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The importance of Chaoborids (*Chaoborus flavicans*) in selection of restoration methods for a eutrophic and dystrophic lake

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The main motivator of this thesis was to discover the importance of *Chaoborus* in a eutrophic and dystrophic lake and evaluate suitable restoration methods to enhance the state of the study lake. The role of *Chaoborus* in the study lake, Lake Jouttenus, was studied with sampling of both the water column and the sediment, echo-surveys, and diet analyses of fish. The sampling was planned as comprehensive, and the sampling stations were distributed across the whole lake area to examine the density and distribution of *Chaoborus*. The deeper areas of the lake were emphasized more in sampling because *Chaoborus* tend to favor those areas. The *Chaoborus* density was calculated with stratified sampling, which gives a more precise mean density estimate than simple random sampling. In addition, fish were caught on four (4) study occasions to find out if they had included *Chaoborus* in their diets.

The mean density of *Chaoborus* in Lake Jouttenus was a little lower than expected, only 271 individuals/m^2 in areas ≥2 m depth. The highest density of larvae was found from mid-depths between 6.0-7.9 m in the sediment. Only the deepest areas (≥8 m depth) had limnetic *Chaoborus* and more limnetic than benthic larvae. The mean length of larvae was 8.4 mm in the sediment and 9.0 mm in the water column. The length distributions appeared to be unimodal. Echo-surveys confirmed that the larvae occurred in the water column only in the deepest area in the north of the lake where the hypolimnion had a low oxygen concentration below 4 m depth. The diet analyses showed that roach and perch had eaten *Chaoborus* but the number of *Chaoborus* was high only in the diet of roach in early July.

In comparison with other studied lakes, the density of *Chaoborus* was the lowest in Lake Jouttenus. In addition, the mean length of benthic *Chaoborus* in Lake Jouttenus was lower than in the other studied humic lakes. The distribution of limnetic *Chaoborus* appeared as restricted to the deepest areas in the lake and elsewhere the larvae occurred only in the sediment. The slow growth of *Chaoborus* and their low mean density could be explained by the lack of an efficient refuge and probably also the lack of resources. The larvae were unable to reach the epilimnion and their prey safely at daytime and/or the amount and quality of food items for the larvae were poor.

The darkness caused by humic substances and low oxygen concentration in the hypolimnion created a refuge for the limnetic larvae only in the deepest area of Lake Jouttenus. Roach and perch ate *Chaoborus* occasionally. However, mass removal of fish is not recommended as it would decrease the predation pressure by fish on *Chaoborus* and increase the risk of *Chaoborus* population growth at the deepest areas and enable their range to extend. Instead of mass removal of fish all methods that aim in reducing the humic substances in the water especially at the lake catchment area might enhance the state of the lake. The clarification of water would diminish the living conditions for *Chaoborus* in long-term and help with controlling the other troubling factors such as *Gonyostomum semen* blooms in the study lake.

Keywords - Avainsanat - Nyckelord
Chaoborus, humic substances, Lake Jouttenus, lake restoration, roach, perch, fish diet

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Tiivistelmä - Referat - Abstract

Tämän maisterityön päämääränä oli selvittää sulkasääsken toukkien (*Chaoborus*) merkitys rehevässä humusvetisessä järvessä ja arvioida soveltuvia kunnostusmenetelmiä järven tilan kohentamiseksi. *Chaoborus*-toukkien roolia tutkimusjärvessä (Jouttenus) tutkittiin näytteenotolla sekä vesipatsaasta että sedimentistä, kaikuluotaamalla ja kalojen ravintoanalyysein. Näytteenotosta suunniteltiin kattava ja näytteenottopisteet jaettiin koko järven pinta-alalle, jotta saataisiin selville *Chaoborus*-toukkien tiheys ja levinneisyys. Järven syviä alueita painotettiin näytteenotossa enemmän, koska sulkasääsken toukat suosivat niitä. Toukkatiheys laskettiin ositetun otannan avulla, joka antaa perusotantaa tarkemman keskitiheysestimaatin. Lisäksi pyydettiin neljänä (4) ajankohtana kaloja, jotta saataisiin selville, käyttävätkö ne ravinnokseen sulkasääsken toukkia.

*Chaoborus*ten keskitiheys Jouttenuksessa oli hieman alhaisempi kuin odotettiin, vain 271 yksilöä/m^2 ≥2 metriä syvillä alueilla. Korkein toukkatiheys löytyi keskisyvyyksiltä 6,0–7,9 m syvyydeltä sedimentistä. Vain syvimmillä alueilla (≥8 m) oli limneettisiä toukkia ja enemmän limneettisiä kuin benttisiä toukkia. Toukkien keskipituus oli 8,4 mm sedimentissä ja 9,0 mm vesipatsaassa. Pituusjakaumat näyttivät yksihuippuisilta. Kaikuluotaustutkimukset vahvistivat, että toukkia esiintyi vesipatsaassa vain järven pohjoisella syvännealueella, missä alusvesi oli vähähappista 4 m alapuolella. Ravintoanalyysit näyttivät, että särjet ja ahvenet olivat syöneet sulkasääsken toukkia, mutta niiden määrä oli suuri ainoastaan särjen ruokavaliossa heinäkuun alussa.

Verrattaessa toisiin tutkittuihin järviin *Chaoborus*-tiheys Jouttenuksessa oli alhaisin. Lisäksi benttisten toukkien keskipituus Jouttenuksessa oli paljon lyhyempi kuin muissa tutkituissa humusjärvissä. Limneettisten *Chaoborus*-toukkien levinneisyys näytti rajoittuvan järven syvännealueeseen ja muualla toukkia oli vain sedimentissä. Toukkien hidas kasvu ja alhainen keskitiheys voisivat selittyä sillä, että järvessä ei ollut riittävän hyvää suojapaikkaa ja toukat saattoivat mahdollisesti kärsiä myös ravintopulasta. Todennäköisesti toukat eivät päässeet turvallisesti päiväsaikaan ruokailemaan päällysveteen ja/tai toukkien ravinnon määrä ja laatu oli alhainen.

Humusaineiden aiheuttaman pimeyden ja alusveden alhaisen happipitoisuuden antama suoja mahdollisti sulkasääsken toukkien esiintymisen vesipatsaassa vain Jouttenuksen syvännealueella. Särki ja ahven söivät ajoittain *Chaoborus*-toukkia. Hoitokalastusta ei kuitenkaan suositella, koska se vähentäisi kalojen aiheuttamaa saalistuspainetta toukkia kohtaan, mikä kasvattaisi riskiä sille, että toukat voisivat lisääntyä järven syvänteellä ja niiden esiintymisalue leviäisi. Hoitokalastuksen sijaan järven tilaa saattaisivat kohentaa kaikki järviveden humuspitoisuutta alentavat kunnostusmenetelmät erityisesti valuma-alueella. Veden kirkastuminen heikentäisi sulkasääsken elinmahdollisuuksia pitkällä tähtäimellä ja auttaisi myös muiden tutkimusjärveä vaivaavien tekijöiden, kuten limalevän esiintymisen, hallinnassa.

Avainsanat - Nyckelord - Keywords
Chaoborus, humus, Jouttenus, järven kunnostus, särki, ahven, kalojen ravinto

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1 Introduction

Eutrophication of lakes and other aquatic systems is a very common problem. Eutrophication causes, for example, oxygen deficiency and blooming of algae, which cause further harmful effects. Especially algae blooms are known for their harmfulness on human recreational activities and their management is considered as important. Food web management in lakes is one of the most used method in lake restoration. Lake food webs have been manipulated via stocking piscivorous fish or removing large amounts of planktivorous fish (e.g. Shapiro et al. 1975; Carpenter et al. 1985; Olin et al. 2006). One of the original ideas in food web management was to decrease the predation pressure on zooplankton via decreasing the density of planktivorous fish, which then should increase the zooplankton biomass in a lake (Carpenter et al. 1985). A strong zooplankton community can control the growth and development of phytoplankton and restrict their blooming (Carpenter et al. 1985). The control of zooplankton biomass via fish predation is an important mechanism for food web manipulations, but there are other important mechanisms too (Horppila et al. 1998).

However, mass removal of fish may not be the optimal restoration method, if, for example, the lake is turbid, its water column has low light conditions, and there occurs invertebrate predators such as the phantom midge (Scheffer 1998; Horppila & Liljendahl-Nurminen 2005). Phantom midges (Chaboridae, *Chaoborus*) are invertebrate predators, which mainly eat zooplankton and spend their egg, larvae, and pupae life stages in aquatic environments (Parma 1971). Previously it was assumed that if a lake has a high fish density then invertebrate planktivory would be low (Carpenter et al. 1985). However, Liljendahl-Nurminen et al. (2003) later found that the consumption of zooplankton by *Chaoborus* can be manifold when compared to the consumption of planktivorous fish even when the density of planktivorous fish was high. In addition, Liljendahl-Nurminen et al. (2005) proved experimentally that *Chaoborus* predation on cladocerans can be stronger than the effect of fish predation. Thus, *Chaoborus* can be the main predators of herbivorous zooplankton and control their population size instead of planktivorous fish and contribute to the formulation of extensive algal blooms.

Fish do predate on *Chaoborus* but their abilities to find the larvae and effectiveness as predators vary with the living conditions in the lake. For example, smelt (*Osmerus eperlanus*) is known to be an effective predator of *Chaoborus* even in lakes with low light conditions or total darkness in the hypolimnion (Horppila et al. 2004). Perch (*Perca fluviatilis*), on the other hand, is in general an effective predator only in good light conditions (e.g. Helfman 1979; Jansen & Mackay 1992). Perch rely more on their vision as predators than, for example, roach (*Rutilus rutilus*) (e.g. Bergman 1988; Diehl 1988) which is more effective in low light conditions (Bohl 1980). In Estlander et al. (2010) perch appeared to be the inferior predator when compared to roach in small dystrophic forest lakes in Southern Finland. In addition, for example, Malinen & Vinni (2019) found that smelt ate *Chaoborus* at Lake Alajärvi whereas perch and vendace (*Coregonus albula*) did not. Regardless, the importance of *Chaoborus* in fish diets is a topic that has not been studied much even though species and lake-specific differences are probably large, and it would also be reasonable for the planning of mass removal of fish as a restoration method.

Mass removal of fish is a popular method for lake restoration even when there is no information about the occurrence of *Chaoborus* in the lake in question. Mass removal of fish can be harmful if it improves the living conditions for *Chaoborus*. If the fish predate on *Chaoborus* and the number of fish predators decreases after mass removal the predation pressure on *Chaoborus* is reduced. This allows the growth of *Chaoborus* population, which can intensify the zooplankton consumption by *Chaoborus*, and decrease the ability of zooplankton to control phytoplankton (Liljendahl-Nurminen et al. 2005). Likewise, even if the fish do not predate on *Chaoborus* the management of the fish assemblage might allow the growth of the *Chaoborus* density and allow them to predate on zooplankton more efficiently as speculated in both Liljendahl-Nurminen et al. (2005) and Malinen & Vinni (2013b). If the fish include *Chaoborus* occasionally in their diet the living conditions of *Chaoborus* will again improve after mass removal of fish, and *Chaoborus* density can in theory grow (cf. Malinen & Vinni 2013b). All things considered; invertebrate predators such as *Chaoborus* can act both as a prey and as a competitor for fish (Liljendahl-Nurminen et al. 2003; Pekcan-Hekim et al. 2006), which makes them as not easily fitting into traditional lake restoration theories. Thus, they also pose a challenge for lake restoration issues because they may be out of reach of lake managers (Horppila & Liljendahl-Nurminen 2005).

Chaoborus, however, need a suitable environment to thrive in. Horppila et al. (2004) found that clay-turbidity and low light conditions offered suitable living conditions for *Chaoborus*. In addition, Liljendahl-Nurminen et al. (2008) tested that high clay-turbidity (visual refuge) and low oxygen concentration (physiological refuge) together provided a refuge efficient enough for *Chaoborus* against fish predators, but alone they did not. Liljendahl-Nurminen et al. (2008) discuss that there is a connection between the depth of a lake, the stratification of the water column, and the low light conditions prevailing in deep areas. The ability of visual predators like fish to detect their prey is altered in different turbidity conditions due to the different behavior of light. According to Hemmings (1966) and Hinshaw (1985) inorganic material reduces the contrast between the prey and its background, which is more important for fish predators than the amount of light in the water column which is reduced by organic turbidity. For example, clay is an inorganic material that scatters light in the water column whereas, for example, humic substances are organic material that influence the absorption of light (Kirk 1994). In addition, low light conditions together with dark-colored or turbid water weakens fish predation and gives an advantage to tactile predators such as *Chaoborus* (Vinyard & O’Brien 1976; Eiane et al. 1997).

Chaoborus perform diurnal vertical migrations that are triggered by the presence of fish and fish predation (e.g. Nilssen 1974; Borkent 1981; Luecke 1986; Dawidowicz et al. 1990). *Chaoborus* can tolerate low oxygen concentrations and take refuge from fish in low oxygenated water layers but they occasionally need to migrate into the epilimnion to prey on zooplankton (Luecke 1986). Thus, clay-turbidity appears to be a key contributor to the coexistence of dense fish and *Chaoborus* populations (e.g. Horppila et al. 2004; Liljendahl-Nurminen et al. 2008). If *Chaoborus* can avoid their predators easily they do not need to burrow into the sediment to safety. Rather the larvae can stay high in the water column closer to their prey and migrate into the epilimnion to predate on zooplankton efficiently at night (Horppila & Liljendahl-Nurminen 2005).

In addition to clay-turbid lakes some humic-watered lakes have been shown to support high densities of *Chaoborus* (Ramcharan et al. 2001; Estlander et al. 2009; Malinen et al. 2011a; Malinen & Vinni 2013a). However, according to Malinen et al. (2011a) the abundant occurrence of *Chaoborus* in humic lakes has not appeared as consistent as in clay-turbid lakes because of the variance observed in larvae density in ostensibly similar humic lakes. For example, Estlander et al. (2009) found the highest water color and the highest density of *Chaoborus* from the same lake among their four humic study lakes. However, the density of *Chaoborus* did not clearly correlate with water color because one of their study lakes had the lightest water color and the second highest density of *Chaoborus* (Estlander et al. 2009). In Estlander et al. (2009) the thickness of the low-oxygenated water layer together with the humic substances seemed to mainly dictate the density of *Chaoborus* and offer the most suitable living conditions for them. Humic substances that color the lake water dark brown and influence the light conditions might not provide as good of a restriction to visibility against the fish predators as, for example, clay-turbidity does (cf. Hemmings 1966; Hinshaw 1985). Regardless, there are numerous dystrophic lakes in Finland that can support even a high *Chaoborus* density because humic lakes often have low oxygen concentration in the hypolimnion.

