plants by animals and the interventions by mankind in the formation of basin and shore line also input and output of material and organisms. More than one discipline is afflicted and the variables investigated by the disciplines are interrelated.

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