The purpose of this thesis is to discover the importance of *Chaoborus* in the humic study lake. One of the main motivators of this thesis is to find suitable restoration methods that can be realized at the study lake. To be able to evaluate relevant and suitable restoration methods for the study lake via a *Chaoborus* survey, the research questions of this thesis are: 1) what is the density of *Chaoborus* in the water column and the sediment; 2) what is their distribution in the lake; and 3) does some fish species include them in their diet to a significant extent? Answers to the research questions were searched with *Chaoborus* sampling from both water column and sediment and with diet analyses of fish.

2 Materials and methods

2.1 Study area

The study area is Lake Jouttenus located in Pirkanmaa, Finland, in the municipality of Ruovesi (Fig. 1). Lake Jouttenus has an area of 1.29 km^2 and its drainage basin is 23 km^2. Most of the catchment area is forest land (16.5 km^2) and only minor parts of the land use consist of agricultural or built-up areas (Makkonen 2013). The maximum depth of Lake Jouttenus is 12.1 m, and the mean depth is 3.05 meters. The deepest basin is in the north (Fig. 2).

Jouttenus forms a lake chain with three other lakes, Lake Myllyjärvi, Lake Rikalanjärvi, and Lake Kaleton, which are located on the western and northwestern side of Jouttenus (see Fig. 1). Waters drain from surrounding land areas into Jouttenus and flow further through these three other lakes. Lake Jouttenus is the largest lake in the lake chain, and it largely determines together with the second largest lake, Lake Rikalanjärvi, the water quality in the two smaller lakes. Thus, if the water quality of Lake Jouttenus and Lake Rikalanjärvi enhances the water quality in the whole lake chain can enhance. Due to this Lake Jouttenus was considered as a potential target for restoration.

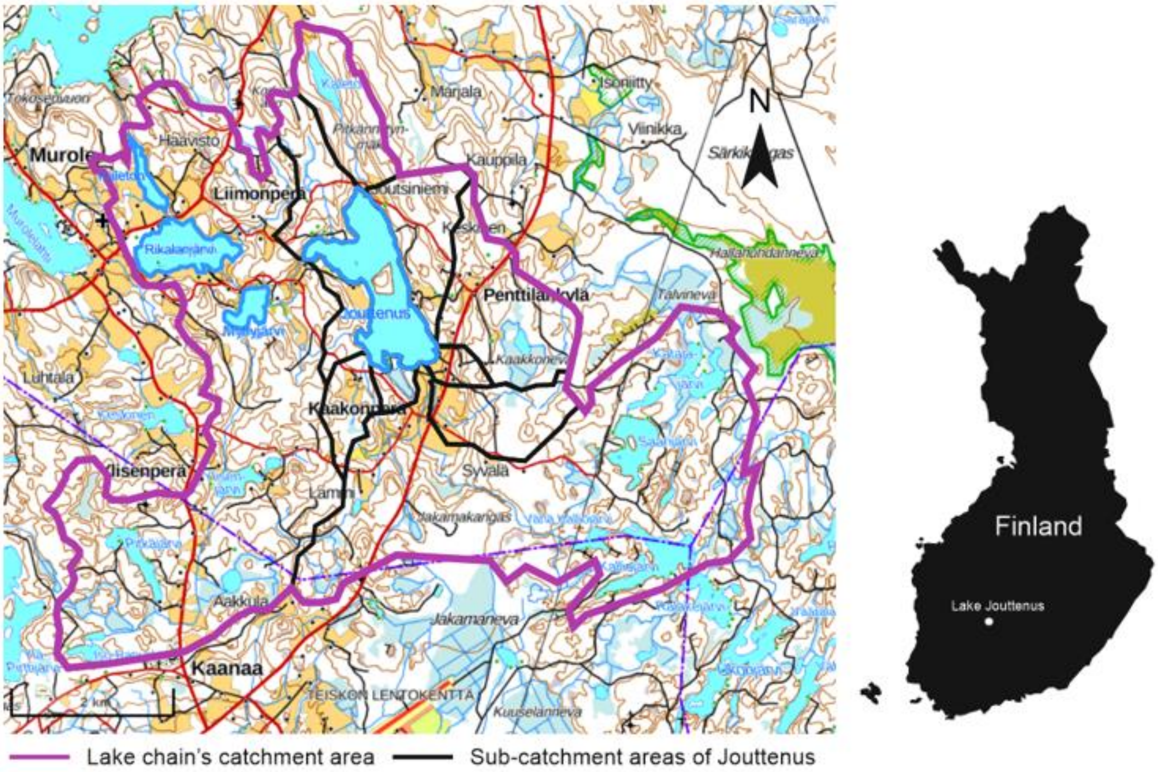


Figure 1. Location of Lake Jouttenus compared to southern half of Finland. The thick blue lines are the shorelines of the lakes belonging to the lake chain. Black lines show the sub-catchment areas of Lake Jouttenus (the eastern half of the whole chain's catchment area). Source: karttapaikka.fi.

Jouttenus is a humic lake that has eutrophicated. According to Makkonen (2013) the external loading caused by human activities has been moderate. However, based on the calculations given in the restoration plan (Makkonen 2013) that considers the whole lake chain the nutrient concentration levels of the waters draining into Lake Jouttenus are estimated to contain twice as high levels of nutrients (phosphorus) as those of natural drainage waters. The excess allochthonous phosphorus causes strong eutrophication in Lake Jouttenus since the lake is prone to eutrophication due to its small size and sheltered location which prevents wind-driven turbulence (Makkonen 2013). Due to little wind-driven mixing the lake water circulates poorly, the autumn overturn in the water column might be incomplete and does not fully circulate the water in the hypolimnion as well (Makkonen 2013). Incomplete overturns cause oxygen deficiency in the deepest water layers.

Oxygen concentration measurements alone suggest that the deepest layers of the water column have low oxygen concentration at least during summer and fall months (Makkonen 2013; Table 1). The release of nutrients follows from degradation activities in the anoxic water layers in the hypolimnion. According to previous measurements the amount of nutrients and iron in the deepest water layers of Lake Jouttenus indeed seem to imply that the oxygen deficiency in the hypolimnion allows nutrients such as phosphorus and ammonium to be released from the sediments (Makkonen 2013; Table 1). This implies that the lake is in a state of internal loading, which has been evident in the lake from the beginning of the 21st century according to Makkonen (2013).

In addition, yearly blooms of blue-green algae reduce the recreational use of Lake Jouttenus. On the 1st of September in 2011, a chlorophyll-a value of 200 µg/l was measured at Lake Jouttenus (Makkonen 2013). Such high chlorophyll-a values suggest extensive algal blooms and the presence of *Gonyostomum semen*, which has been observed at the lake on several previous years (Makkonen 2013; Järvi-meriwiki). There are also some records that the local lake shore residents have reported worsened conditions at the lake already in the 1950s (Makkonen 2013). Furthermore, a local lake protection association has been actively promoting lake restoration matters at Lake Jouttenus. The association has, for example, built a floating raft with an aeration device on top of the deepest area of the lake to help water circulation there and organized several small-scale restoration projects.

Table 1. Measurements taken on the 7th of September 2020 from Lake Jouttenus. (C = a verified result over or below an alarm limit, L = a result below a determination limit, CODMn = chemical oxygen demand, P = phosphorus, PP = phosphate phosphorus, N = nitrogen, Fe = iron). Source: Hertta-database.

Variable	0-2 m	1 m	5 m	11 m	Unit
Temperature		16.3	15.4	8.3	°C
Oxygen, dissolved		8.7	7.2	L 0.2	mg/l
Degree of oxygen saturation		89	73	L 1	sat. %
Turbidity		12	12	51	FNU
Conductivity		4.8	4.8	8.2	mS/m
Alkalinity		0.21	0.22	0.62	mmol/l
pH		7.2	6.9	6.3	
Color		44			mg/l Pt
Total N		830	660	4100	µg/l
Ammonium N		L 5	L 5	7	µg/l
Total P		4	32	C 3000	µg/l
PP		35	33	310	µg/l
Fe		620	750	C 27000	µg/l
Chlorophyll-a	49				µg/l
CODMn		8.2	8.1	20	mg/l
Visibility depth	0.5				m

Furthermore, an experimental gillnet fishing was recommended in the restoration plan to discover further possible restoration methods. The local summerhouse owners have reported that Lake Jouttenus is at least inhabited by perch, roach, ruffe (*Gymnocephalus cernua*), pikeperch (*Sander lucioperca*), bleak (*Alburnus alburnus*), pike (*Esox lucius*), and bream (*Abramis brama*). However, there is no data from the fish assemblage of Lake Jouttenus, and thus, the possibilities of mass fish removal as a restoration method for Lake Jouttenus cannot be evaluated yet. It was also mentioned in the restoration plan that a *Chaoborus* survey should be arranged before considering the mass fish removal method. Therefore, a *Chaoborus* survey was planned and implemented at Lake Jouttenus in the summer of 2021. Previous water quality data, morphological characteristics of the lake, and preliminary echo sounding observations from Lake Jouttenus before this study all suggested that the lake could be inhabited by a dense *Chaoborus* population and thus, Lake Jouttenus was selected as the study area.

With the survey, the abundance and distribution of *Chaoborus* and their significance to implementing further lake restoration methods can be discovered.

Finally, according to Salmela et al. (2021) *Chaoborus flavicans* is the only lake dwelling *Chaoborus* species living together with even high densities of fish in larger lakes. Thus, the species occurring in Lake Jouttenus is probably *C. flavicans*, and from now on *C. flavicans* is the only species of *Chaoborus* that will be discussed about in this thesis so it will be referred to as *Chaoborus* in short.

2.2 Sampling

To examine the density and distribution of *Chaoborus* in a lake sampling needs to be comprehensive and sampling stations need to be distributed across the whole lake area. Samples need to be taken from both the sediment and the water column. Stratified sampling, which is based on depth zones, was used for *Chaoborus* sampling. Sampling stations were selected randomly within depth zones. The depth zones acted as the strata used in stratified sampling. According to previous studies larvae density increases as the depth of the lake increases and there are typically no larvae at the shallower depth zones (Liljendahl-Nurminen et al. 2002). Thus, the shallowest depth zone from 0.0 to 1.9 m was excluded from this study. The sampling stations were inside depth zones 2.0-3.9, 4.0-5.9, 6.0-7.9, and ≥8 meters. In total there were 14 sampling stations (Fig. 2).

Calculations with stratified sampling required the areas of each depth zone. The depth contours for Lake Jouttenus existed only on an old map and they were drawn on a newer, more updated map taken from Karttapaikka.fi website (Fig. 2). Depth contours (2, 4, 6, 8, and 10) relevant to this study were drawn. Then the new map was printed in A3 and all the depth zones were cut out and weighed. The islands were cut out too since they needed to be excluded from the weights. A ratio between the lake area in hectares and its weight in grams was calculated for the whole lake. With the help of this ratio, an area for each depth zone was calculated (see Table 2).

Table 2. The areas of each depth zone (ha) and their percentage value of the whole lake area based on the calculations and weighing. The area for the whole lake was 127.5 ha (the value from weighing).

Depth zone (m)	**0.0-1.9**	**2.0-3.9**	**4.0-5.9**	**6.0-7.9**	**≥8.0**
Area (ha)	31.2	61.1	24.2	9.0	2.0
Area (% of whole lake)	24 %	48 %	19 %	7 %	2 %

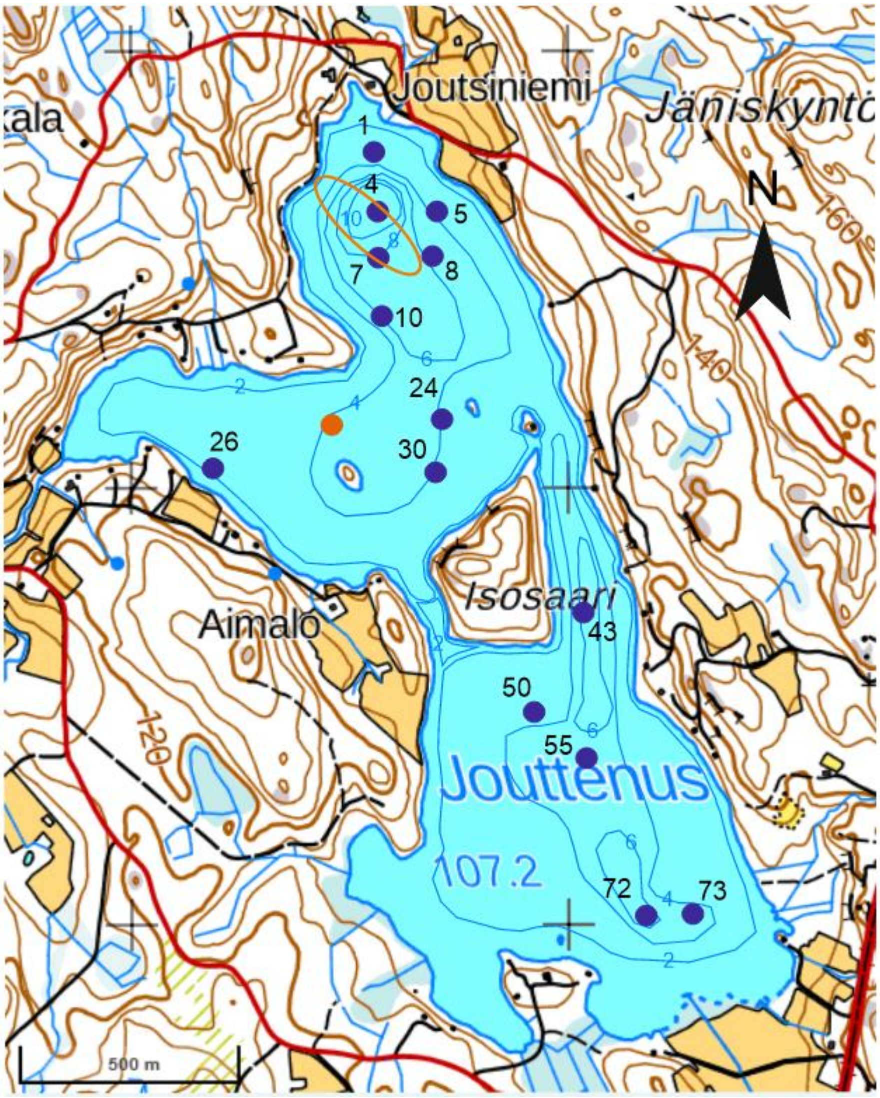


Figure 2. A map of depth contours, *Chaoborus* sampling stations (dark blue dots), visibility depth measuring station (orange dot), and gillnet locations (area marked with orange circle) at the study lake. Aeration device raft approx. at sampling station 4. Source: karttapaikka.fi, adapted with depth contour lines and sampling/measuring stations.

The field work was carried out on the 22nd of June 2021 (see Table 3 for more detailed schedule). Sampling was done with a two-meter-high plankton net (mesh size 183 µm and diameter 50 cm) for water column sampling and with an Ekman sampler (sample size 231 cm^2) for sediment sampling. The sediment samples were sifted through a sieve cloth (mesh size 500 µm) before collecting the sample sediment into plastic sample bottles. The plankton net was hauled through the whole water column from bottom to surface. One haul from each sampling station was taken with the net and with the Ekman sampler. All samples were preserved in small plastic bottles and frozen afterwards for analysis.

Table 3. A more detailed schedule of the sampling done at Lake Jouttenus. RL=Reetta Lehto, MV=Mika Vinni, locals = people from the lake chain protection association (Jouttenuksen järviketjun suojeluyhdistys ry).

Sampling	**Method and equipment**	**Date**	**Sampler**
Chaoborus larvae and pupae	Plankton net (whole water column bottom to surface) and Ekman sampler (sediment)	22nd of June and 12th of August 2021	RL, MV
Oxygen and temperature	Water column, every meter	22nd of June and 12th of August 2021	RL, MV
Visibility	Secchi disk	From May to September	MV, locals
Echo sounding	Double-beamed CHIRP 200 kHz	From June to October	Locals
Fish	Gillnet (Nordic), 3 locations: shore, pelagic surface, pelagic deep	4 times; between 13th of June and 26th of September 2021; day and night catch	Locals
Diet analyses	Scoring method (perciforms), volume/percentage method (cyprinids); microscope, visual analysis	See above	MV

To compliment the *Chaoborus* survey, water temperature and oxygen concentration in the water column were measured on five sampling stations: 4 (depth 12.0 m), 26 (depth 2.8 m), 43 (depth 6.0 m), 55 (depth 4.8 m), and 72 (depth 6.0 m; Fig. 2, Table 3). The measurements were taken every meter from surface to bottom. The sampling stations were located across the whole lake area to grasp possible differences or similarities in temperature and oxygen concentration. These measurements were made to confirm that there were no large differences between the different basins of the lake in water temperature and oxygen concentration. In addition, Secchi disk depth was measured once near the orange dot (Fig. 2).

The locals conducted echo-surveys at the lake from the end of May to October and measured Secchi disk depth through the study period. Equipment used in echo sounding was Garmin’s double-beamed Chirp with a 200 kHz frequency. Echo sounding was done at the deepest basin in the north (depth 12.1 m) and at the deepest southern basin (depth >6 meters). Dense *Chaoborus* swarms are relatively easy to recognize with echo sounding equipment (Malinen et al. 2005a). The echo sounder draws the population that usually forms dense flat swarms, a kind of a mattress formation, into the water column. This “mattress” can be at different depths depending on the living conditions at the lake. The changes in the density of limnetic *Chaoborus* can be roughly estimated with echo sounding (Eckmann 1998; Knudsen et al. 2006). Results from visibility measurements were saved in an online data website, and echo-surveys were recorded with videos or pictures and saved for further analysis.

To examine if the abundance of *Chaoborus* is controlled by fish the locals had caught fish for diet analyses. The fish were caught with Nordic gillnets from different depths in June, July, August, and September (Fig. 2). The length of the Nordic gillnets was 30 m, and they had 12 panels (panel length 2.5 m and height 1.5 m) of different sizes of mesh (from 5 to 55 mm, from knot to knot; Appelberg et al. 1995). The different mesh sizes are in a random order in the gillnet (Appelberg et al. 1995). On each study occasion the nets were set from morning to early evening and from evening to morning. The day catch included only daylight hours and the night catch included the dark and twilight periods. One of the gillnets was located near the shore bank (water depth 3 m, the net reached the bottom). Another net was in the pelagic (headline depth 1 m), and the third net was also in the pelagic but set deeper (headline depth 4 meters). The deep net was set only in June and September due to the low oxygen concentration prevailing in the deep layer below 4 m depth. After each fishing effort, the caught fish were frozen and delivered to a laboratory for further analyses.

Another field trip to the study lake was made on the 12th of August 2021. Additional water temperature, oxygen concentration, and Secchi disk depth measurements were taken on that day for comparison. Furthermore, a few net hauls were done to catch *Chaoborus* larvae and/or pupae to determine their approximate emergence period at the study lake.

2.3 Laboratory analyses

To investigate the abundance and distribution of *Chaoborus* in Lake Jouttenus, the samples were analyzed later in a laboratory. First, the samples were melted and prepared by collecting all *Chaoborus*. The *Chaoborus* were then counted and approximately two hundred larvae from the water column and ca. one hundred larvae from the sediment samples were measured with a microscope. After each sample, the wet weight of all *Chaoborus* in each sample were weighed (accuracy 0.001 grams or more). All the information was collected into table format for further statistical analyses. After weighing, Chaoborids were preserved just in case in ethanol.

The diet analyses for fish were conducted in a laboratory. The sample fish were melted from the freezer and their guts were prepared. The gut insides were analyzed visually and scored accordingly under a microscope. For the diet analyses the stomach and esophagus of perch were analyzed with a scoring method (Windell 1971) and the first third of the long intestine of cyprinids were analyzed with a volume/percentage method (Vøllestad 1985; Rask 1989). Only the first third of the long intestine was analyzed because there the eaten food items are not as fully ingested, and they are easier to recognize. First, the fullness of the gut was estimated and then the food items were recognized and determined. The analyses reveal what the sample fish have eaten before getting caught by the net.

2.4 Statistical analyses

The density estimates for *Chaoborus* larvae were calculated with stratified sampling in which the area of each depth zone was used as stratum weights. The idea of stratified sampling is to move as much as possible of the variation in larvae density data between the strata. In allocation of sampling effort, deeper strata were emphasized more than the shallower strata because Chaoborids tend to occur in deeper, preferentially hypoxic, or anoxic parts of lakes (Liljendahl-Nurminen et al. 2002). In most cases the precision of the mean density estimate is higher with stratified sampling compared with the simple random sampling (Pahkinen & Lehtonen 1989). The advantages of stratified sampling are amplified if there is preliminary information how the variance or density varies in the strata. Here the information of the preference of *Chaoborus* larvae for deeper areas was utilized. Stratified sampling enables an unbiased computing of the estimate of the mean even though the sample sizes vary in relation to the areas of the strata.

These following equations (Cochran 1977; Pahkinen & Lehtonen 1989) were applied in computing the estimate for mean *Chaoborus* density estimate with stratified sampling:

$\bar{y} = \sum_{h=1}^{L} w_h \bar{y}_h$ (1) $v(\bar{y}) = \sum_{h=1}^{L} {w_h}^2 v(\bar{y}_h)$ (2), in which

$\bar{y}_h$ = mean of sample in hth stratum

L = the number of strata

$v(\bar{y}_h)$ = the variance of the mean density estimate in hth stratum, calculated with form (4), and in this form n, s^2, and $\bar{y}$ are then stratum specific

w_h = the stratum weight of the hth stratum = $\frac{A_h}{A}$ (3), in which

A_h = the area of hth stratum

A = the area of the study area

The estimate for the mean of variance, which describes the precision of the mean of the sample, can be calculated with:

$v(\bar{y}) = \frac{s^2(1-\frac{n}{N})}{n}$ (4), in which

s^2 = the variance of the sample = $\frac{\sum_{i=1}^{n}(y_i-\bar{y})^2}{n-1}$ (5) and

N = the size of the population (the amount of sampling units fitting inside the study area)

n = sample size

Typically, in a finite population the correction factor $(1-\frac{n}{N})$ is usually very close to one and thus it can be ignored. Then the variance of the mean density estimate is sample variance divided with sample size. Mean larvae density estimates were also calculated for each depth zone and for both water column and sediment. In addition, 95% confidence intervals were calculated for the mean larvae density estimate based on the Poisson distribution (Jolly & Hampton 1990). To test the significance of the calculation method the confidence intervals were also calculated with normal distribution. Confidence intervals help with analyzing uncertainty of the density estimates.

The density estimates (individuals/m^2) for each sampling station were calculated for both limnetic and benthic *Chaoborus* and analyzed with the R-program. The data was managed with the SiZer -package in a procedure where the limnetic density data were all pooled together, and the densities were viewed in relation to the depth of each sampling station. The threshold or breakpoint in density in relation to depth was searched with SiZer because there seemed to be a clear breakpoint in density of limnetic density data (Sonderegger et al. 2009). The theoretical background for these piecewise linear regressions is presented in Toms and Lesperance (2003). The benthic density data was managed separately in the same way.

The data from *Chaoborus* sampling was also used to form length distributions for limnetic and benthic larvae. The mean lengths for both benthic and limnetic larvae and their 95% confidence intervals with normal distribution were also calculated. The difference of *Chaoborus* larvae lengths in different habitats and also the lengths of roach and perch that had eaten *Chaoborus* were tested with a t-test. The difference of including *Chaoborus* in diet between the fish species was tested with a Pearson’s Chi-Square test.

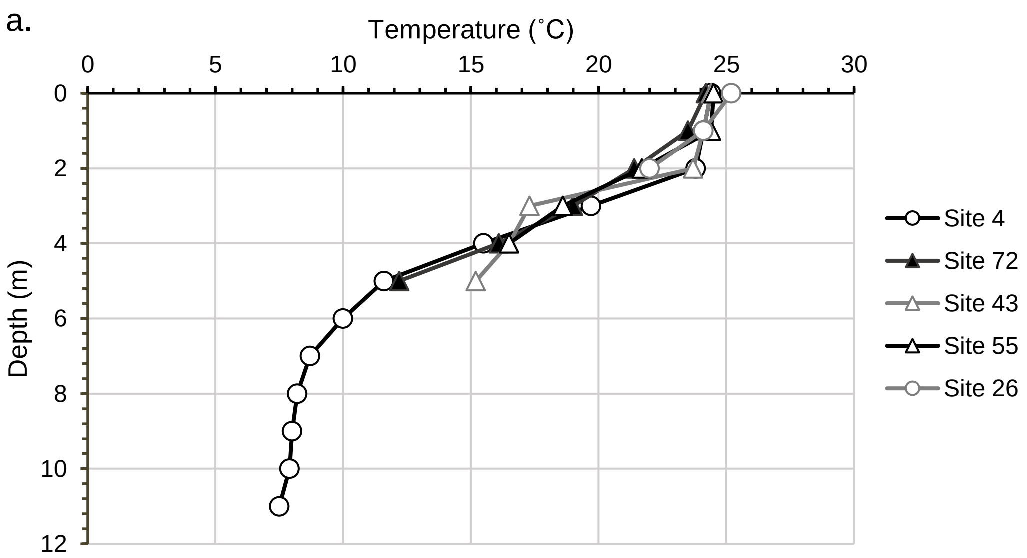
3 Results

3.1 Temperature stratification, concentration of dissolved oxygen, and Secchi disk depth

Temperature and oxygen concentration profiles were relatively similar in the whole lake (Fig. 3). In June, on the field day, the surface water temperature was 25 °C and oxygen concentration was 9.8 mg/l in the epilimnion. Temperature and oxygen concentration also decreased in the water column towards the hypolimnion relatively similarly. These measurements suggested that there were no large differences between the different basins of the lake. However, the temperature and oxygen concentration values had some variation between the sampling stations. For example, in 3 m depth there were variation of 3-7 mg/l in oxygen concentration and 17-20 °C in temperature in June (Fig. 3a and 3b).

Temperature decreased quite steadily through the water column from a maximum of 25.2 °C in the epilimnion to a minimum of 7.5 °C in the hypolimnion (Fig. 3a). The coldest water was measured in the deepest basin (station 4) and the warmest near the littoral area where the water was 2.8 m deep (station 26).

Oxygen concentration was highest in the epilimnion at all stations and lowest at the northern basin >7 m depth and at the southern basin >4 m depth (Fig. 3b). Sampling station 4 was located at the deepest area in the north, and the depth of the water column was 11.8 m there. Oxygen deficiency or hypoxia started at



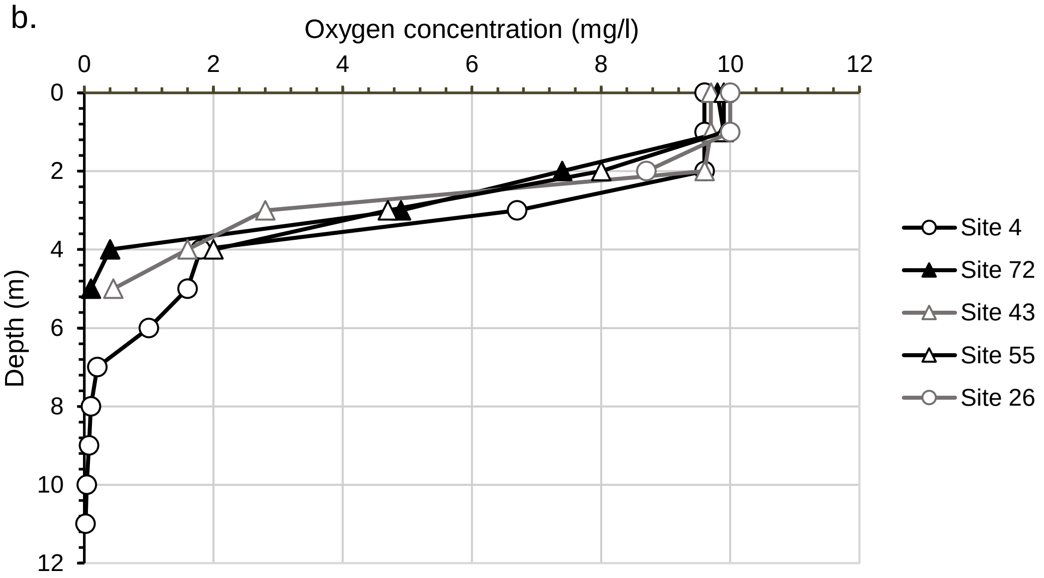


Figure 3. Profiles of temperature (a.) and oxygen concentration (b.) on June 22nd, 2021. Stations were purposedly chosen to cover the whole lake area to find possible differences between these variables.

station 4 already from 4 m depth making the water column hypoxic and basically anoxic from 7 m depth onwards (Fig. 3b).

On the 12th of August, the surface water temperature had decreased a little (21 °C), but the oxygen conditions were even weaker (Fig. 4). In August, at all sampling stations (from where the measurements were taken again) there were *ca.* 0.7-2.8 mg/l of oxygen in 3.5 m depth and below 4 m there were almost no oxygen at all. In June, at sampling station 4, oxygen concentration in 4 m depth was 1.8 mg/l but in August the concentration had dropped to only 0.2 mg/l. In circa 4 m depth there was a sharp transition in temperature (station 4). Compared with June, the stratification had become stronger at the deepest areas in the north in August (at station 4). In addition, the water in the more southern station 43 was noticeably warmer in 5 m depth than in the north at station 4. In August, at sampling station 43 the water temperature in 5 m depth was 17 °C when at station 4 the water temperature was 12.7 °C. Furthermore, near the aeration device raft (next to station 4) the air had a very strong odor of hydrogen sulfide.

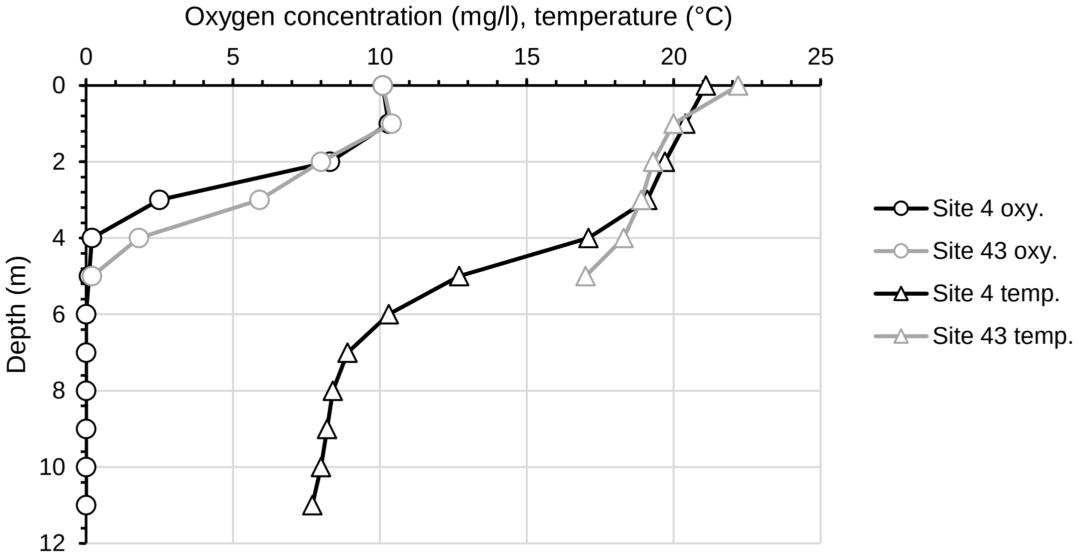


Figure 4. Oxygen concentration (left) and temperature (right) profiles in stations 4 and 43 on the 12th of August. In August, the oxygen concentration was lower below 4 m depth and temperature in the epilimnion had decreased when compared to measurements from June.

The Secchi disk depth was 1.05 m on the first field day on the 22nd of June, and on the 12th of August, it was 1.2 meters. Secchi disk depth values stayed between 0.8 and 1.3 m through the whole study period (Fig. 5). Variation in Secchi disk depth was quite strong, which was probably due to rainfall and algal turbidity. In previous years, measurements of Secchi disk depths have been approximately on the same level (Makkonen 2013).

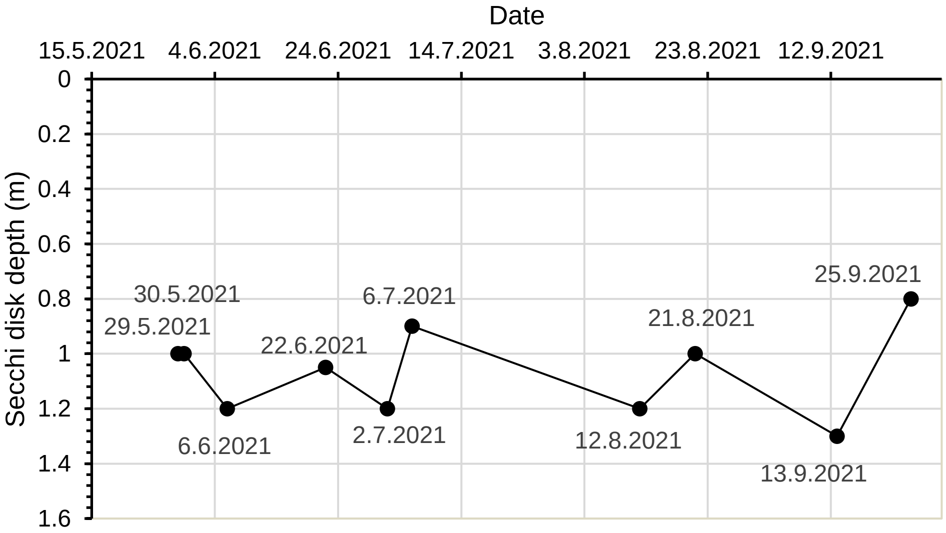


Figure 5. Secchi disk depth measurements from the study lake from the end of May to late September.

3.2 Density and distribution of *Chaoborus*

3.2.1 *Chaoborus* density in the summer of 2021

Considering the whole study lake, at depth zones over 2 m deep the mean larvae density was 271 individuals/m^2 as the weighted average. If the depth zone of 0.0-1.9 m is considered too, and the *Chaoborus* density is expected to be zero, then the mean larvae density would be 202 individuals/m^2. It was assumed that *Chaoborus* do not occur at the shallow littoral areas, and thus they were excluded, and no sampling was executed there. The whole population was calculated to consist of 26 154 individuals in the study lake. Most of the *Chaoborus* occurred in the sediment (22 814 individuals) and the rest (3 340 individuals) in the water column.

The highest density of larvae in the whole study lake was within depth zone 6.0-7.9 m where the mean density was over 1400 individuals/m^2 (Fig 6). The larvae were mostly burrowed into the sediment (1277 individuals/m^2). In the same depth zone, the mean larvae density in the water column was considerably smaller, only 152 individuals/m^2 (Fig. 6).

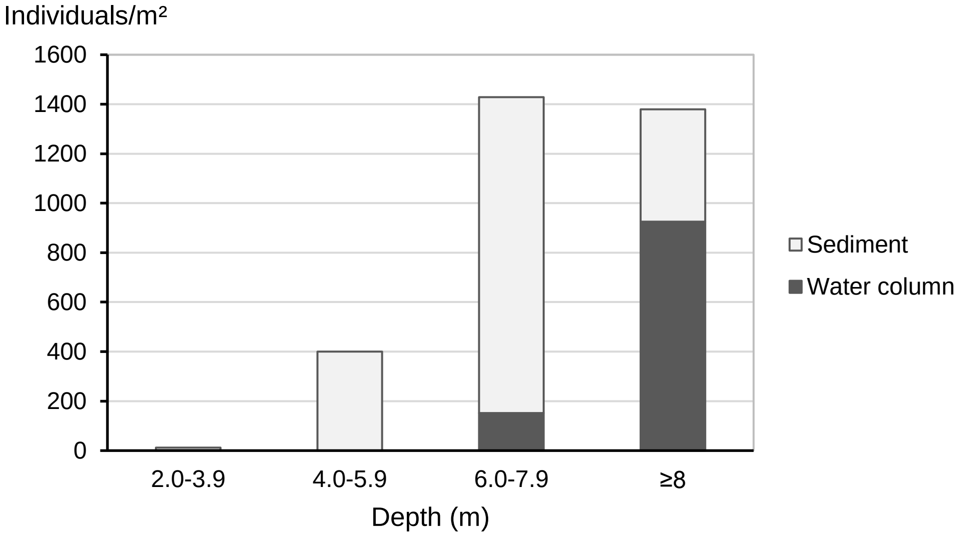


Figure 6. The mean density of *Chaoborus* larvae in the studied depth zones. The highest density was found from the mid-depths in the sediment and only the deepest zones had limnetic larvae. Larvae were scarce in the shallow depths, where they were burrowed into the sediment (2.0-5.9 meters).

The 95% confidence intervals for the density estimate according to Poisson distribution were 160-411 individuals/m^2, and according to normal distribution they were 148-394 individuals/m^2. These two confidence intervals were quite similar (Fig. 7). The primary suggestion for the calculation is the Poisson distribution because it can manage with unique, high values, and the density of *Chaoborus* data is skewed towards the right. For example, the sampling station 10 had a very high and a unique number of larva individuals (Fig. 8b, Table 4). Due to the skewness normal distribution might produce more unrealistic confidence intervals, for example, the lower boundary could go below zero. Since there is no exact previous scientific knowledge of the most suitable distribution for *Chaoborus*, the impact of the different distributions was tested here (Fig. 7). The selection of the used distribution did not have a great effect on the calculation of the confidence intervals.

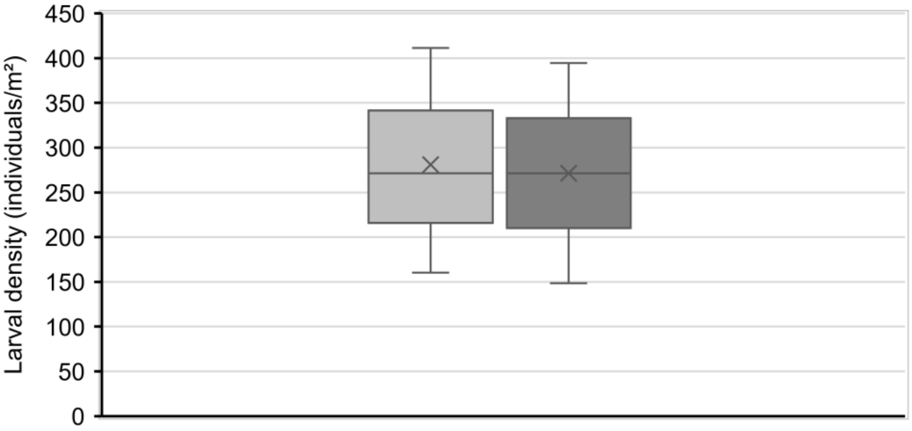


Figure 7. The 95% confidence intervals according to the Poisson distribution (light gray) and normal distribution (dark gray). X = median, line inside the box = mean larvae density (271 ind./m^2), and whiskers = 95% confidence intervals.

3.2.2 The relationship between depth and larvae density

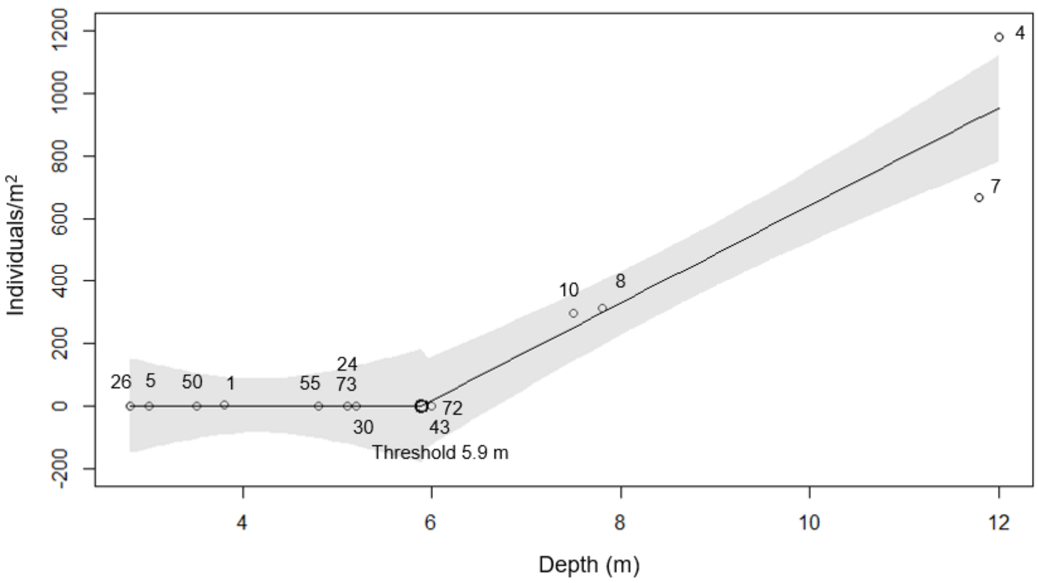
Chaoborus were lacking from most of the samples taken from the water column. The greatest numbers of *Chaoborus* in the water column occurred in the deepest water layers at sampling stations 4 and 7. At sampling station 4 (depth 12 m), the *Chaoborus* density was 1182 individuals/m^2 in the water column, and at sampling station 7 the density was 668 individuals/m^2 (Table 4, Fig. 8a). The mean larvae density in the water column of the deepest layers (areas ≥8 m deep) was 925 individuals/m^2 and in the sediment at the same depth zone it was 454 individuals/m^2 (Fig. 6). At sampling station 4 there were 433 individuals/m^2 larvae and at station 7 there were 476 individuals/m^2 in the sediment (Table 4, Fig. 8b).

Table 4. The density (individuals/m^2) of *Chaoborus* in the study lake at each sampling station. Net = *Chaoborus* samples from the water column, Ekman = *Chaoborus* samples from the sediment, and Total = Net + Ekman.

Station	Depth	Net	Ekman	Total
26	2.8	0.0	0.0	0.0
5	3.0	0.0	0.0	0.0
50	3.5	0.0	0.0	0.0
1	3.8	5.0	43.0	48.0
55	4.8	0.0	519.0	519.0
24	5.1	0.0	433.0	433.0
73	5.1	0.0	130.0	130.0
30	5.2	0.0	519.0	519.0
43	6.0	0.0	909.0	909.0
72	6.0	0.0	519.0	519.0
10	7.5	296.0	2943.0	3239.0
8	7.8	311.0	736.0	1047.0
7	11.8	668.0	476.0	1144.0
4	12.0	1182.0	433.0	1615.0

At sampling station 10, in 7.5 m depth, the densest swarm of larvae (over 2900 individuals/m^2) occurred in the sediment (Fig. 8b). Other sampling stations in the mid-depths had high densities of benthic larvae too, and all in all most of the larvae were benthic when considering the whole study lake (Fig. 6 & 8, Table 4).

a. water column



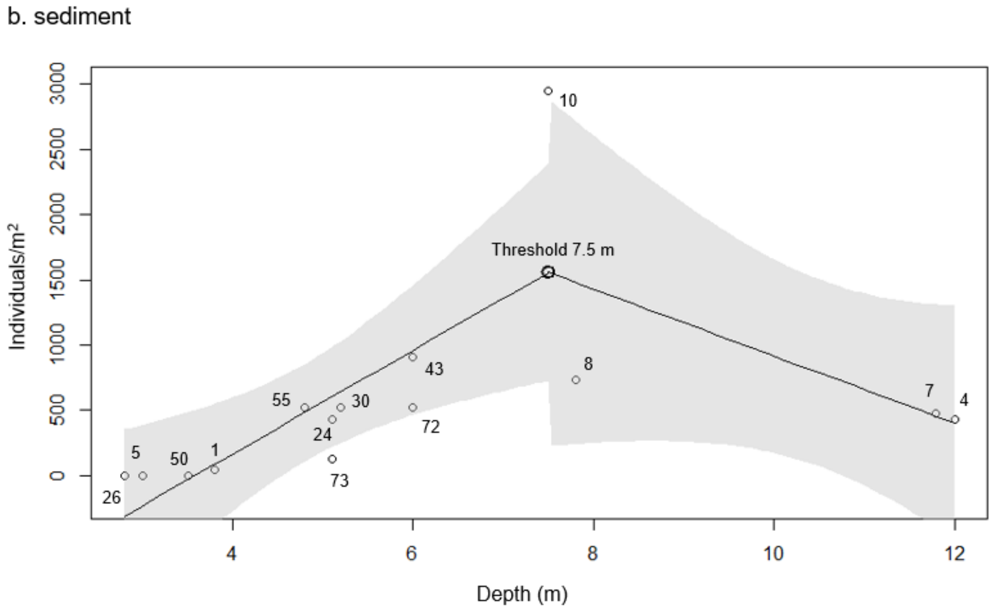
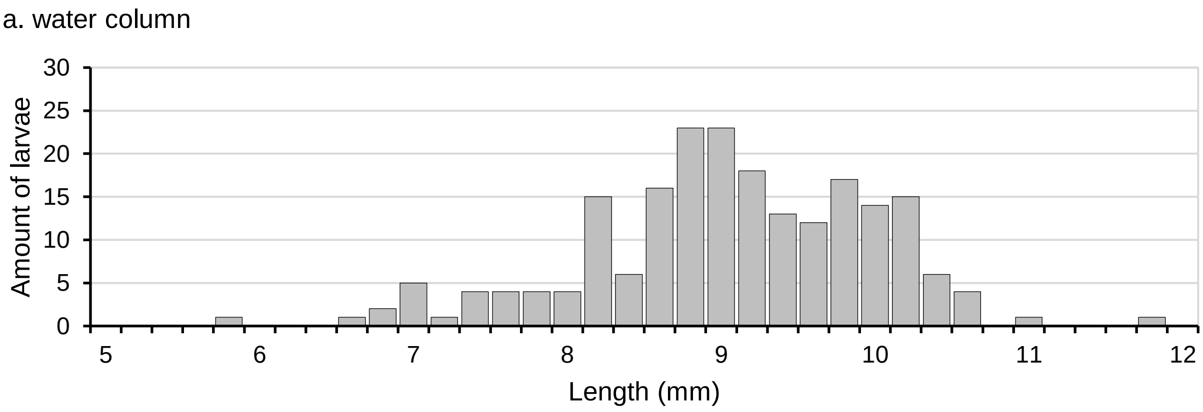


Figure 8. The density of *Chaoborus* (individuals/m^2) in the water column (a.) and in the sediment (b.) at each sampling station. Threshold means the depth of the water column where the number of larvae starts to grow (a.) and after growing decrease again (b.). Threshold depth in water column was 5.9 m and 7.5 m in sediment. The gray area is the bandwidth of the curve.

At the shallow zones, between depths of 2.0-3.9 m, both limnetic and benthic larvae were scarce (Fig. 6, 8a & 8b). The sampling stations 1, 5, 26, and 50 at the shallow depth zone had zero or only a few *Chaoborus* individuals (Table 4). No *Chaoborus* pupae were found from any of the samples.

3.2.3 Length distribution and mean length of *Chaoborus*

The mean length for benthic larvae was 8.4 mm and for limnetic larvae it was 9.0 millimeters (Fig. 9). Both length distributions appeared to be unimodal.



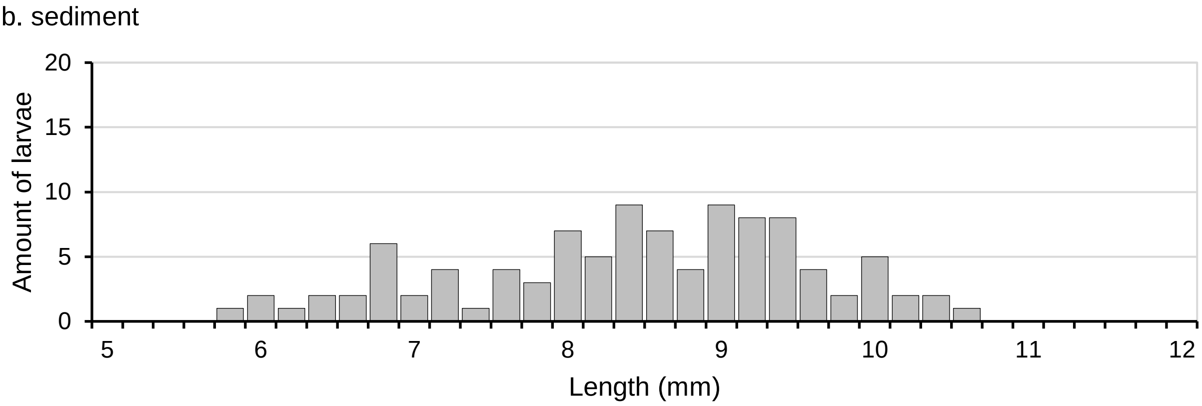


Figure 9. Length distribution of *Chaoborus* larvae a. in the water column and b. in the sediment. Mean length of larvae in the sediment was 8.4 mm (n = 101) and for larvae in the water column it was 9.0 mm (n = 210).

The length data was tested with a t-test, which showed that the mean length of the limnetic and the benthic larvae differed significantly (p = <0.001). When the confidence intervals for mean lengths were calculated the confidence intervals according to normal distribution for limnetic larvae were 8.9-9.1 mm, and 8.2-8.6 mm for benthic larvae (Fig. 10). The confidence intervals were very short, and thus the sample size (n=210 water column; n=101 sediment) had been large enough for the precise estimation of the mean (Fig. 10).

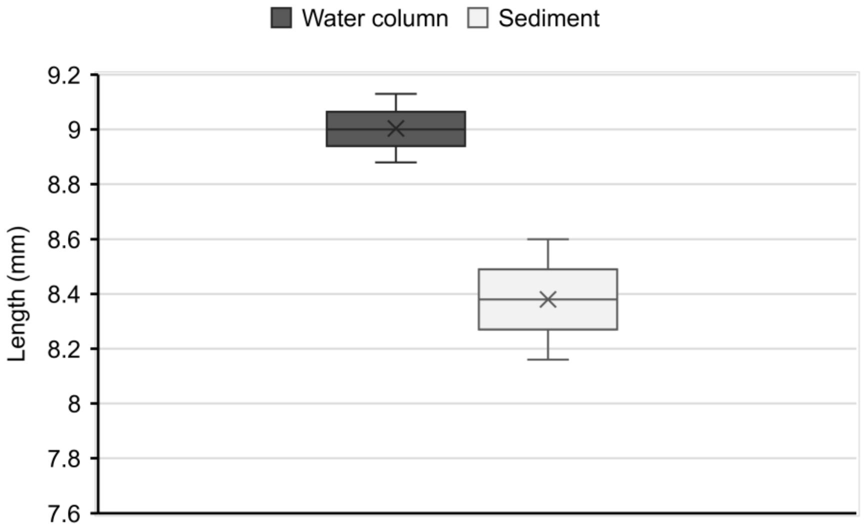


Figure 10. The lengths of the larvae in both habitats and their 95% confidence intervals according to normal distribution. There is a clear gap between the minimum of water column and the maximum of sediment. X = median, line inside the box = mean length of larvae, and whiskers = 95% confidence intervals.

3.2.4 Echo sounding observations and distribution of larvae

Echo sounding at the study lake's southern basin (max. depth only >6 m) did not reveal any limnetic larvae swarms. However, the locals surveyed the southern basin only two times while they did many repeating surveys at different occasions at the northern basin.

Echo sounding at the northern basin (max. depth >12 m) revealed that the limnetic *Chaoborus* population was usually located between 5.5-8.5 m depth in June and for the first half of July (Fig. 11a). Larvae were most abundant during this period as the population was drawn as yellow-orange by the echo sounder. A common sight during June to early July at daytime was that a dense *Chaoborus* population appeared on the screen of the echo sounder when water depth was over 6 meters. The upper boundary of the larvae layer was located at 6 m and it reached the sediment as a dense mat. When deeper water layers (over 10 m) were reached, the population did not usually reach the bottom anymore (Fig. 11a). Thus, between 10-12 m there were very little larvae and the echo sounder drew only sparsely located blue dots, or no larvae at all.

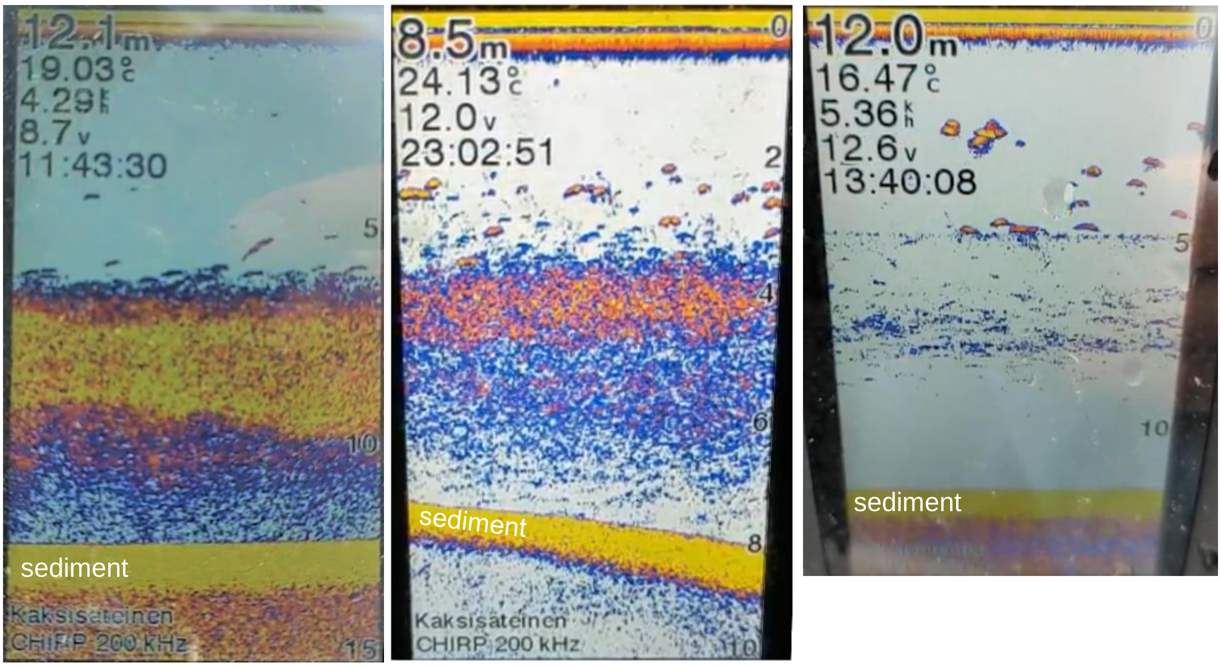


Figure 11. Pictures of the echo sounder's screen. Swarm of larvae on the 6th of June (a.) at daytime, on the 6th of July (b.) at nighttime, and on the 21st of August (c.) at daytime. The swarm of limnetic *Chaoborus* is the yellow-orange coloration and blue dotting in the water. The densest swarm of larvae is between *ca.* 6-9 m (yellow-orange coloration). The larger spots on top of the larvae swarms are fish.

Echo sounding observations made at night at the northern basin revealed that the limnetic larvae population had reached a shallower depth between *ca.* 3-5 meters. The larvae had clearly started to migrate upwards toward the epilimnion already at 11 pm (Fig. 11b). Once the larvae population had reached only 1.9 m depth at nighttime and the whole depth of the water column was only 5 meters. The population was dense yellow-orange and it was clearly closer to the littoral zone. These observations demonstrate both the vertical and horizontal migration behavior of the larvae.

Starting approximately from the last half of July to the last half of August the swarms of limnetic larvae seemed sparser on the echo sounder's screen at the northern basin. Instead of the typical yellow-orange coloration, the swarm was drawn on the screen mostly as sparsely located blue dots (Fig. 11c). The depth of this blue coloration was the same as previously *ca.* 5.5-8.5 meters. The decrease in the density of limnetic *Chaoborus* was also supported by the plankton net hauls done on the 12th of August because the deep layers were basically empty when only few small *Chaoborus* larvae (length *ca.* 5 mm) were caught then in the first place.

From early September (5th of Sept.) the echo sounder was again drawing a dense yellow-orange swarm of limnetic larvae in the water column at the deepest areas of the northern basin. The larvae were located close the bottom sediment in all videos. In late September echo-surveys showed sometimes a dense limnetic larvae population and sometimes only sparse, blue dotting, which suggested that the occurrence of the limnetic population fluctuated. Last observations made on October 17th showed again only sparse blue dotting.

3.3 Diet analyses of roach and perch

3.3.1 *Chaoborus* in the diets of roach and perch

Only a small portion of the diets of roach and perch consisted of *Chaoborus* larvae in June (Fig. 12 & 13). In early July, however, over 50% of the daytime diet and almost 90% of the nighttime diet of roach consisted only of *Chaoborus* larvae

(Fig. 12). In early July, perch had eaten *Chaoborus* both during the day and at night (Fig. 13). On the 31st of July and 1st of August, larger perch individuals had eaten also *Chaoborus* at night, though, the share of larvae from the total diet is very small. Smaller perch did not eat *Chaoborus* at all in July-August (Fig. 13). Both pupae and larvae of *Chaoborus* were found in the diet of roach at nighttime on the 1st of August. No *Chaoborus* larvae or pupae were found from the diet of roach or perch in September (Fig. 12 & 13).

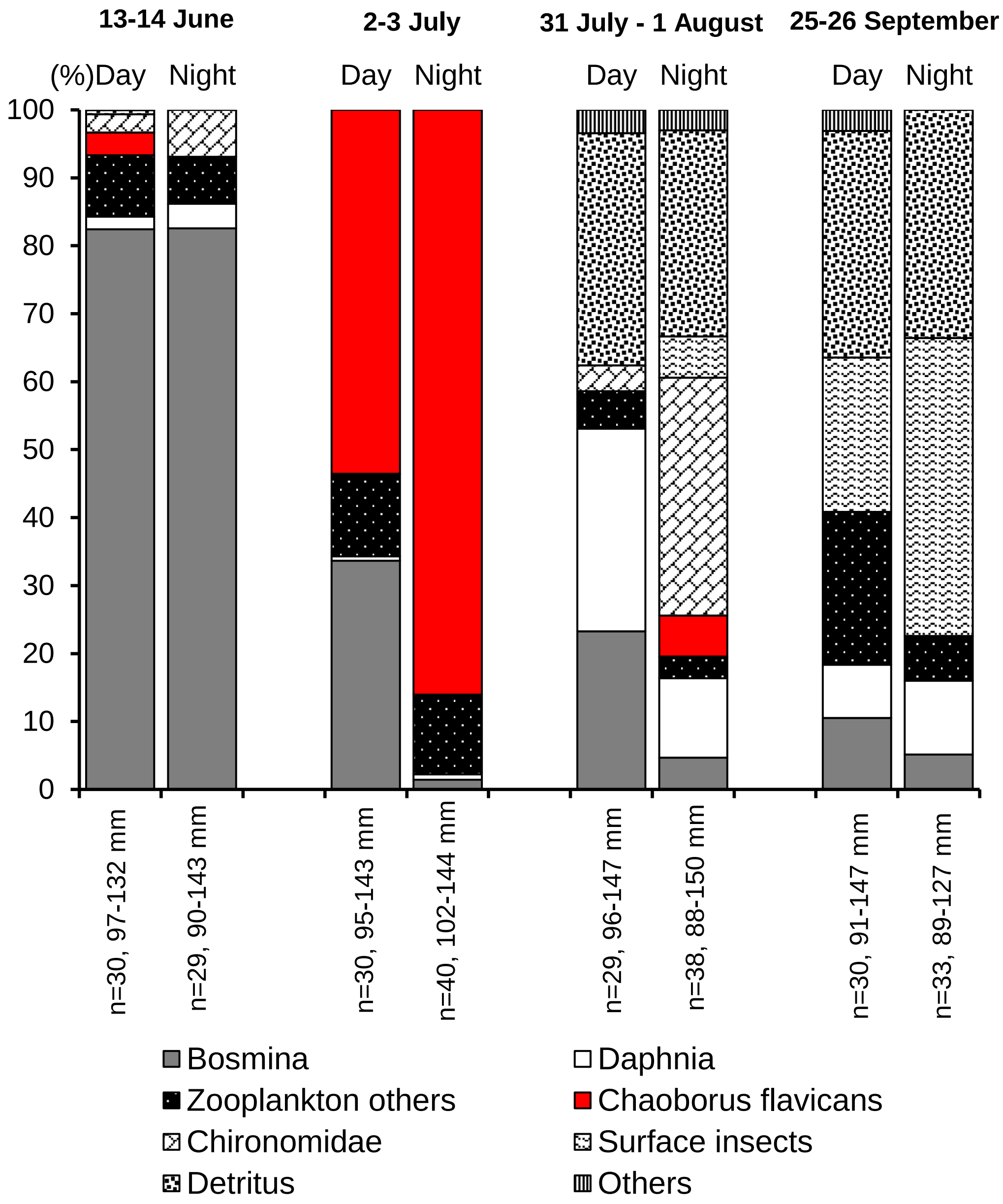


Figure 12. The gut contents of roach analyzed with a volume/percentage method from the long intestine. It appears that *Chaoborus* larvae were popular food items in early July.

The total number of roach that had eaten *Chaoborus* was 52 (total number of studied roach = 259) and for perch it was 8 (total number of studied perch = 206). They were tested with a Pearson's Chi-Square test. The difference between the two species was significant in relation to including *Chaoborus* into their diets (Chi=26.8, df=1, p<0.001). In addition, the lengths of roach and perch that had eaten *Chaoborus* did not differ significantly (two-sided t-test, t=-1.556, df=58, p=ns).

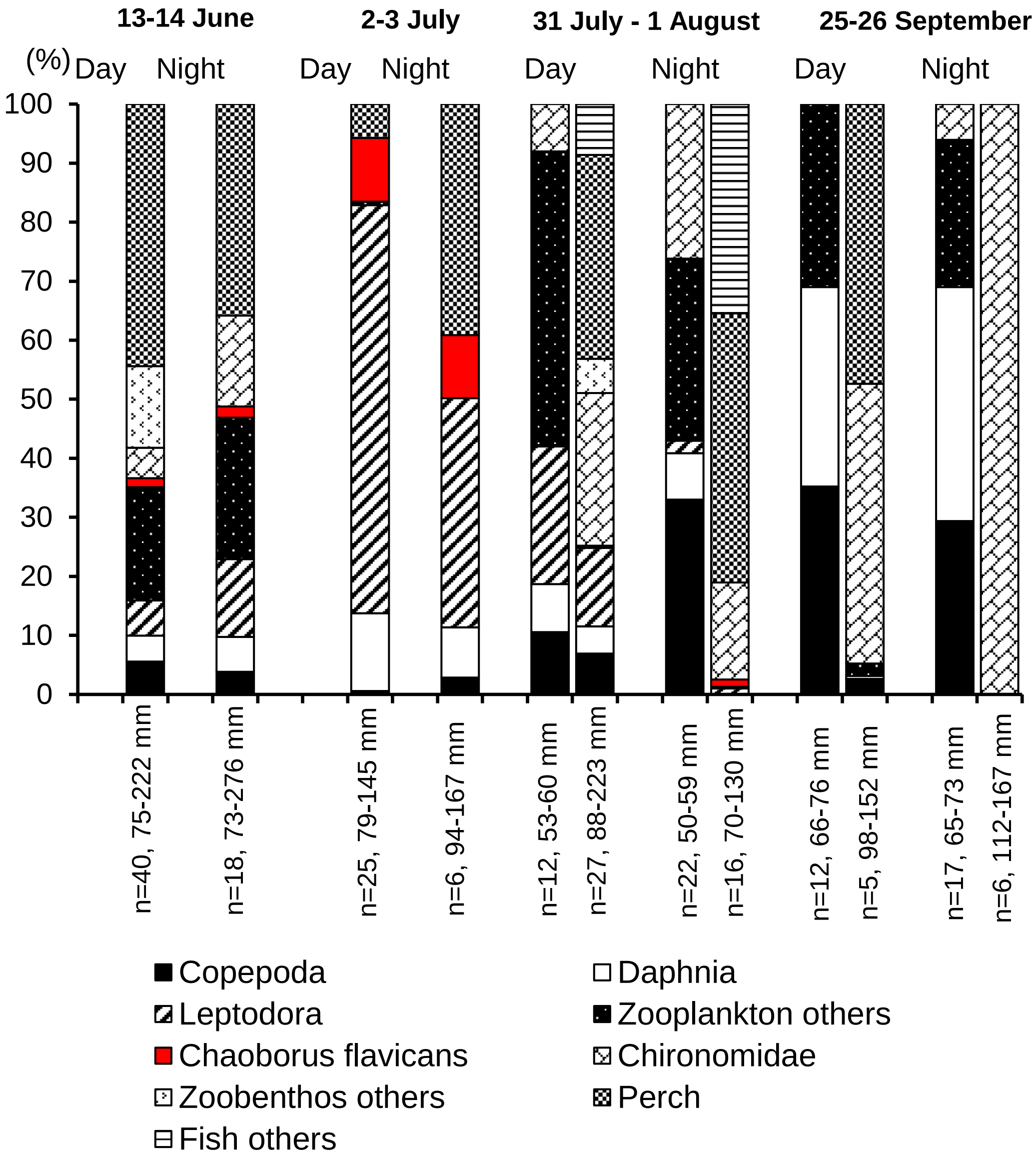


Figure 13. The gut contents of perch analyzed with a scoring method from the esophagus and stomach. *Chaoborus* larvae seemed to be less important food items for perch in general when compared to roach.

3.3.2 Other food items

It appears that roach favored cladocerans and more specifically bosminids in their diet in June (Fig. 12). Other zooplankton (*Copepoda*, *Leptodora*, *Chydorus*) and *Chironomidae* formed nearly the rest of the diet of roach. Perch had eaten a lot of other perch both during the day and at night in June (Fig. 13). Other popular food items were other zooplankton (*Chydorus*, *Limnosida*, *Diaphanosida*, *Sida*, *Eurycercus, Leptodora*), and *Chironomidae*. Perch had also eaten daphnids, copepods, and other zoobenthos (*Asellus*, *Notonectidae*). Other zoobenthos was also present in the diet of perch during the day (Fig. 13).

In early July, bosminids and other zooplankton were popular in the diets of roach (Fig. 12). *Leptodora* was the most common food item in perch stomachs during the day and at night in early July (Fig. 13). Cannibalism was found, too, because especially at night perch had eaten other perch (Fig. 13). Daphnids and copepods were also included in the early July diet of perch.

In July-August, the roaches had eaten again a lot of cladocerans dominantly daphnids (Fig. 12). The roach had also eaten quite a lot of detritus both during the day and at night. Furthermore, the roaches had eaten a lot of *Chironomidae*, and surface insects at night, and a bit of other zooplankton and other food items such as *Ostracoda* and plant material during the day and at night (Fig. 12). In July-August, the guts of the larger perch individuals consisted of either smaller perch or other fish (bream, roach) both during the day and at night (Fig. 13). The diet of small perch consisted mostly of other zooplankton and *Leptodora* during the day. At night, they had eaten mostly copepods and *Chironomidae*. Larger perch individuals had eaten *Chironomidae* and *Leptodora* too.

In late September, the diet of roach consisted mostly of detritus and surface insects during the day and at night, and less of cladocerans (Fig. 12). In September, large perch individuals had eaten mostly smaller perch during the day and *Chironomidae* during the day and at night too (Fig. 13). The diet of the caught smaller perch consisted of other zooplankton, daphnids, and copepods both during the day and at night (Fig. 13). At night, the smaller perch also ate some *Chironomidae*.

4 Discussion

4.1 *Chaoborus* in Lake Jouttenus

4.1.1 Comparison of *Chaoborus* density and findings of distribution

The mean density of *Chaoborus* in Lake Jouttenus was the lowest when the density of *Chaoborus* was compared to other studied lakes (Fig. 14). The densities in other lakes might be higher because they are clay-turbid lakes and Jouttenus is a humic lake. Clay-turbidity together with depth, low light conditions, and low oxygen concentration in the hypolimnion supports the high density of *Chaoborus* (Liljendahl-Nurminen et al. 2002; Horppila & Liljendahl-Nurminen 2005). However, Lakes Piilolammi and Pannujärvi are humic lakes, and they have the highest densities of *Chaoborus* in figure 14. The high density of Lake Piilolammi probably resulted from an efficient refuge for the larvae and from a fish assemblage, which did not include efficient *Chaoborus* predators (Malinen et al. 2011a). At Lake Pannujärvi the high density could be explained by exceptionally low oxygen concentration in the hypolimnion (Malinen & Vinni 2013a).

However, Lakes Sahajärvi and Kytäjärvi are clay-turbid lakes and their *Chaoborus* density was 276 individuals/m^2 in Lake Sahajärvi (Malinen et al. 2008) and 260 individuals/m^2 in Lake Kytäjärvi in areas over 1.5 m depth (Malinen et al. 2011b). It should be noted that even though the mean density of *Chaoborus* in the humic study lake, Lake Jouttenus, was 271 individuals/m^2 it was in areas ≥2 meters. When the number of *Chaoborus* was expected to be zero in depth zones 0.0-1.9 m the mean density of larvae in Lake Jouttenus was estimated as 202 individuals/m^2 in the whole lake. Thus, the depth zones used in the calculations can also influence the mean density estimates. In addition, the amount and distribution of sampling stations might influence the mean larvae density estimates. The sampling of *Chaoborus* was weighted to emphasize the deeper areas with stratified sampling at Lake Jouttenus, but, for example, in the study including Lakes Sahajärvi and Hunttijärvi the sampling was not weighted to emphasize the deeper areas of the lakes (Malinen et al. 2008).

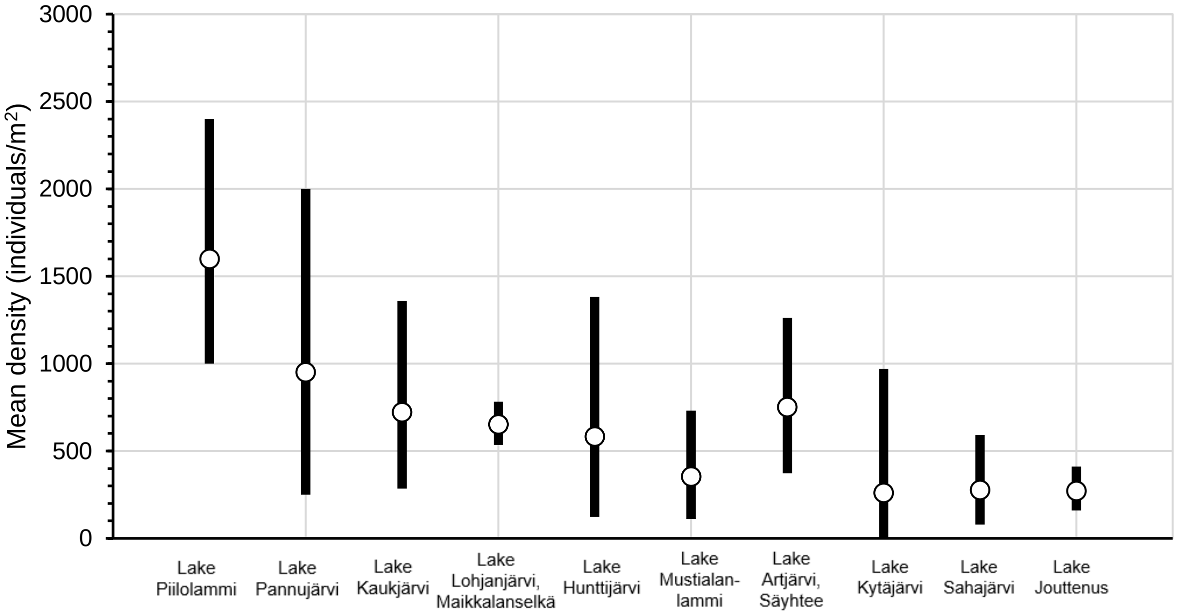


Figure 14. The mean density estimates for *Chaoborus* larvae in areas >1.5 m depth and their 95% confidence intervals in several other studied Finnish lakes (Malinen & Vinni 2013a). The other lakes are clay-turbid except Lakes Pannujärvi, Piilolammi, and Jouttenus are humic lakes. The density values include *Chaoborus* densities both in the water column and the sediment. At Lake Piilolammi the sediment was sampled with a Kajak sampler (Malinen et al. 2011a) and not with an Ekman sampler as in the other studies.

It should be also noted that the lakes from other studies in figures 14 and 15 had an exceptionally high density of *Chaoborus*. Thus, these lakes do not represent the average densities of *Chaoborus* in lakes and here the relatively low density of *Chaoborus* in Lake Jouttenus was compared to these lakes with a particularly high *Chaoborus* density. The results of these other studies are, however, comparable with the results of this current study since the sampling has been conducted with the same equipment and methods. The sampling has also been done approximately at the same time in May-June in all studies.

Surprisingly, the greatest larvae density (>1400 individuals/m^2) was found from depth zones between 6.0-7.9 m at Lake Jouttenus. Most of the larvae were burrowed into the sediment in this depth zone regardless of hypoxia and darkness in the water column. Humic substances might not provide as good of a visual refuge as clay-turbidity and low oxygen concentration does not provide enough refuge by itself (Pekcan-Hekim et al. 2006; Liljendahl-Nurminen et al. 2008). Thus, it appears that the hiding behavior of the *Chaoborus* and their relatively low density at Lake Jouttenus could be partly explained by the lack of a strong refuge.

Perhaps studies considering watercolor and *Chaoborus* could shed light on the efficiency of humic substances as providing visual refuge for *Chaoborus*.

Furthermore, the limnetic larvae were quite restricted to the deep areas of the northern basin (max. depth 12.1 m), which had the lowest oxygen concentration and the least amount of light in the water column. Thus, the avoidance of predators appeared to be the easiest for *Chaoborus* at the deepest areas in the north where indeed the highest density of limnetic *Chaoborus* clearly occurred. Deep sampling stations (areas ≥8 m) had less benthic than limnetic larvae, and the water column was hypoxic starting from 4 m depth or even anoxic near the bottom, which allows the larvae to stay in the water column (cf. Pekcan-Hekim et al. 2006; Liljendahl-Nurminen et al. 2008). However, the area of this depth zone (areas ≥8 m) is only 2.05 ha, which is 2% of the whole lake area. Perhaps, if the deep area in Lake Jouttenus would have been larger and/or deeper, the mean *Chaoborus* density could have been higher and their range larger. However, in agreement with previous studies, depth together with low light conditions and hypoxia created the most suitable refuge conditions for the limnetic *Chaoborus* in lake Jouttenus.

The lack of a strong refuge could also explain why the southern lake basin had no limnetic *Chaoborus*. In the southern basin good light conditions do not provide enough shelter from fish predation because the basin is not deep enough and there are no refuge zones of low oxygen concentration since the epilimnion almost reaches the bottom. Liljendahl-Nurminen et al. (2008) argued that depth favors *Chaoborus* because stratification contributes to the formation of refuge zones and a daytime refuge provides better possibilities for finding prey. For example, in a study from Lake Vähä-Tiilijärvi the *Chaoborus* population was shown to be very scarce due to good light conditions in the water column (Malinen & Vinni 2020). However, in Estlander et al. (2009) the density of *Chaoborus* did not clearly correlate with water color because from their four study lakes the lightest water color and the second highest density of *Chaoborus* occurred in the same lake. In Estlander et al. (2009) the low oxygen concentration together with high water color seemed to mainly dictate the density of *Chaoborus*. Thus, depth creates darkness and enables both the lake to stratify and the larvae to migrate in the water column, which allow the larvae to escape from their predators (cf. Liljendahl-Nurminen et al. 2008; Malinen & Vinni 2020). Thus, *Chaoborus* in the southern basin of Lake Jouttenus were hiding in the sediment because burrowing allowed their survival.

4.1.2 Comparison of *Chaoborus* mean lengths

The benthic *Chaoborus* in Lake Jouttenus were shorter than in other studied Finnish lakes when mean lengths of benthic larvae were compared (Fig. 15). Lakes Pannujärvi, Suolijärvi (Hyvinkää), and Piilolammi are humic lakes too like Lake Jouttenus, but the others are clay-turbid lakes. In general, the mean length of benthic larvae in humic lakes has been a little shorter when compared to clay-turbid lakes. However, the mean length of benthic *Chaoborus* in Lake Jouttenus was very short when compared to the other studied lakes in Malinen and Vinni (2013a). It might suggest that the amount of prey was low, or the larvae were forced to hide constantly, and they could not get to their prey, and thus, the larvae grew slowly due to lack of resources. The nutritional situation could also have been so weak in Lake Jouttenus that even the relatively small *Chaoborus* population was too dense or overly dense and caused the lack of resources. For example, in Malinen and Vinni (2017) the short lengths of *Chaoborus* were

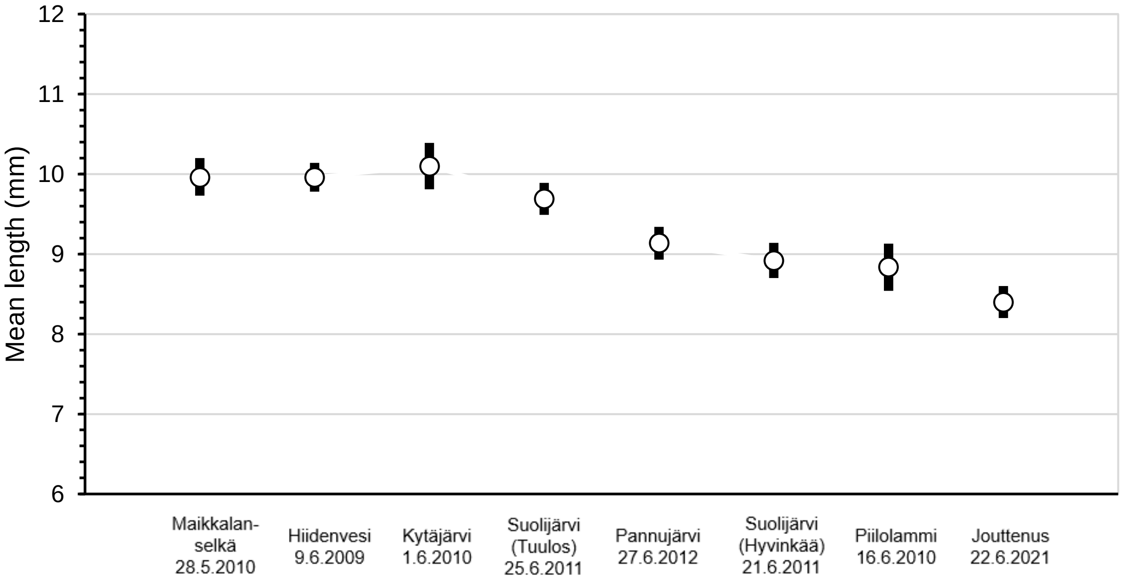


Figure 15. The mean length of *Chaoborus* larvae in the sediment and their 95% confidence intervals in several studied Finnish lakes. Lakes Pannujärvi, Suolijärvi (Hyvinkää) and Piilolammi are humic lakes too like Lake Jouttenus. The rest are clay-turbid lakes. The mean lengths of *Chaoborus* in other lakes from Malinen and Vinni (2013a).

probably the consequence of an overly dense population of *Chaoborus* which could have caused the lack of resources. However, there was not a zooplankton survey coupled to this current study, which could have shed light on the nutritional situation and on the zooplankton assembly of Lake Jouttenus.

At Lake Jouttenus, the length distributions of *Chaoborus* were unimodal both for benthic and limnetic larvae, which suggested that there was probably only one main emergence period. On the 22nd of June, no pupae were found, which indicates that the beginning of the hatching period was not close yet. On the 12th of August, the deep areas of the north were practically empty of larvae, which suggested that the hatching had happened between the field days and shortly before the second field day because only a few larvae from the new generation were caught. It has been noted that *Chaoborus* in clay-turbid lakes hatch earlier than in humic lakes (e.g. Malinen et al. 2011a; Malinen & Vinni 2013a). For example, in the study of Liljendahl-Nurminen et al. (2002) at the clay-turbid Lake Hiidenvesi the highest *Chaoborus* biomass occurred in May-June and the new larvae generation inhabited the lake already in late July and early August. In Lake Jouttenus, however, the highest limnetic larvae biomass occurred approximately in June-July and the new generation appeared on early September. Thus, the life cycle of larvae in these lakes differ by approximately a month from each other.

The length of the larvae life cycle is influenced by variables such as water temperature, the amount and quality of food items, burrowing behavior, and the length of the daylight period (Parma 1971). *Chaoborus* might not grow as quickly in humic lakes than in clay-turbid lakes because as formerly mentioned humic substances do not provide as an efficient refuge from predators as clay-turbidity together with low light and low oxygen conditions (cf. Horppila & Liljendahl-Nurminen 2005; Pekcan-Hekim et al. 2006; Liljendahl-Nurminen et al. 2008). Thus, an efficient refuge allows the larvae to graze on food items and to stay in the water column. Parma (1971) also discusses that larvae do not grow while burrowed into the sediment, which appears to be the situation in Lake Jouttenus because the *Chaoborus* larvae were very short and hiding a lot in the sediment. In addition, Stenson (1990) found that acidic conditions (pH min. 4.5; max. 4.8) together with weak nutritional resources influenced the size of *Chaoborus* larvae significantly via producing stress. Between years 2019 and 2020 the value of pH had varied between 5.9-7.1 in 5 m depth in Lake Jouttenus (Hertta-database). Further studies on pH and *Chaoborus* could provide information on the effects of acidity to larvae lengths in humic lakes in general.

4.1.3 Other findings

Echo-surveys made at night suggested that *Chaoborus* migrated vertically in Lake Jouttenus. *Chaoborus* migrated towards the epilimnion at night to predate on zooplankton, and at dawn swam back into the hypolimnion to the refuge zone. Similar migration behavior has been observed in several other studies (Malueg & Hasler 1966; Parma 1971; Sardella & Carter 1983; Horppila et al. 2000). In addition, the larvae did not occur immediately at 4 m depth when the oxygen concentration decreased to unsuitable levels for fish rather the larvae mat usually occurred slightly deeper at 5 m depth. This probably resulted from the risk of fish predation too since fish can search for prey even in low oxygen concentration (e.g. Rahel & Nutzman 1994; Malinen et al. 2005b; Horppila et al. 2009).

An echo-survey done at mid-July at night revealed that limnetic *Chaoborus* occurred at a quite shallow depth near the littoral zone at Lake Jouttenus. These may not have been *Chaoborus* larvae but rather pupae. Parma (1971) observed that *Chaoborus* pupae can burrow into the firmer sediment near the littoral zone while the larvae do not burrow into the shallows. However, at least Liljendahl-Nurminen et al. (2002) did not find significant correlation between the sediment quality and larvae density at Lake Hiidenvesi. Borutsky (1939) and Wood (1956) suggested that *Chaoborus* might migrate to shallower depths due to the approaching of emergence period and hatching. In addition, in echo-surveys done between late September and mid-October *Chaoborus* appeared to gather again into the deepest areas of Lake Jouttenus. Then *Chaoborus* density decreased which probably resulted from the burrowing of the new generation for overwintering as *Chaoborus* tend to accumulate in deep areas in fall (Borutsky 1939; Wood 1956). Thus, the observations from Lake Jouttenus would be compatible with previous observations about pupae migrating to shallower depths in late summer and larvae accumulating into deep areas of lakes in fall (Borutsky 1939; Wood 1956; Parma 1971).

In addition, annual variance in the population size of *Chaoborus* should be considered together with the results of this current study. Repeated studies between 1999-2017 from Lake Hiidenvesi have shown that the annual variation of the *Chaoborus* population size can be large (Malinen & Vinni 2017). According to Malinen and Vinni (2017) most of the variation can be quite well explained by calculating the thermal sum of plus degree-days. In general, weather conditions appeared to explain the variance because after warm summers the density of *Chaoborus* was high and after cool summers the density was low (Malinen & Vinni 2017). The size of the parent population and the following new larvae population do not appear to have a connection that would explain the size of the new emerging generation (Malinen & Vinni 2017). However, they discussed that in case of a low larvae density, this connection cannot be fully excluded because the lowest density was 690 individuals/m^2 in 2017 (Malinen & Vinni 2017). This is the case with Lake Jouttenus because the mean density estimate was 271 individuals/m^2 there. It should be also noted that Lake Jouttenus is a completely different kind of lake in comparison to Lake Hiidenvesi.

Therefore, the warm and sunny weather in the early summer of 2021 might have a positive impact on the development of the following *Chaoborus* year class (cf. Malinen & Vinni 2017). Thus, the next generation might be even more abundant in the next summer of 2022. Similarly, the size of the *Chaoborus* population this year in 2021 at Lake Jouttenus might be smaller than on average and give misleading results. Furthermore, many other factors such as changes in biological interactions or in abiotic variables could influence the size of the *Chaoborus* population. Only monitoring and repeated studies could give reliable answers and take several varying environmental factors into consideration.

4.2 Is the size of *Chaoborus* population controlled by fish?

The gillnets set for catching fish for diet analyses suggested that fish avoided the water column below 4 m depth at the pelagic. One of the gillnets was set to the deep pelagic in June and September and only few fish were caught with those gillnets. The fish were caught near the net’s headline, which was in 4 m depth and rest of the net below was empty. Other gillnets near the shore bank and at the epilimnion of the pelagic caught more fish. Furthermore, the oxygen measurements near the aeration device raft at the deep pelagic in the north suggested that the oxygen concentration below 5 m depth generated practically anoxic living conditions for fish. The aeration device appears not to circulate water efficiently enough to provide any benefits for the oxygen conditions there. Thus, the fish seemed to avoid the low oxygen concentration at the deep pelagic where the density of *Chaoborus* was the highest. However, the low oxygen concentration did not fully restrict the fish that were searching for prey (cf. Rahel & Nutzman 1994; Malinen et al. 2005b; Horppila et al. 2009).

The diet analyses of this current study suggested that *Chaoborus* represented a relatively important food item for roach at least in July. In July at nighttime, almost all the gut contents of roach consisted of *Chaoborus*. Only two roach individuals had also other food items in their guts. However, roach did not include *Chaoborus* abundantly in their diet during the whole study period. Perch did not seem to include *Chaoborus* significantly into their diet because the number of perch individuals that had eaten *Chaoborus* was low in all study occasions and if perch had eaten *Chaoborus* there were also other food items in the guts too. Roach had eaten more *Chaoborus* than perch, which might result from perch relying more on its vision as a predator than roach (Helfman 1979; Bergman 1988; Diehl 1988; Jansen & Mackay 1992). Thus, perch appeared to be more restricted by the humic substances and low light conditions prevailing in Lake Jouttenus than roach (cf. Estlander et al. 2010). All things considered; it is possible that roach slightly controlled the *Chaoborus* population in Lake Jouttenus, but perch did not. However, the situation could change if the fish density changed in Lake Jouttenus, for example, after a mass removal of fish. At least the presence of fish and the kairomones they produce might scare the *Chaoborus* forcing them to hide (Dawidowicz et al. 1990; Tjossem 1990). Furthermore, the kairomones produced by fish may already alone force the larvae to hide, and thus, if fish were removed *Chaoborus* might stay in the water column, which could change the state of the lake for worse.

It should be also noted that in this current study the number of studied roach and perch varied between species and study occasions. There were not enough different sized perch to be studied on every occasion because the number of studied perch varied between different catches from 6 to 40 individuals. The number of studied roaches was more stable since they varied only between 29-40 individuals. Furthermore, the locals had taken some of the largest perch and they were not analyzed at all, which might influence the results. In addition, a significant percentage of the caught fish (over 95%) were perch or roach, and other fishes (bream, bleak) represented a very small portion of the catch. Some of the bream and bleak individuals that were analyzed had eaten *Chaoborus*. However, due to the lack of results any proper conclusions cannot be drawn on how all the other fish species than roach and perch included *Chaoborus* in their diets. The gillnets were not set evenly across the lake area and thus, for example, fishes that occur mostly in shallow depth zones might be missing due to low capture percentage rate. This study was planned to emphasize the diets of the fish occurring at the pelagic area of the northern basin because the densest *Chaoborus* population most likely occurred there (cf. Liljendahl-Nurminen et al. 2002).

4.3 The plague of Lake Jouttenus?

It appears that *Chaoborus* might not be behind of the yearly blooming of blue-green algae during summer months. However, a strong bloom of *Gonyostomum semen* was detected below 2 m depth on the 12th of August, which suggested that the plague of Lake Jouttenus might not be the blooming of blue-green algae but rather the blooming of a mixotrophic organism *G. semen*. At Lake Jouttenus high values of chlorophyll-a have been connected to the blooming of *G. semen* (Makkonen 2013), and the species has been observed at the lake on several other years too (Järvi-meriwiki).

G. semen typically thrives in environments that resemble the conditions of Lake Jouttenus. Allochthonous humic substances draining from the surrounding forested catchment area provide a rich source of dissolved organic carbon (DOC) for the use of organisms such as *G. semen* (Trigal et al. 2011). The seeping of phosphorus from the bottom sediment at Lake Jouttenus also favors *G. semen* since it can migrate vertically between water layers (Salonen & Rosenberg 2000). Vertical migration allows *G. semen* cells to reach the phosphorus rich water in the hypolimnion of Lake Jouttenus where it can gain nutrients scarce in the epilimnion and avoid metabolic losses (Salonen & Rosenberg 2000). Furthermore, mixotrophy provides *G. semen* advantage over other algae since it can switch from autotrophy during the day to heterotrophy at night. During a bloom, *G. semen* cells can form 95% of the whole phytoplankton biomass (Rask et al. 1998; Hehmann et al. 2001), and recurring blooms are typical for it if it has once reached a high biomass (Hehmann et al. 2001; Willén 2003).

Trigal et al. (2011) tried to establish possible connections between *Chaoborus* and blooming of *G. semen* in humic lakes because these two organisms seem to prefer similar habitats. However, due to the difficulty of interpreting the causality of food web interactions and availability of resources in their study lakes Trigal et al. (2011) could not establish clear causality between the two organisms. In addition, Malinen & Vinni (2013a) discussed that there is little scientific evidence of the interaction of *G. semen* and *Chaoborus*, but they suggested these two organisms could in theory benefit from the presence of the other in the same lake.

At Lake Jouttenus the *Chaoborus* population was not very dense, although, the blooming of *G. semen* seemed intensive. In the study of Trigal et al. (2011) the high biomass of *G. semen* was accompanied by a large *Chaoborus* population, however, all lakes that had large *Chaoborus* population did not have a high *G. semen* biomass. Thus, it does not seem very likely that there is a clear, straight-forward benefitting interaction between these two organisms, but further studies could help in formulating their possible relationship.

4.4 Recommendations for restoration

From the perspective of restoration methods roach would have been the targeted species if mass removal of fish would be an option. However, it is not recommended to remove roach since they ate *Chaoborus* at least at the time when the density of larvae was the highest. There is a possibility that in theory *Chaoborus* population could grow and expand their range if the number of fish predators decreased significantly as speculated by Malinen & Vinni (2013b). Liljendahl-Nurminen et al. (2005) also speculated that a more abundant occurrence of *Chaoborus* could enhance the blooming of blue-green algae via the increased predation pressure on zooplankton, which reduces the predation pressure on phytoplankton. This could be the case in Lake Jouttenus. Other fishes might also influence the density of *Chaoborus* population by predating on the larvae. However, without proper results of the effects of the other fishes it is not recommended to realize a mass removal of fish.

Monitoring studies of stocking smelt in lakes troubled by *Chaoborus* have shown changes in the behavior of the larvae. For example, Lake Alajärvi was previously bothered by *Chaoborus* and extensive blooms of blue-green algae, however, after the successful stocking of smelt the behavior of *Chaoborus* changed more towards hiding deep in the water column and burrowing into the sediment (Malinen & Vinni 2019). The diet analyses of fish caught from Lake Alajärvi have shown that adult smelts ate *Chaoborus* effectively whereas other former dominant fishes (perch and vendace) did not (Malinen & Vinni 2019). Smelt, however, requires cold water temperatures and enough oxygen in the water column (Dembinski 1971; Nellbring 1989). In case of Lake Jouttenus smelt stocking will not be an option because the oxygen conditions are so poor. In addition, the dark-colored water of Lake Jouttenus may promote the warming of the water temperature to a such level during summer months which is not preferred by smelt (cf. Dembinski 1971; Nellbring 1989). Thus, smelt that prefers colder water temperatures may not even be able to adapt to the living conditions in Lake Jouttenus.

All restoration methods that aim to reduce the humic substances and nutrients draining from the catchment area of the lake could be the most efficient methods for Lake Jouttenus. The enhancement in visibility depth and water clarification would influence both *Chaoborus* and *G. semen* in long-term. The *Chaoborus* would become more prone to fish predation when their daytime refuge in the hypolimnion of the deep areas would be less effective. Vertical migration would become more dangerous for the larvae, they could not be able to grow or graze zooplankton as efficiently as before, and they would need to hide even more than before. Thus, the population would stay as sparse as possible. *G. semen*, on the other hand, benefits from the abundant inputs of dissolved organic carbon draining from the surrounding catchment area (Trigal et al. 2011). If the amount of DOC decreases, *G. semen* would not be in such an advantageous position when compared to other algae species in the lake and would not be able to bloom as often or as extensively. This would benefit the overall recreational usage of the study lake.

One of the most challenging issues concerning the restoration of Lake Jouttenus is to assure the landowners to commit to reducing the external loading from the catchment area. Several restoration methods have been proposed in the restoration plan (Makkonen 2013), but according to the locals none of them have been realized. It is highly recommended to implement water protection measures that decrease the inflow of DOC and nutrients from the catchment area to enhance the visibility depth of the lake. It is not recommended to invest in projects that demand large funding if they provide benefits only in short-term and the results will not be lasting. Some restoration methods such as chemical treatments could provide a rapid change in the state of a lake, but they would most probably require repeating treatments in Finnish conditions (cf. Oravainen 2005). Furthermore, environmental conditions, and lake hydrology and morphology have been found to influence the success of chemical treatments in lakes (e.g. Bakker et al. 2016; Huser et al. 2016). Thus, background studies of the treated lake and careful planning are of grave importance when selecting a treatment. Longevity of the results is not self-evident and monitoring studies are needed to assess the results (cf. Bakker et al. 2016; Huser et al. 2016). Furthermore, the rapid and drastic changes in the living conditions of the lake organisms could possibly cause harm to the ecological functioning of the lake (Bakker et al. 2016). Hence, financial investments should be targeted towards lasting solutions that would not compromise neither the recreational nor the ecological values of Lake Jouttenus.

5 Conclusions

The mean density of *Chaoborus* in Lake Jouttenus was a little lower than expected, only 271 individuals/m^2 in areas over 2 m depth. Most of the *Chaoborus* were hiding in the sediment between 6.0-7.9 meters. The highest density of limnetic *Chaoborus* was found from areas ≥8 m depth. The lack of an efficient refuge could explain both the low mean density and the distribution of *Chaoborus* (Pekcan-Hekim et al. 2006; Liljendahl-Nurminen et al. 2008). Low light conditions and low oxygen concentration created a safe refuge for limnetic larvae only in the deepest areas (≥8 m depth) of the lake in the north. In the shallower depth zones (<6 m depth) or in the southern lake basin of Lake Jouttenus, the larvae were probably too exposed to fish, and thus the daytime distribution of *Chaoborus* was restricted only to the sediment.

In addition, the lack of resources could also explain the low mean density and the low mean length of benthic *Chaoborus* in Lake Jouttenus (cf. Malinen & Vinni 2017). The mean length of benthic *Chaoborus* was lower in Lake Jouttenus than in the other studied *Chaoborus* lakes. Slow growth may result from the lack of resources alone, or also from, for example, the larvae not being able to reach their prey in the epilimnion at daytime since they were too exposed to fish predators in water column, which could have caused the lack of resources too.

The low oxygen concentration in the hypolimnion of the study lake did not fully restrict the fish from predating on *Chaoborus* (cf. Rahel & Nutzman 1994; Malinen et al. 2005b; Horppila et al. 2009). The diet analyses revealed that perch did not include *Chaoborus* in its diet to a significant extent, but roach ate *Chaoborus* when the density of larvae were at its highest. However, if the fish density decreased the density of *Chaoborus* could increase, and thus, mass removal of fish is not recommended.

All things considered; it appears that *Chaoborus* are not behind the yearly blooming of blue-green algae in Lake Jouttenus. The internal loading of the lake and the loading of nutrients and humic substances from the catchment area could possibly be the main reasons behind the blooms of blue-green algae and *G. semen* during summer season at the study lake (cf. Makkonen 2013).

All restoration methods aiming at reducing the external loading of humic substances and nutrients especially from the lake catchment area could be the most efficient methods for Lake Jouttenus. In best case scenario restoration methods can improve the state of the study lake and preserve both recreational and ecological values. Financial losses can be avoided if only the most suitable and effective restoration methods will be implemented. Frankly, it would be pointless to invest into expensive short-term methods because they will not provide positive lasting changes. While long-term plans have the highest probabilities to be successful, the results will, however, most probably and unfortunately be achieved in the distant future. The results of this current study can help with planning restoration projects on the national level because many lakes in Finland are dystrophic and have a hypoxic hypolimnion.

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